BIOENG-458
Next-generation biomaterials

Prof. Li Tang

Lecture 2 Nano-biomaterials

Spring 2025

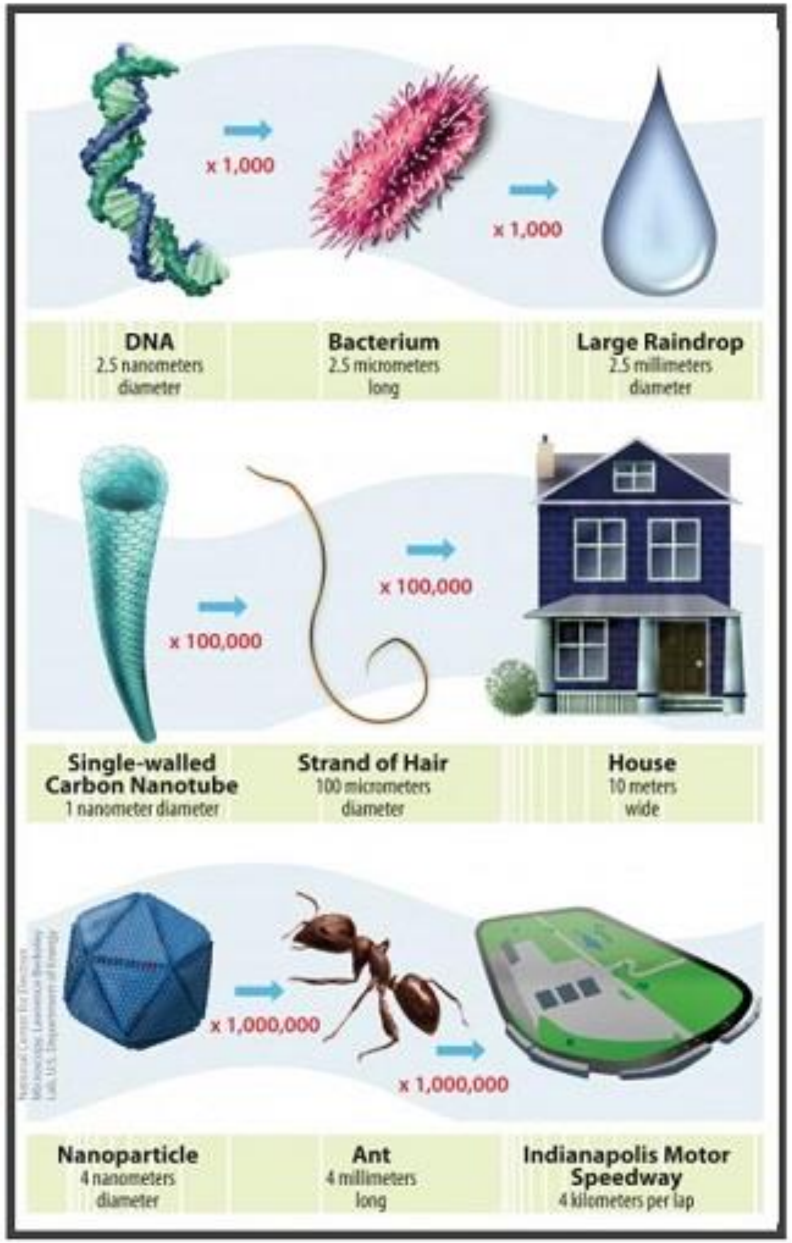
What is nano size?

The prefix "nano" means one-billionth, or 10^{-9}; therefore, **one nanometer is one-billionth of a meter**.

here are some examples:

- A sheet of paper is about 100,000 nanometers thick.
- A strand of human DNA is 2.5 nanometers in diameter.
- There are 25,400,000 nanometers in one inch.
- A human hair is approximately 80,000–100,000 nanometers wide.
- A single gold atom is about a third of a nanometer in diameter.

On a comparative scale, a sphere with a diameter of 1 nanometer is to a softball as **a softball is to the Earth**.



Credit: National Center for Electron Microscopy, Lawrence Berkeley National Laboratory, U.S. Department of Energy

[Just How Small Is “Nano”? | National Nanotechnology Initiative]

What is so special about “Nano”?

Scale at which quantum effects dominate

Macroscale: Inert, Yellow

Credit: Nkauj Vang, University of Minnesota

Nanoscale: Catalytic, Red/Purple

What Is So Special about “Nano”? | National Nanotechnology Initiative

What Is So Special about “Nano”?

- **Scale at which quantum effects dominate**
- **Scale at which surface behavior plays a larger role**
- **Scale at which much biology occurs**

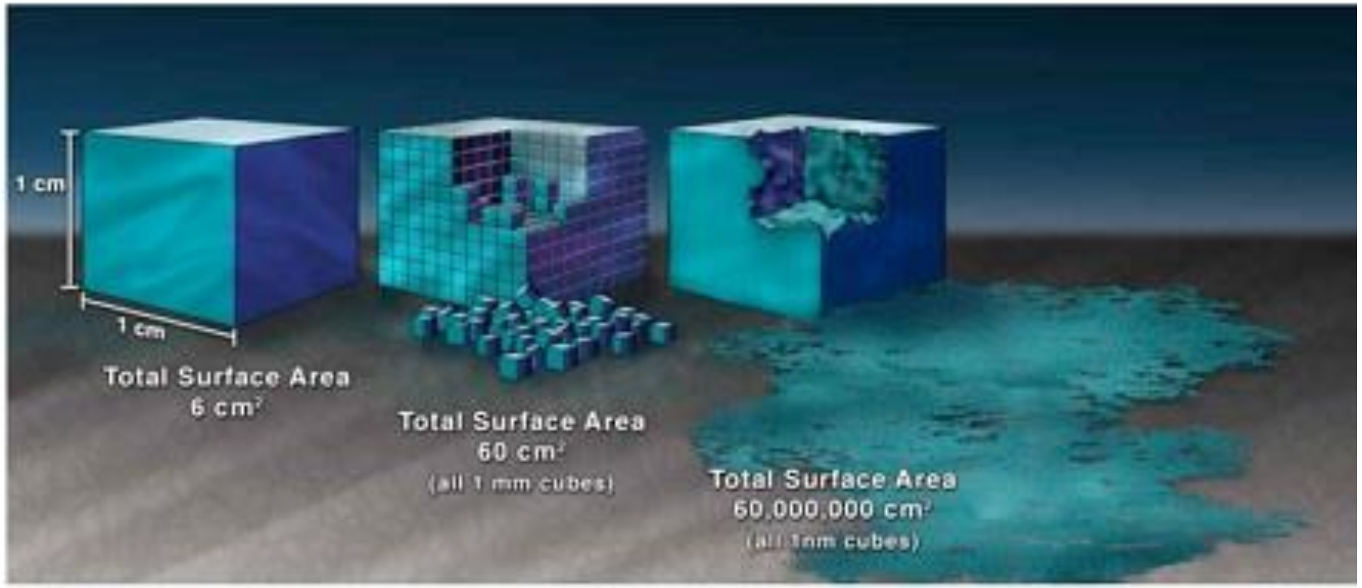
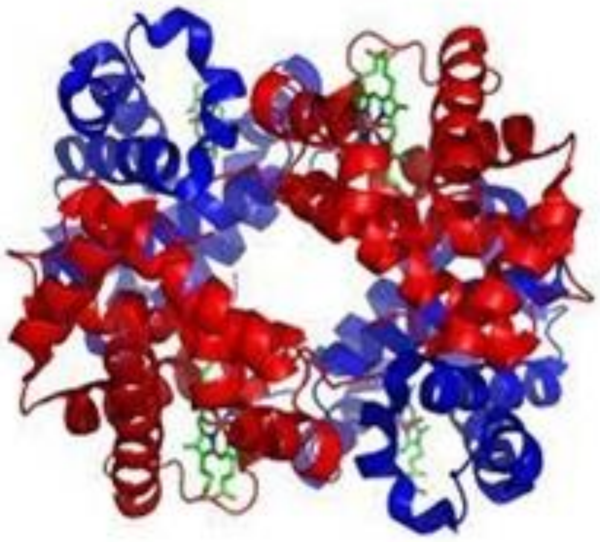


Illustration demonstrating the effect of the increased surface area provided by nanostructured materials.



Computer simulation of hemoglobin, a naturally occurring nanoscale protein that is found in blood. (Image: Centers for Disease Control)

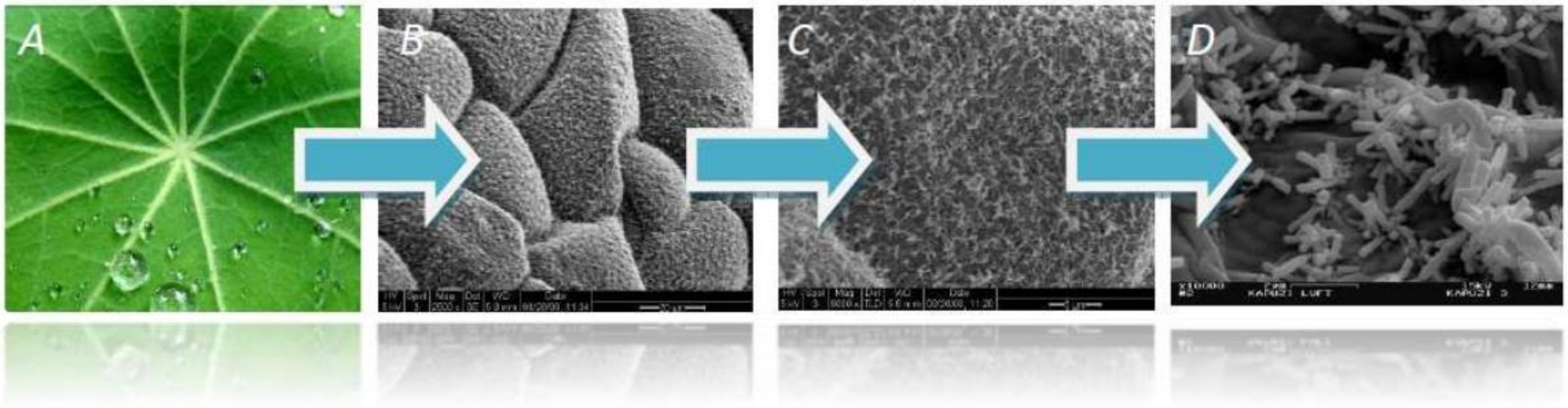
hemoglobin, the protein that carries oxygen through the body, is 5.5 nanometers in diameter.

What Is So Special about “Nano”? | National Nanotechnology Initiative

Does nature make nanomaterials in the biological systems?

Modified from "NATURAL NANOMATERIALS" Shreyas Nangalia

Nanostructures of Lotus leaf



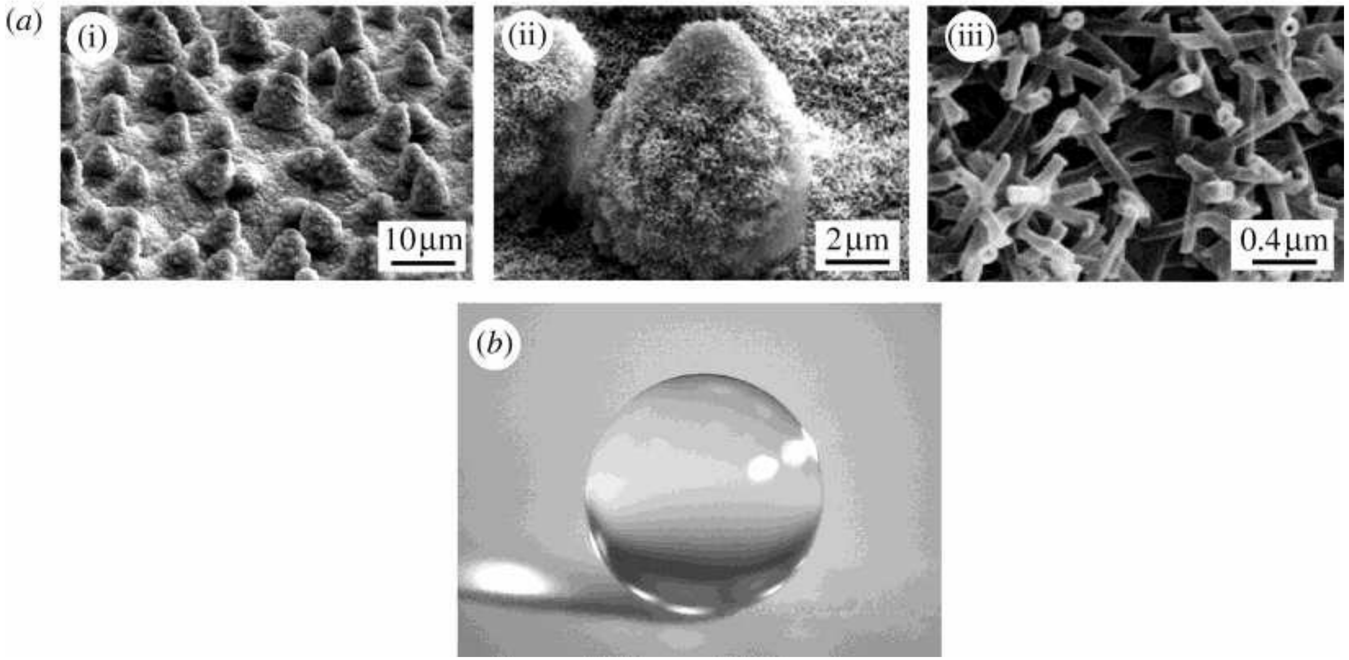
"Lotus effect"

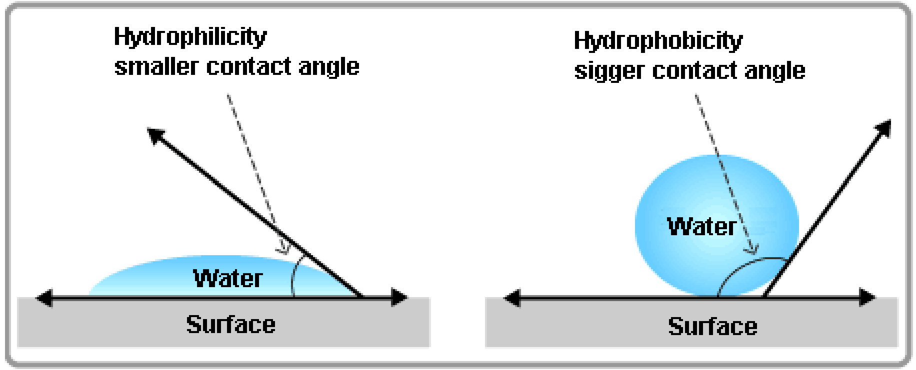
Self-cleaning properties of the lotus plant are the combination of the micro/nano-structures of the leaves and of the epidermal cells on its rough surface, which are covered with wax crystals

Modified from "NATURAL NANOMATERIALS" Shreyas Nangalia

Self Cleaning effect

• Large contact angle due to epicuticula wax and micro-/nano-meterscale bumps on the leaf
• The epicuticula wax provides the low surface free energy, and the micrometer-scale bumps brings a large extent of air trapping when contacting with water, which is essential for superhydrophobicity.





Superhydrophobic: Contact angle > 120°

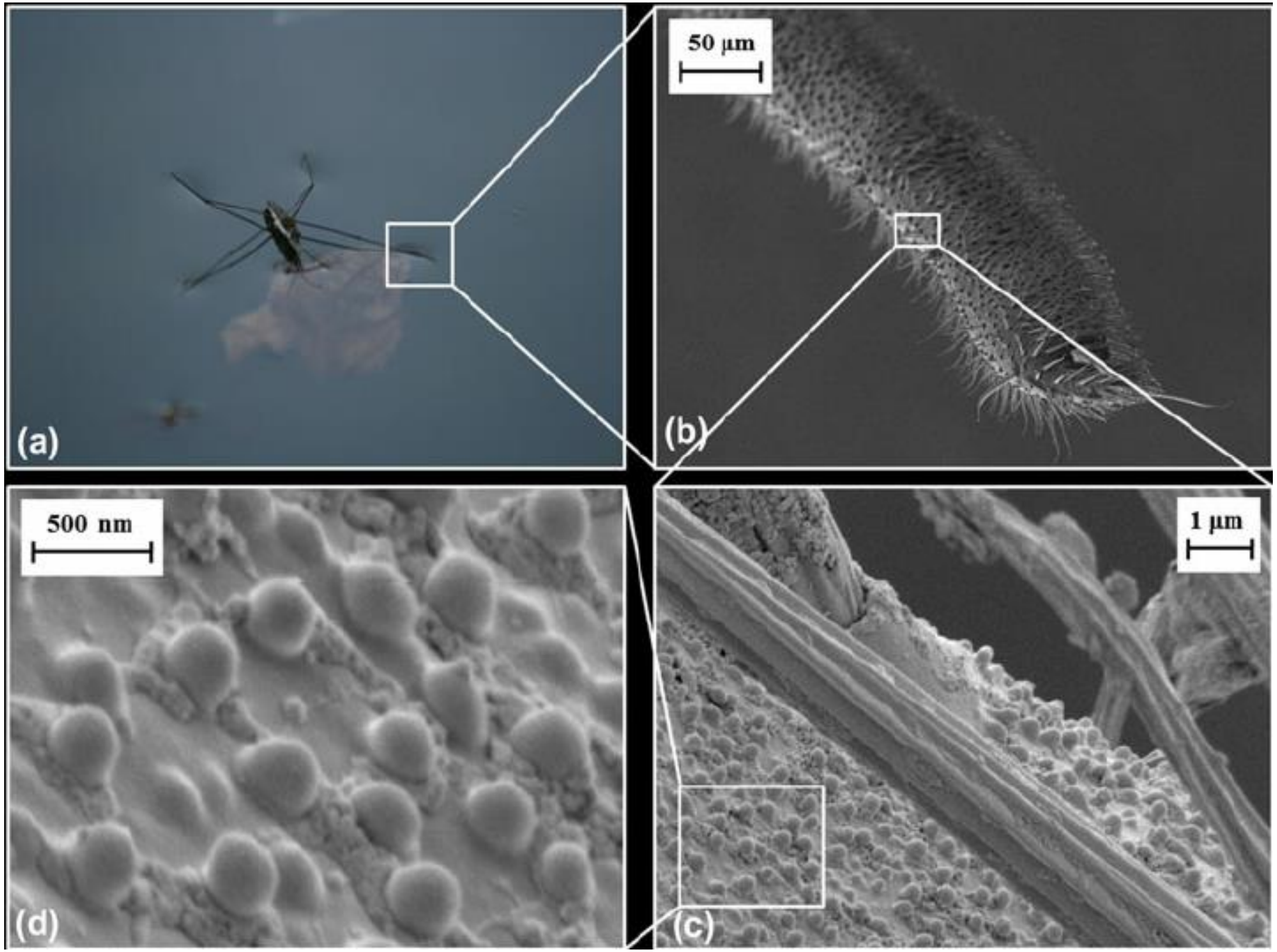
Bhushan B Phil. Trans. R. Soc. A 2009;367:1445-1486

http://www.arc-flash.com.tw/our_tec2-5-en.htm

Self Cleaning effect - applications

Treatments, coatings, paints, roof tiles, fabrics and other surfaces that can stay dry and clean themselves in the same way as the lotus leaf.

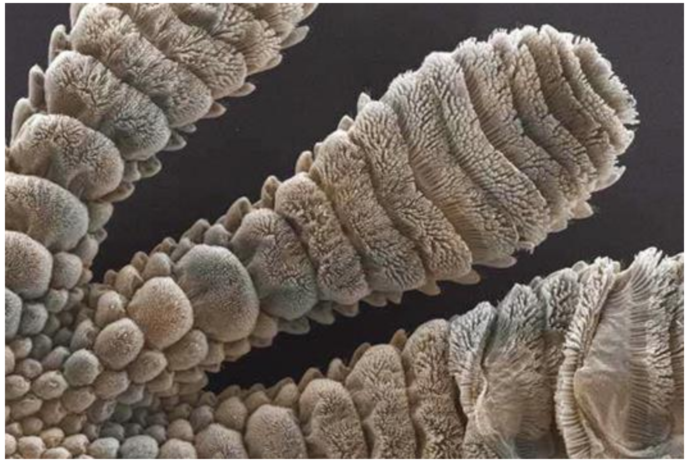
Natural nano-structure in the biological systems



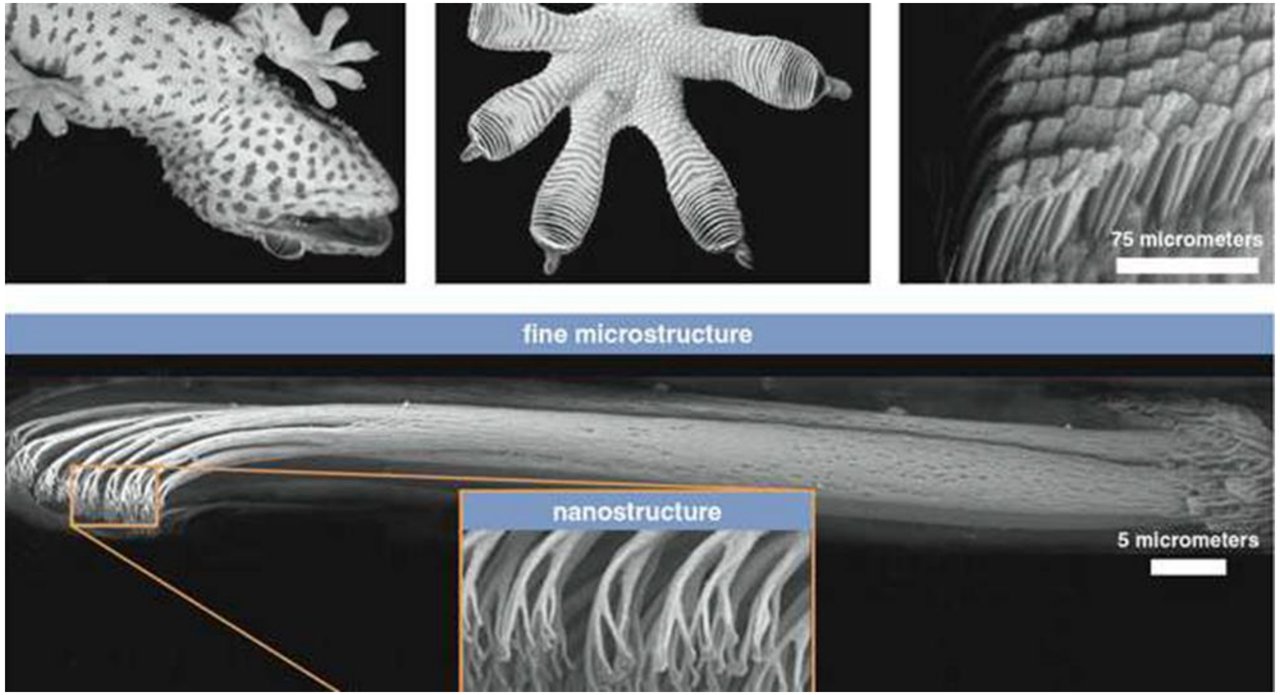
Two-level hierarchical structure of the **water strider'**s leg, which exhibits microstructures with fine nanoscale grooves under the FESEM eye

https://www.researchgate.net/figure/258797975_fig4_FIG-5-Two-level-hierarchical-structure-of-the-water-strider-'-s-leg-which-exhibits

Natural nano-structure in the biological systems

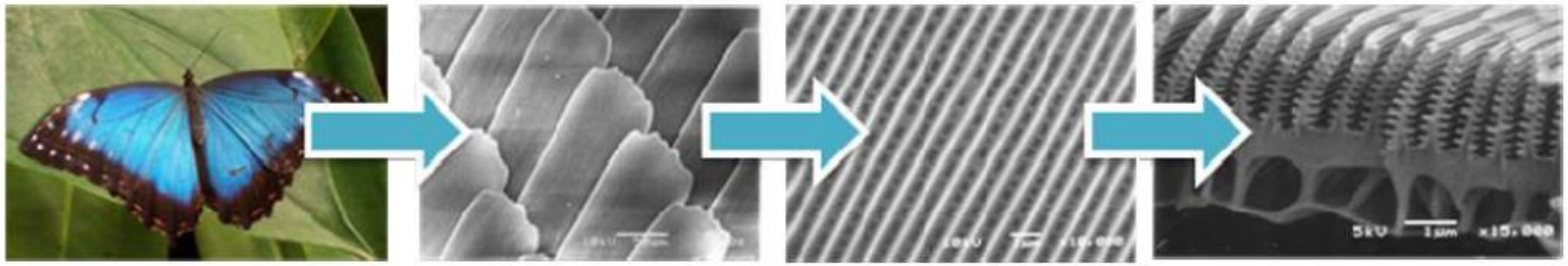


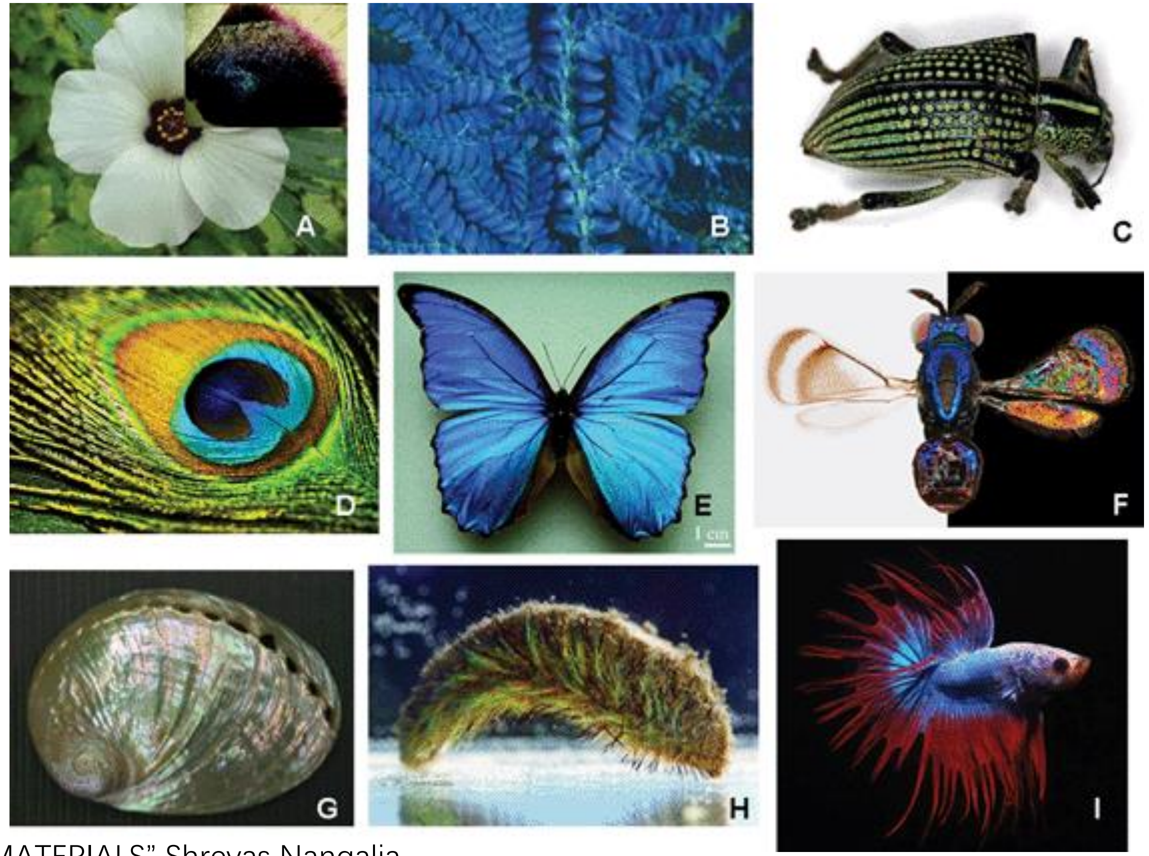
Gecko's feet



Modified from "NATURAL NANOMATERIALS" Shreyas Nangalia

Natural nano-structure in the biological systems





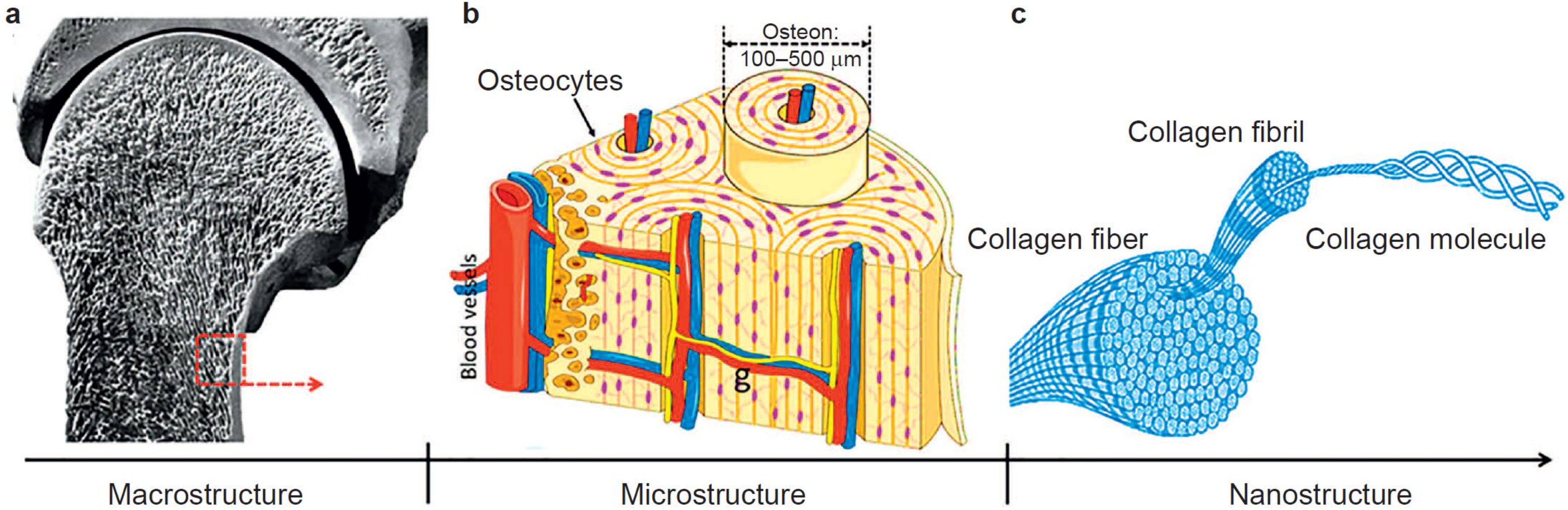
Structural coloration

http://rsbl.royalsocietypublishing.org/content/6/1/128

Modified from "NATURAL NANOMATERIALS" Shreyas Nangalia

Natural nano-materials inside our body

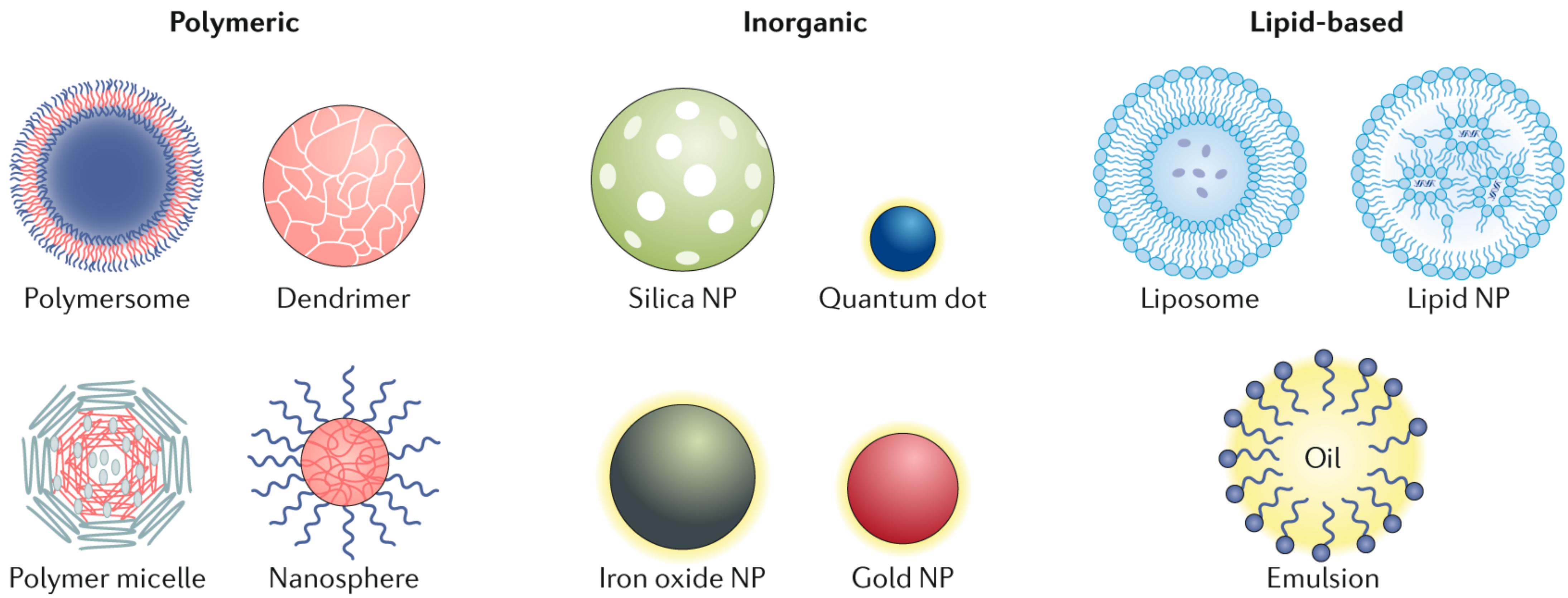
Bone is a nanomaterials composed of organic (mainly collagen) and inorganic (mainly nano-hydroxyapatite) components, with a hierarchical structure ranging from nanoscale to macroscale.



(a) At the macroscopic level, bone consists of a dense shell of cortical bone with porous cancellous bone at both ends.
(b) Repeating osteon units within cortical bone. **In the osteons, 20–30 concentric layers of collagen fibers**, called lamellae, are arranged at surrounding the central canal, which contain blood vessels and nerves.
(c) Collagen fibers (100–2 000 nm) are composed of collagen fibrils. The tertiary structure of collagen fibrils includes a 67 nm periodicity and 40 nm gaps between collagen molecules. The hydroxyapatite (HA) crystals are embedded in these gaps between collagen molecules and increase the rigidity of the bone.

Citation: Bone Research (2015) 3, 15029; doi:10.1038/boneres.2015.29

Types of Nano-biomaterials



Polymeric

- Precise control of particle characteristics
- Payload flexibility for hydrophilic and hydrophobic cargo
- Easy surface modification
- Possibility for aggregation and toxicity

Inorganic

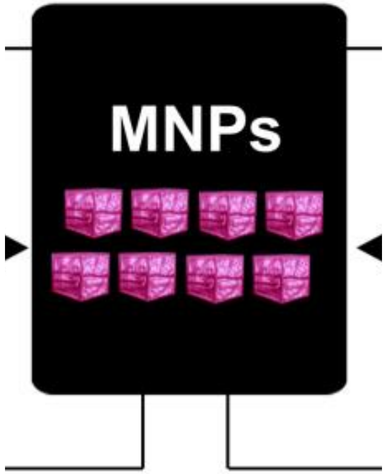
- Unique electrical, magnetic and optical properties
- Variability in size, structure and geometry
- Well suited for theranostic applications
- Toxicity and solubility limitations

Lipid-based

- Formulation simplicity with a range of physicochemical properties
- High bioavailability
- Payload flexibility
- Low encapsulation efficiency

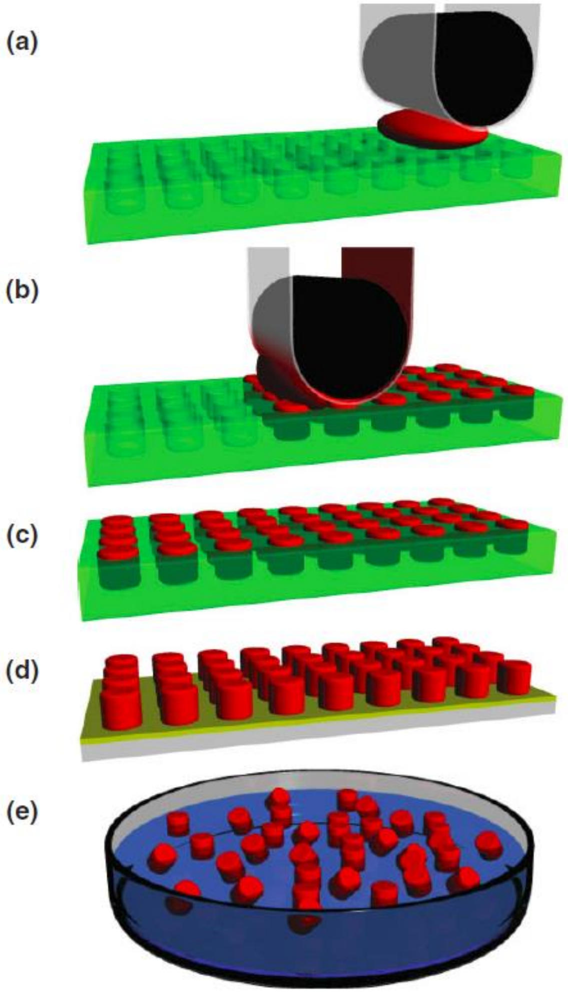
Nature Reviews Drug Discovery **volume 20**, pages101–124 (2021)

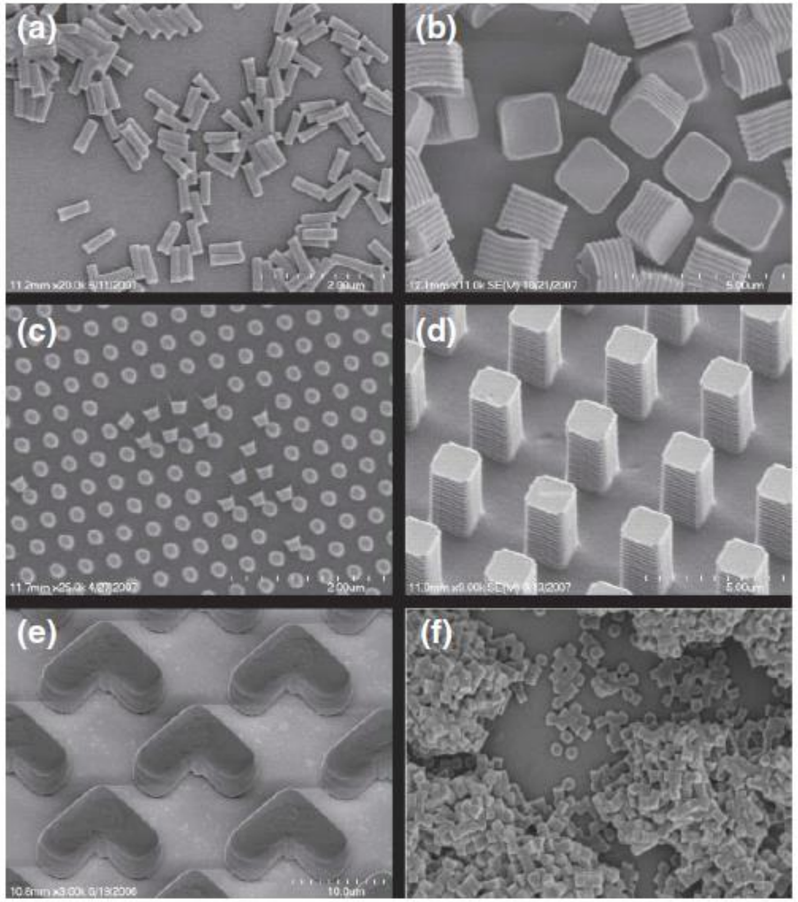
How to synthesize nanomaterials?

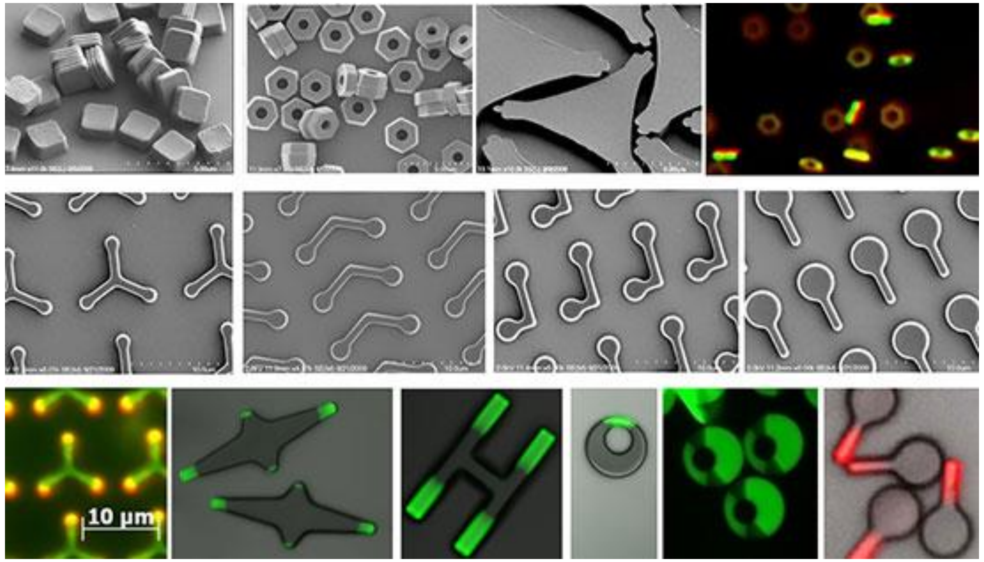


http://www.epmagazine.org/storage/237/en-state-of-the-art-for-nanoparticles-design.aspx

Top down: an example

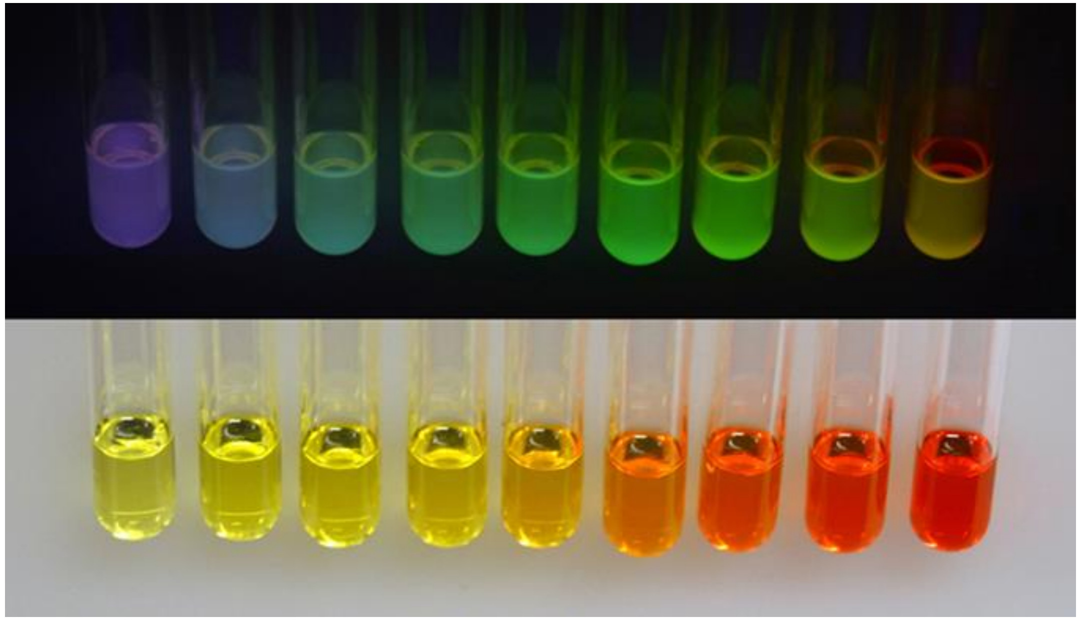




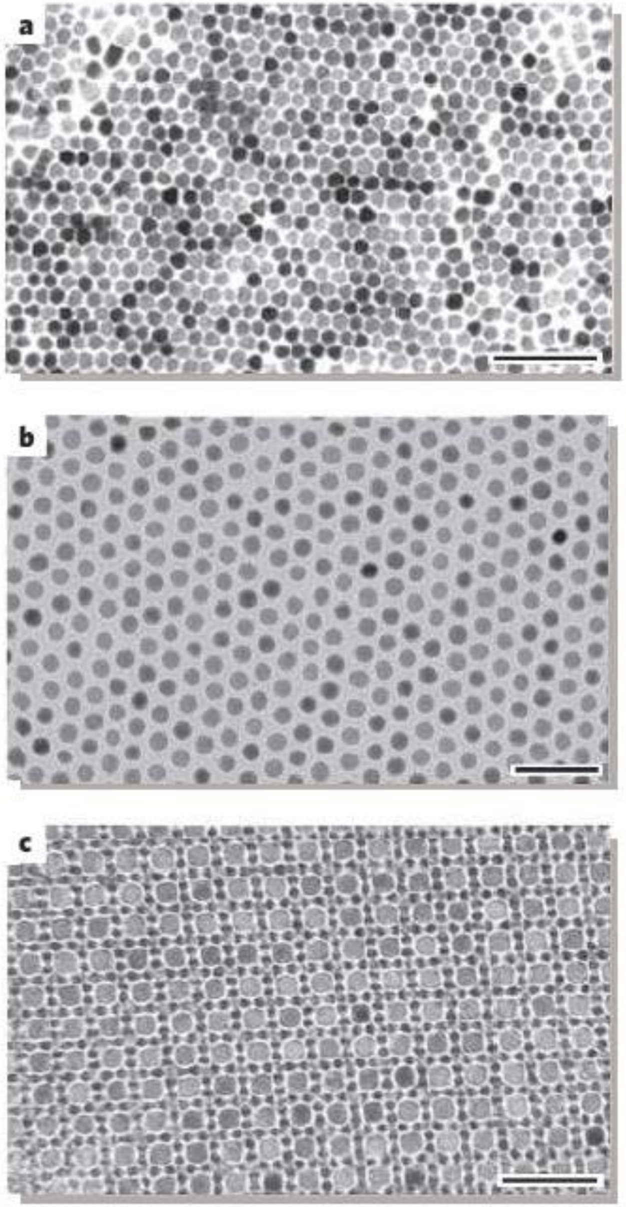


Wiley Interdiscip Rev Nanomed Nanobiotechnol. 2009 July 1; 1(4): 391–404.

Bottom up: Monodisperse colloidal nanocrystals synthesized under kinetic size control



http://education.mrsec.wisc.edu/150.htm



Scale bars, 50 nm.

Nature **437**, 664-670 (2005)

Bottom up: Monodisperse colloidal nanocrystals synthesized under kinetic size control

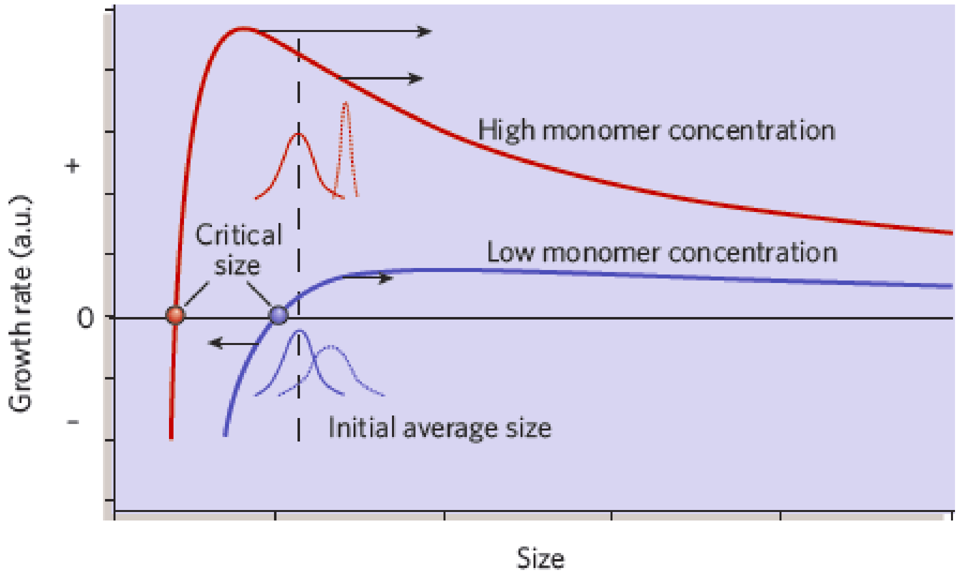
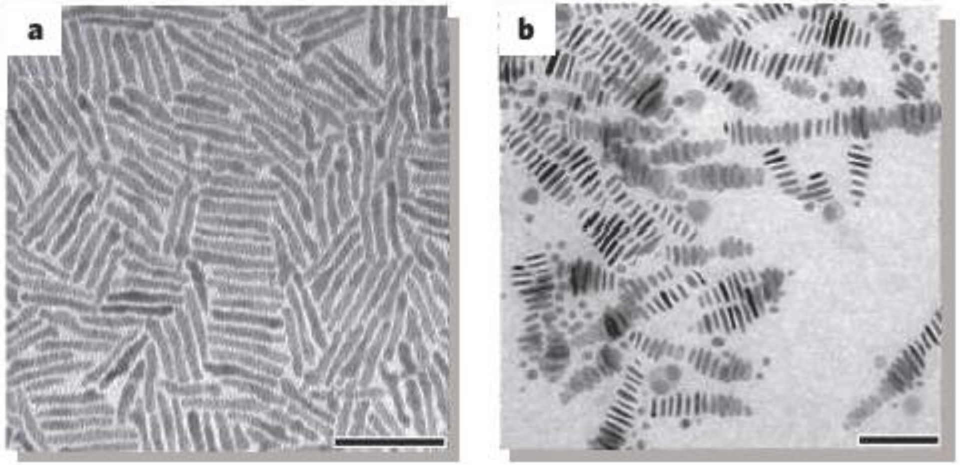


Figure 2 | Size-distribution focusing. The growth process of nanocrystals can occur in two different modes, 'focusing' and 'defocusing', depending upon the concentration of the monomer present. A critical size exists at any given monomer concentration. At a high monomer concentration, the critical size is small so that all the particles grow. In this situation, smaller particles grow faster than the larger ones, and as a result, the size distribution can be focused down to one that is nearly monodisperse. If the monomer concentration is below a critical threshold, small nanocrystals are depleted as larger ones grow and the size distribution broadens, or defocuses. The preparation of nearly monodisperse spherical particles can be achieved by arresting the reaction while it is still in the focusing regime, with a large concentration of monomer still present. a.u., arbitrary units.

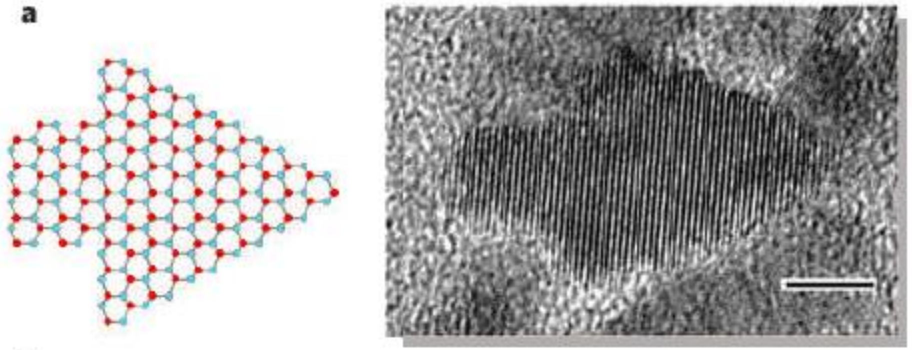
Nature **437**, 664-670 (2005)

Bottom up: Anisotropic growth of nanocrystals by kinetic shape control and selective adhesion

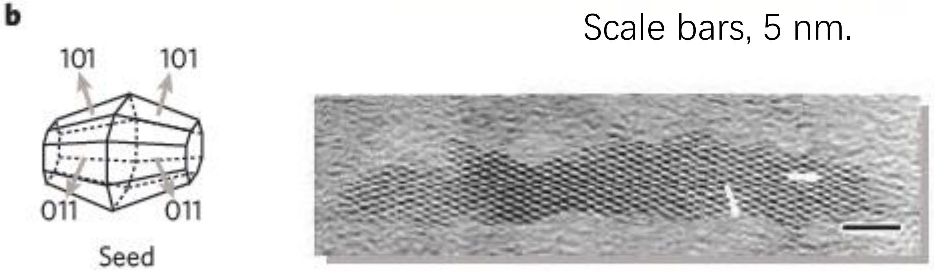


Scale bars, 50 nm.

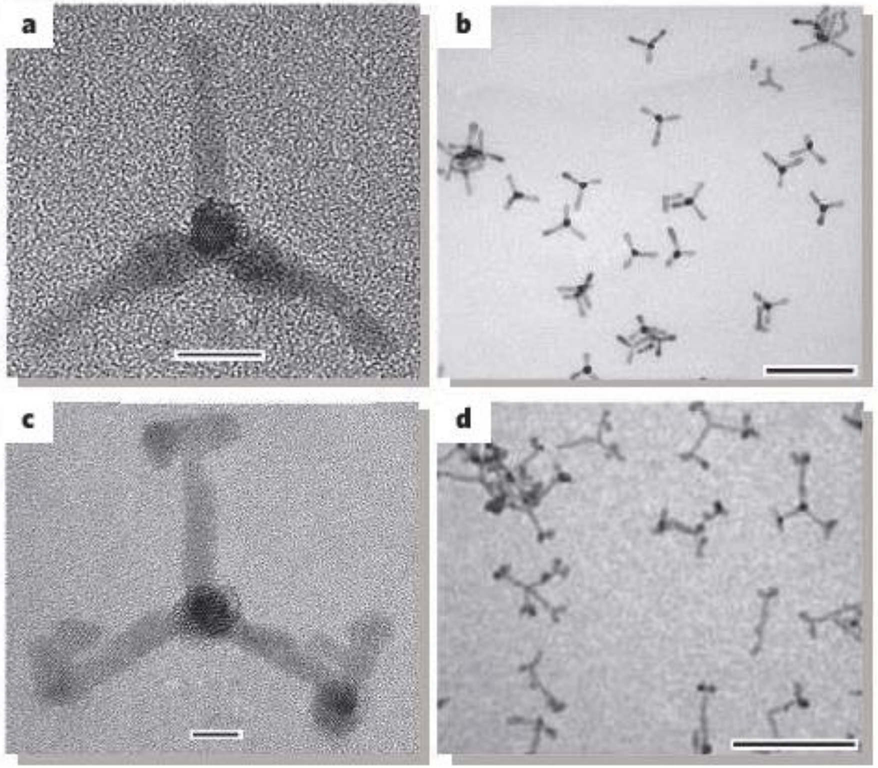
Scale bars, 100 nm.



Scale bars, 5 nm.



Scale bars, 3 nm.



Scale bars, 5 nm.

Scale bars, 100 nm.

Nature **437**, 664-670 (2005)

Bottom up: Shape control of colloidal nanocrystals

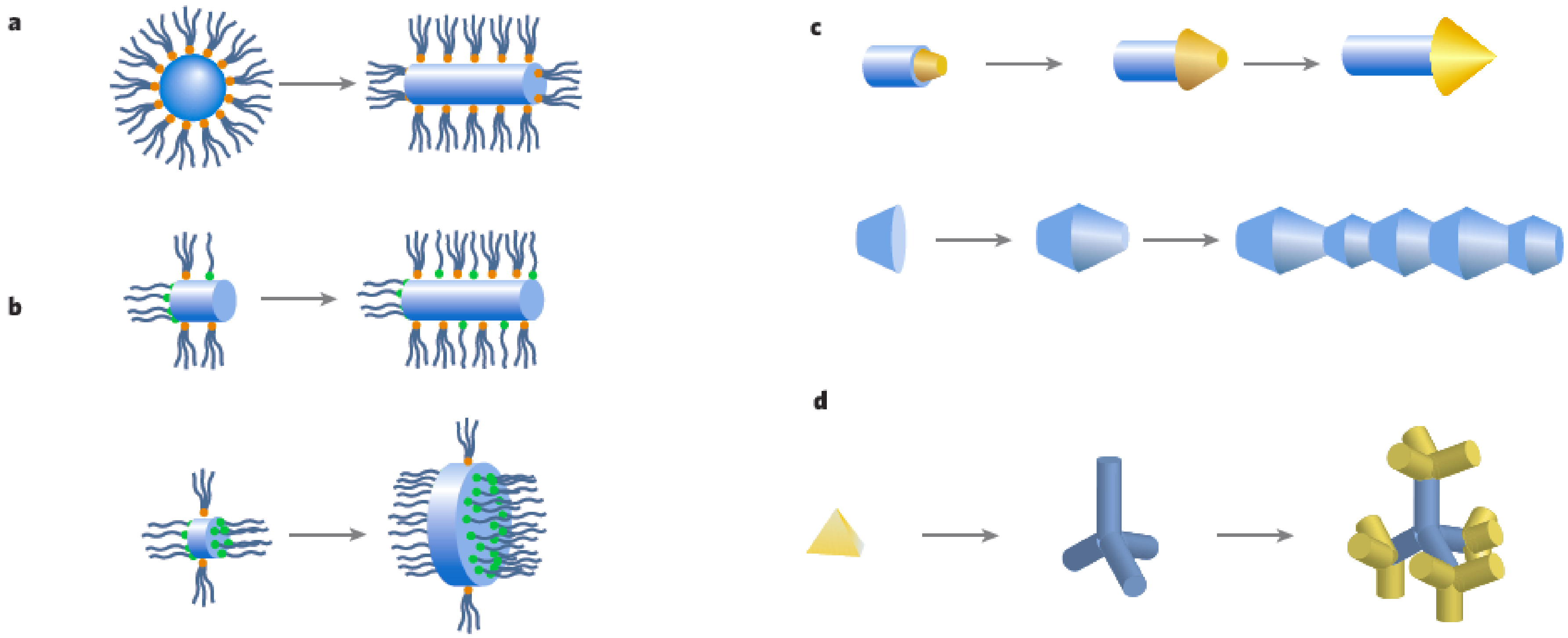


Figure 1 | Shape control of colloidal nanocrystals.
a, Kinetic shape control at high growth rate. The high-energy facets grow more quickly than low energy facets in a kinetic regime.
b, Kinetic shape control through selective adhesion.
c, More intricate shapes result from sequential elimination of a high-energy facet.
d, Controlled branching of nanocrystals. The existence of two or more crystal structures in different domains of the same crystal, coupled with the manipulation of surface energy at the nanoscale, can be exploited to produce branched inorganic nanostructures such as tetrapods.

Nature **437**, 664-670 (2005)

Bottom up: organic nanoparticles

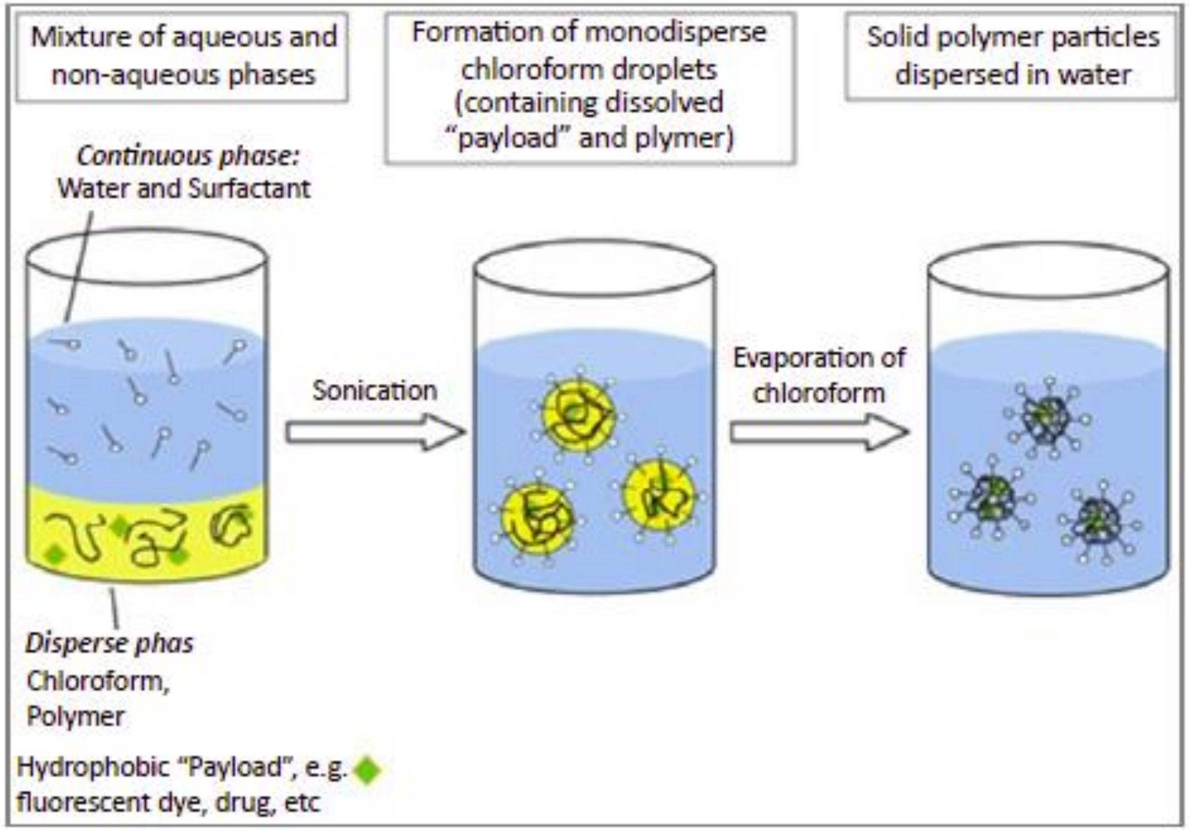
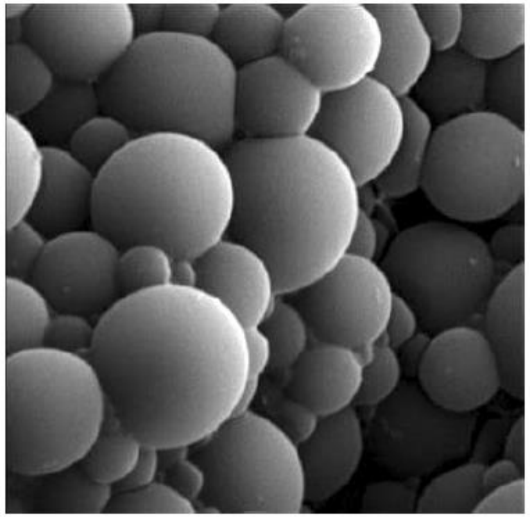
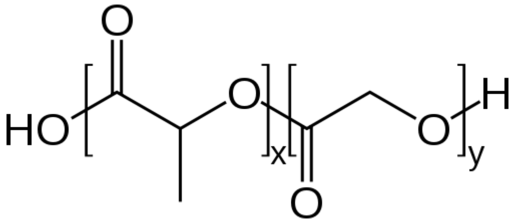


Figure 2: Emulsification process (Source: Musyanovych et al. (2012) [104]).

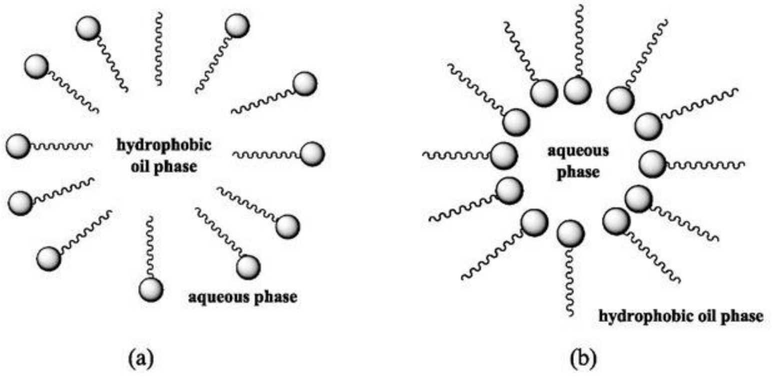


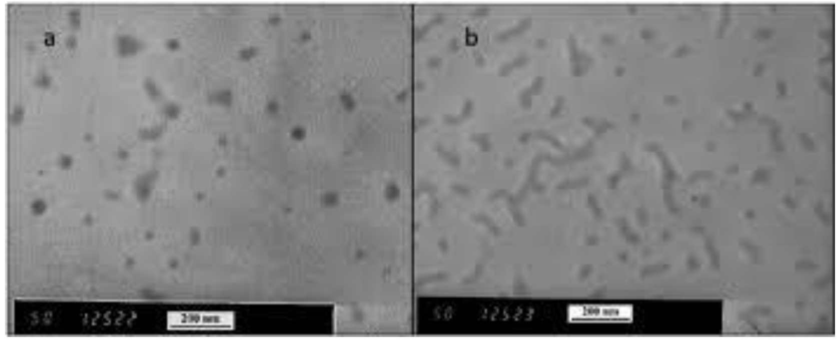
PLGA-drug microshpere



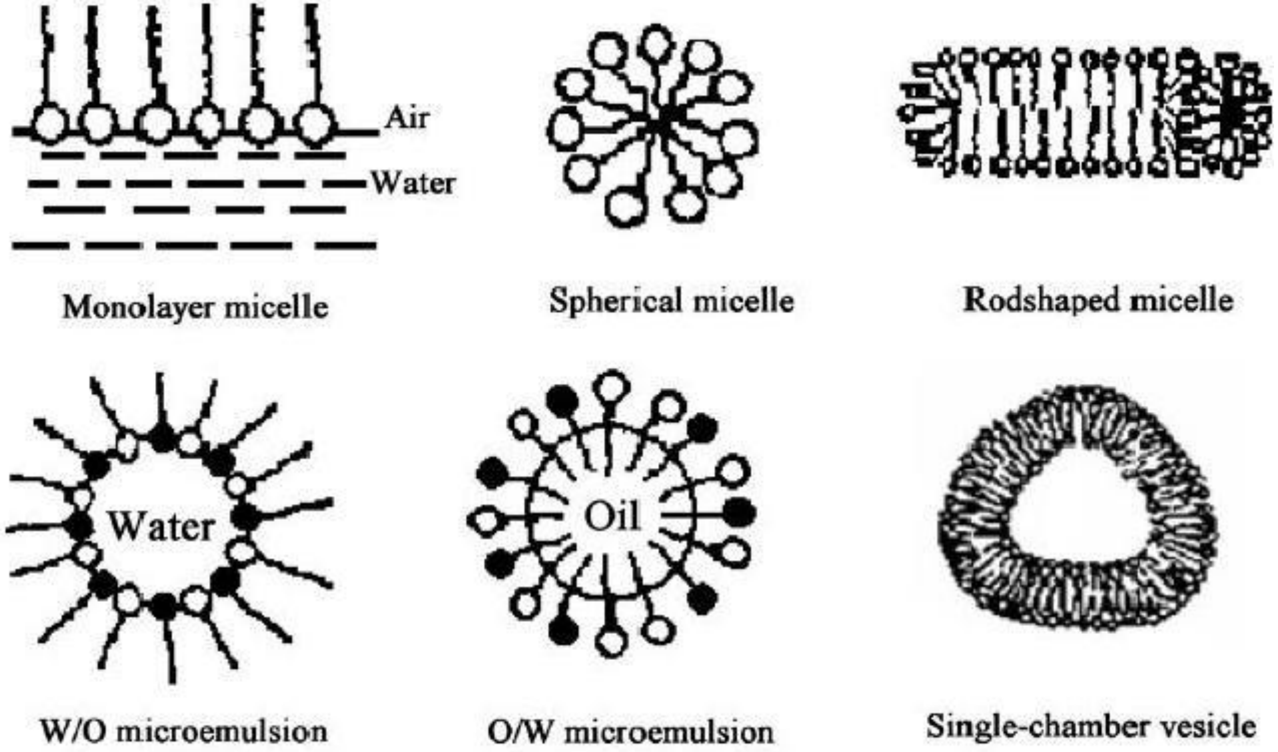
Kumar et al., J Nanomater Mol Nanotechnol 2014, 3:4

Bottom up: organic nanoparticle – micelle



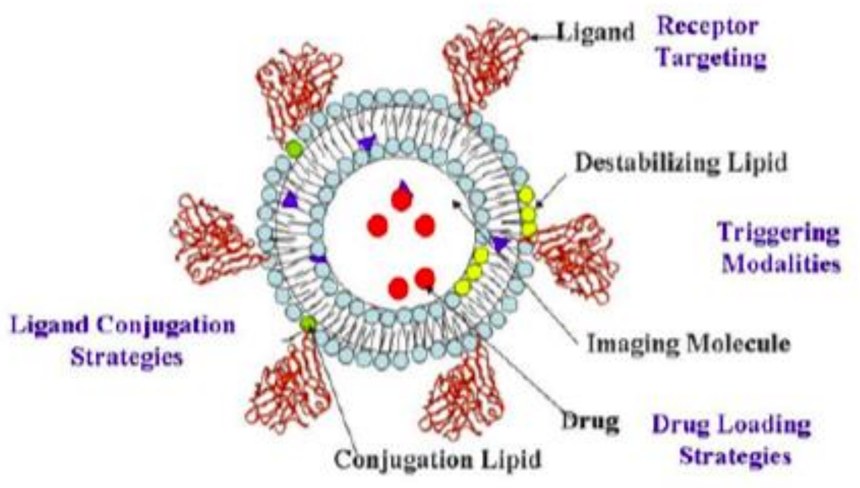


Biomater. Sci., 2016, 4, 511-521



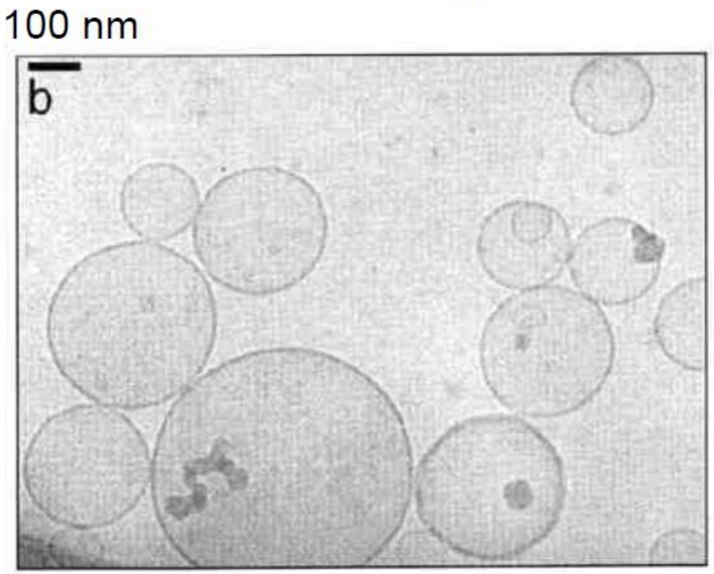
http://www.zeepedia.com/read.php?microemulsion_techniques_significance_of_packing_parameter_synthetic_strategies_in_chemistry&b=132&c=7

Bottom up: organic nanoparticles – liposome



Lipid-based Nanoparticles (LIPOSOMES) For Clinical Applications

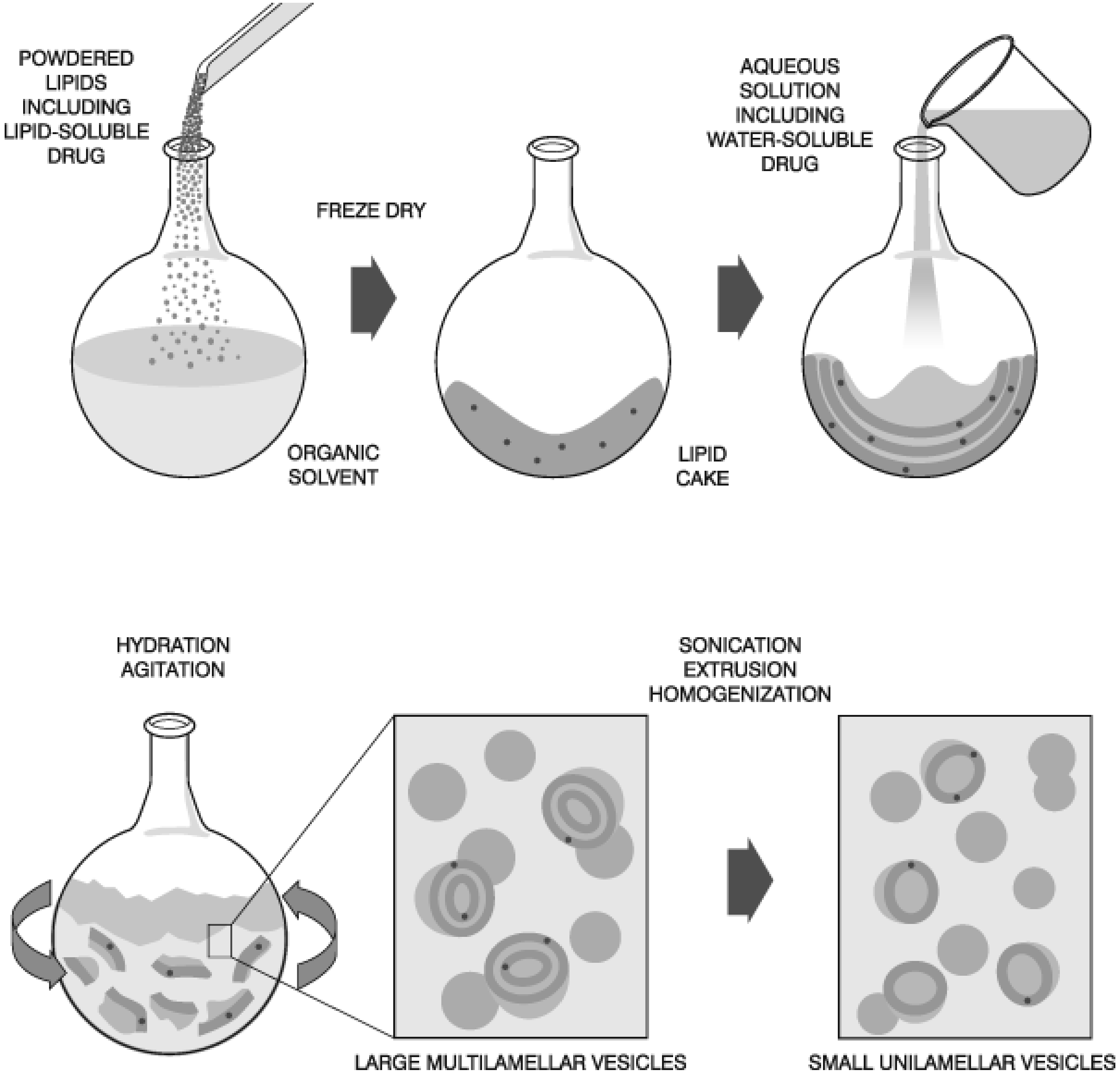
Figure 7: Design principle of an ideal multifunctional lipid-based nanoparticle for targeted and triggered drug delivery. Liposomes consist of a matrix phospholipid, a destabilizing (pore forming) phospholipid and conjugation lipid (green), ligand (brown) attached via the conjugation lipid. The nanoparticle is loaded with a chemotherapeutic agent (red) and an imaging agent in the aqueous milieu. (Source: Puri et al. (2009) [107]).



(Bergstrand and Edwards, *Langmuir*. **17**, 3245-3253 (2001))

Kumar et al., J Nanomater Mol Nanotechnol 2014, 3:4

Synthesis of liposome



https://avantilipids.com/tech-support/liposome-preparation/

Bottom up: organic nanoparticle – dendrimer

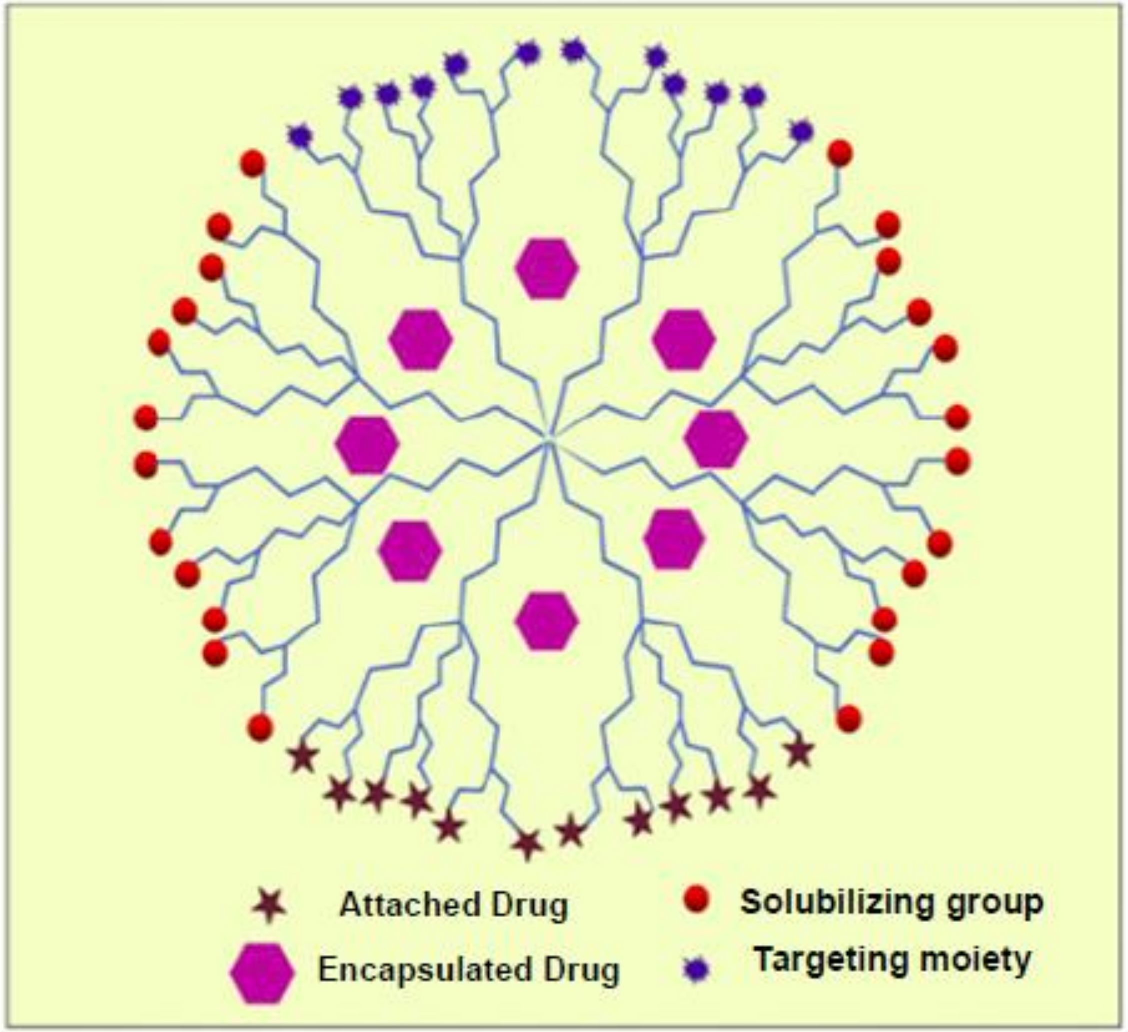


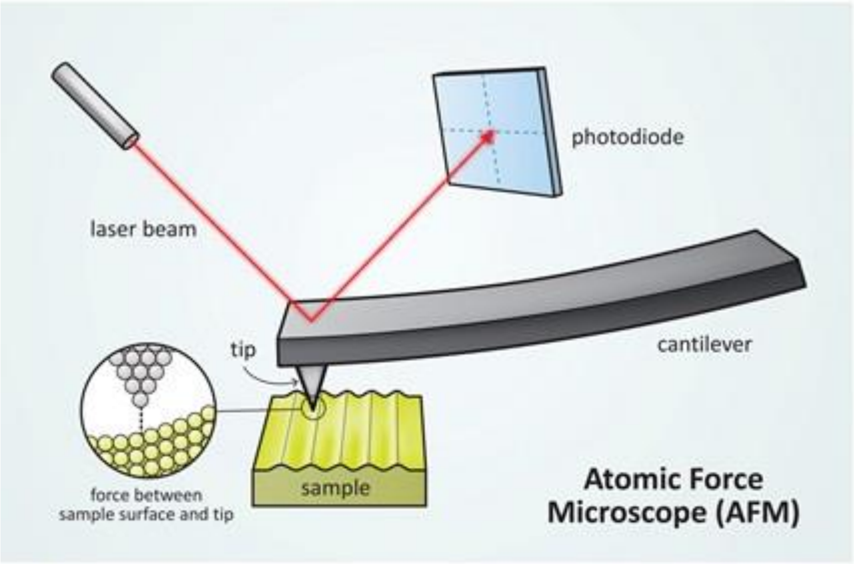
Figure 8: Dendrimer structures (Source: Sakthivel et al. (2003) [108]).

Kumar et al., J Nanomater Mol Nanotechnol 2014, 3:4

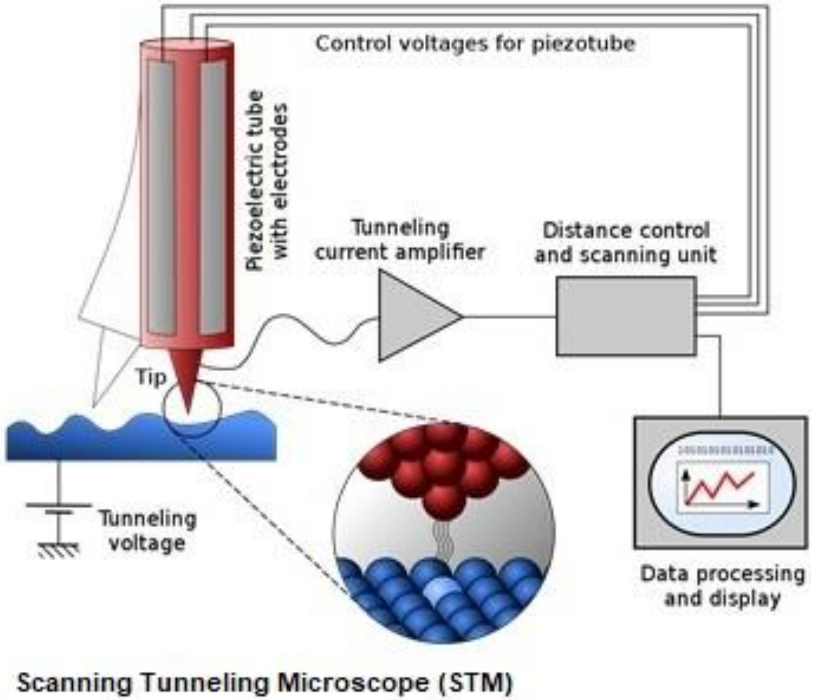
How to see what is going on in the “nano” world?

As early as the 1930s, scientists have been able to image at the nanoscale using instruments such as the scanning electron microscope, the transmission electron microscope, and the field ion microscope, , but these techniques can require extensive sample preparation and are expensive.

The invention of the scanning tunneling microscope (STM) and the atomic force microscope (AFM) in the 1980s is widely credited with opening up the field of nanotechnology.



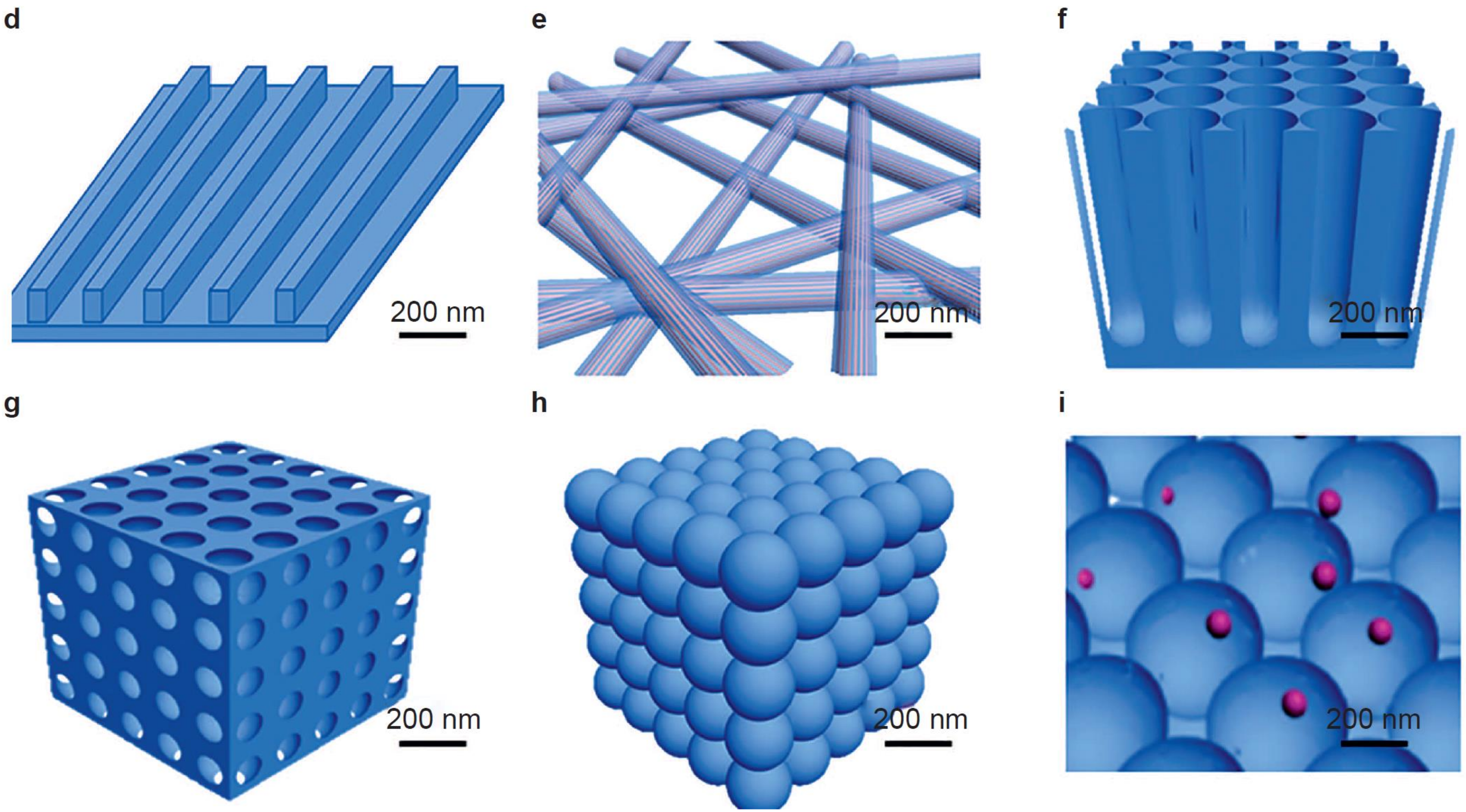
Credit: Emily Maletz/NISE Network



Credit: Michael Schmid and Grzegorz Pietrzak

Working at the Nanoscale | National Nanotechnology Initiative

Nanostructured scaffolds



Nanostructures with features of nanopattern (d), nanofibers (e), nanotubers (f), nanopores (g), nanospheres (h), and nanocomposites (i) with structural components with a feature size in the nanoscale.

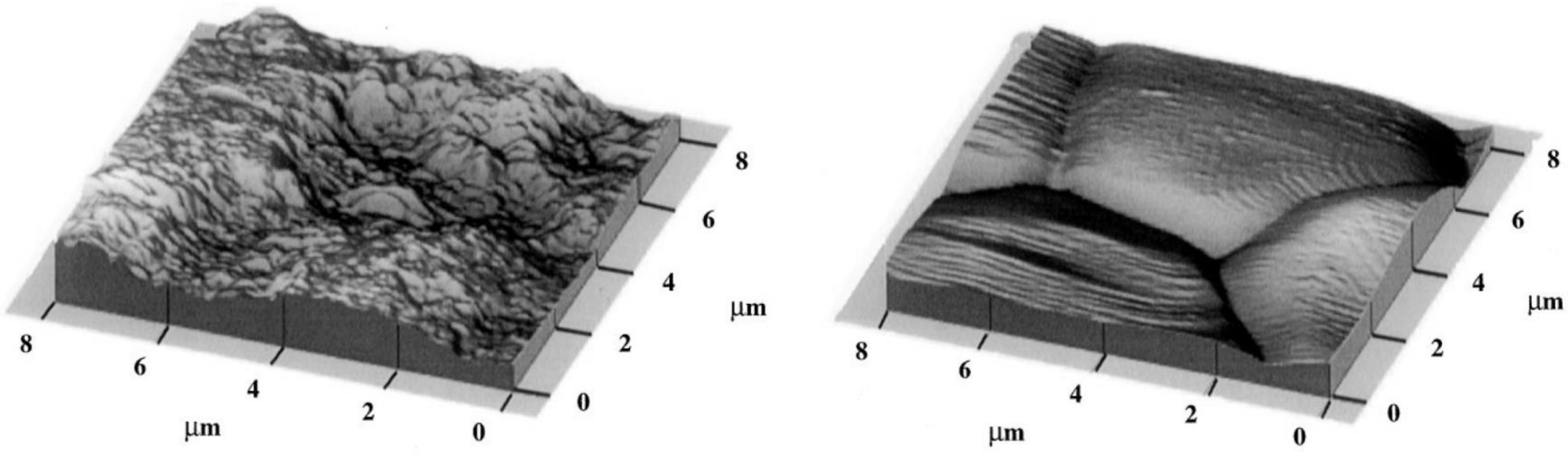
Citation: Bone Research (2015) 3, 15029; doi:10.1038/boneres.2015.29

Nanophase and Conventional Scaffold

Atomic Force Micrographs (AFM) of Nanophase and Conventional Titania

- Nanophase titania samples were obtained by sintering (in air at 103C/min) the 32 nm grain size titania compacts from room temperature to a final temperature of either 600 or 800 C and by maintaining the 600 or 800 C temperature, respectively, for 2 h to obtain materials with grain sizes less than 100 nm.

- Conventional titania samples were obtained by sintering (in air at 103 C/min) the 32 nm titania compacts from room temperature to a final temperature of 1200 C and by maintaining this temperature for 2 h to obtain materials with grain sizes greater than 100 nm.

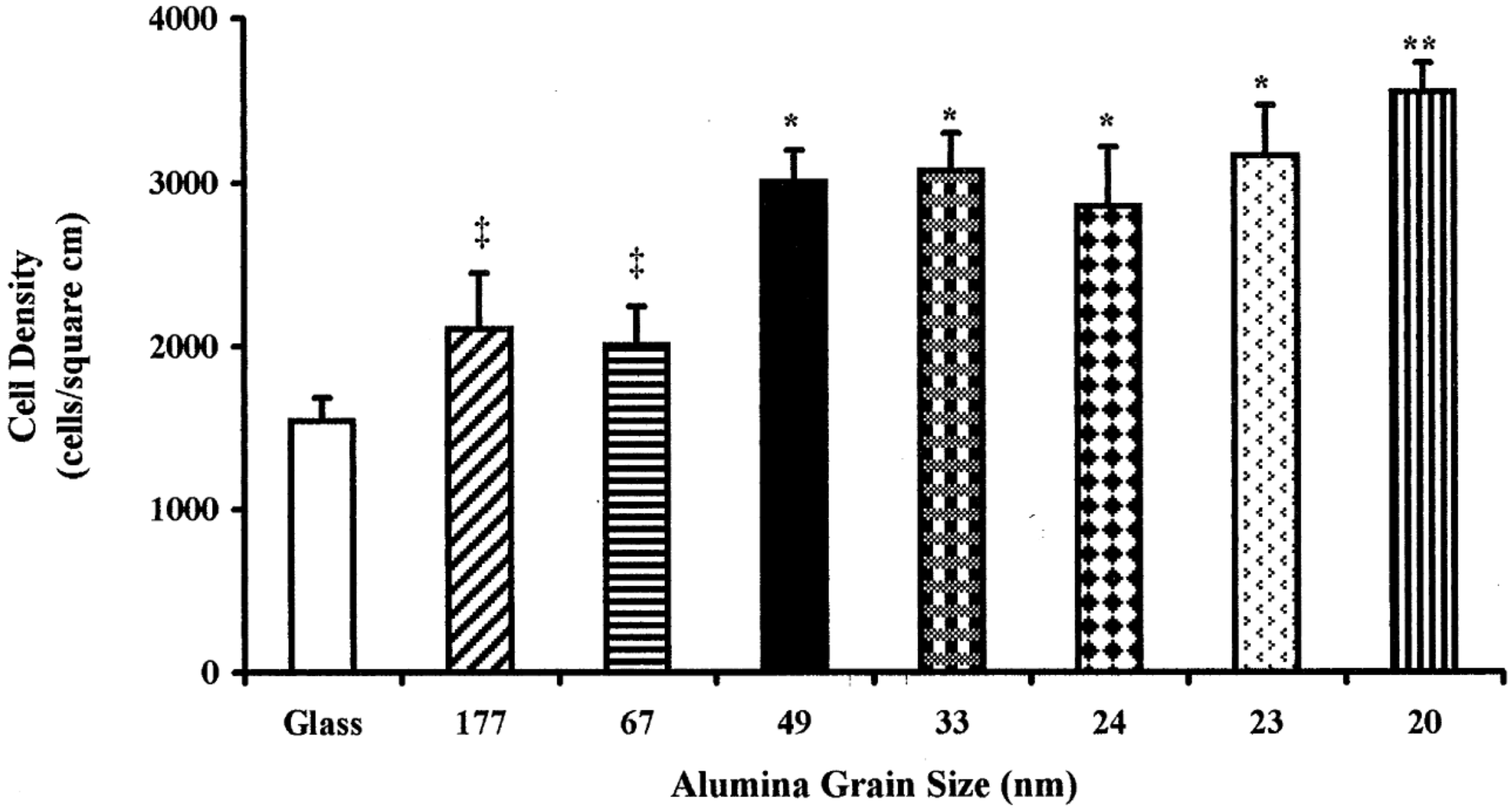


(a) 32 nm grain size titania (nanophase)

(b) 2.12 µm grain size titania (conventional)

Biomaterials 20 (1999) 1221-1227

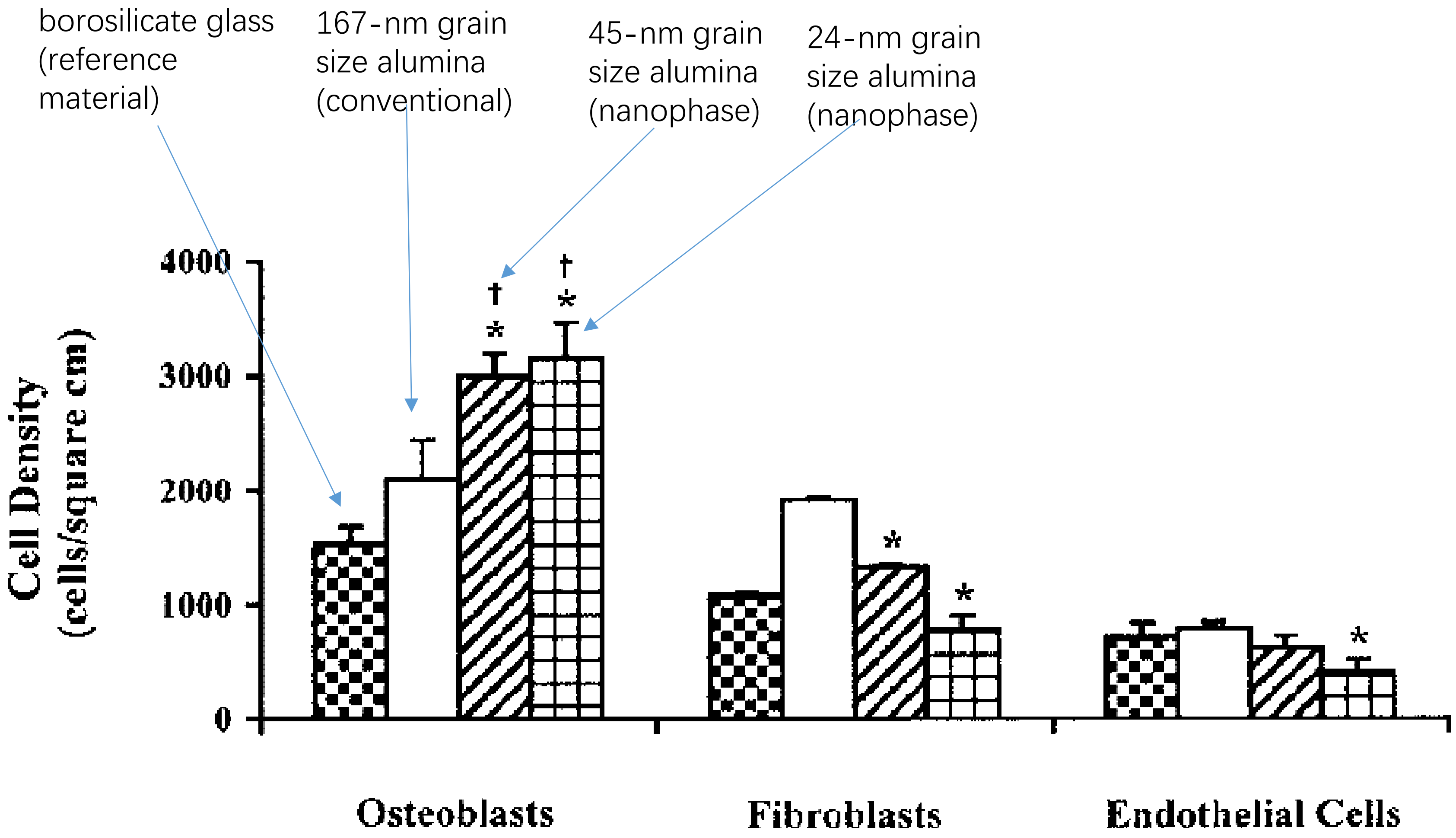
Effect of nanosize feature of scaffold



Osteoblast adhesion on alumina is dependent on the grain size.

Biomaterials 20 (1999) 1221-1227

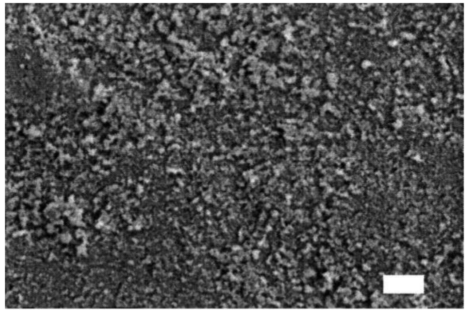
Effect of nanosize feature of Nanophase Alumina



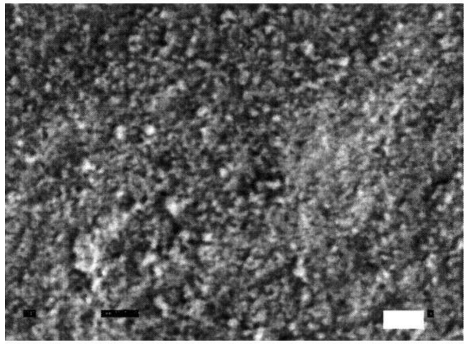
osteoblast adhesion is enhanced on nanophase alumina

T. J. Webster, C. Ergun, R. H. Doremus, R.W. Siegel, and R. Bizios, Journal of Biomedical Materials Research 51:475-483 (2000)

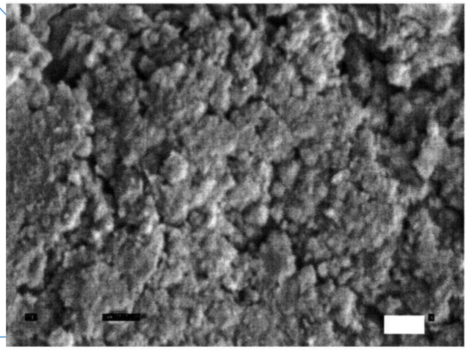
Enhanced osteoblast adhesion on nanofiber alumina



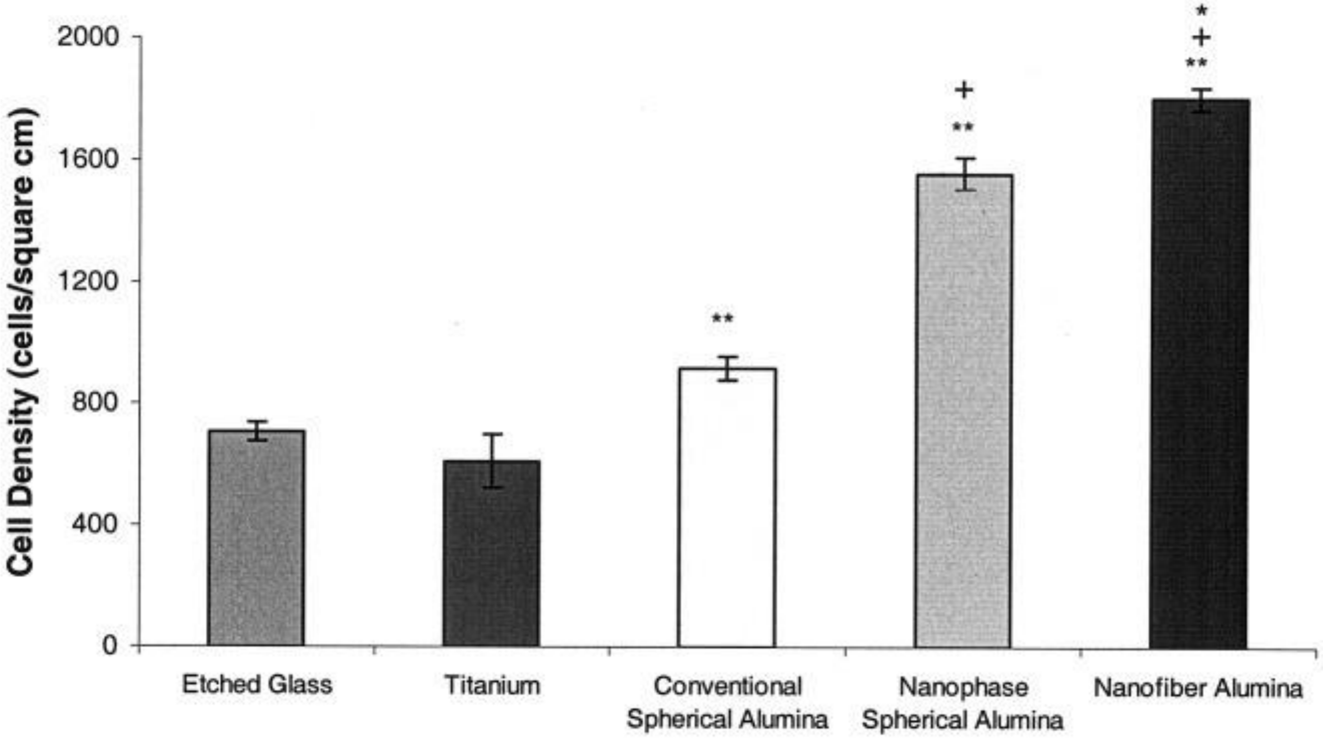
A) Conventional Spherical Alumina Compact



B) Nanophase Spherical Alumina Compact



C) Nanofiber Alumina Compact



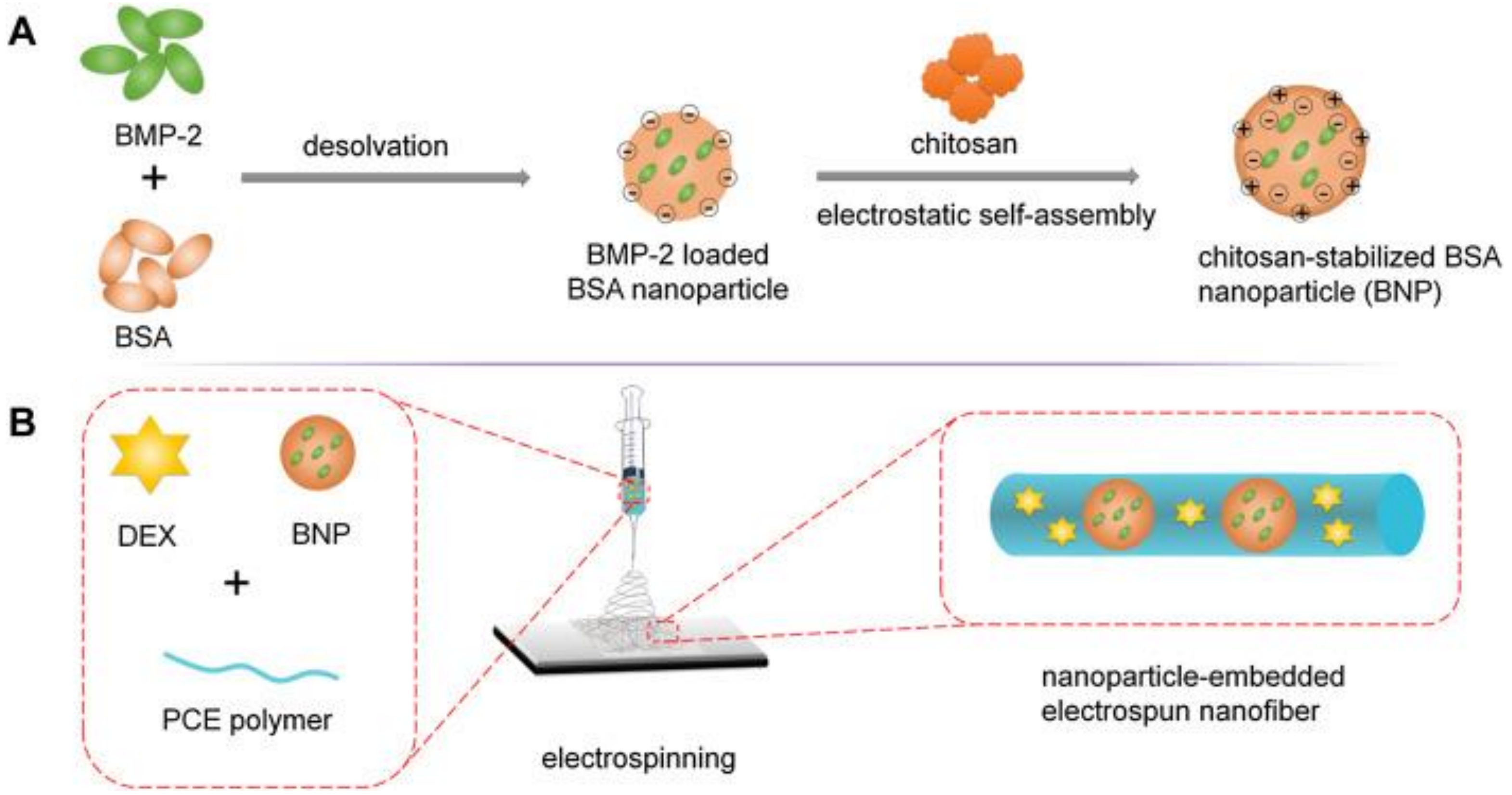
Enhanced osteoblast adhesion on nanofiber alumina

Medical and Biological Engineering and Computing 41:372-375 (2003)
J. Biomed. Mater. Res., 67A: 1284-1293. doi:10.1002/jbm.a.20011

Nanoparticle-embedded electrospun nanofiber scaffold for bone tissue engineering

bone morphogenetic protein-2 (BMP-2):
To obtain osteoinductivity and enhance repair of critical-sized bone defects, an efficient method is to utilize bone morphogenetic protein-2 (BMP-2) therapies this protein may lose bioactivity over a short time due to its short half-life, which limits its local delivery, and does not always exhibit efficacy in bone defect repair *in vivo*

dexamethasone (DEX):
promoting the differentiation of mesenchymal stem cells (MSCs) toward the osteogenic lineage, but the direct use of DEX has been limited mainly due to its toxic side effects



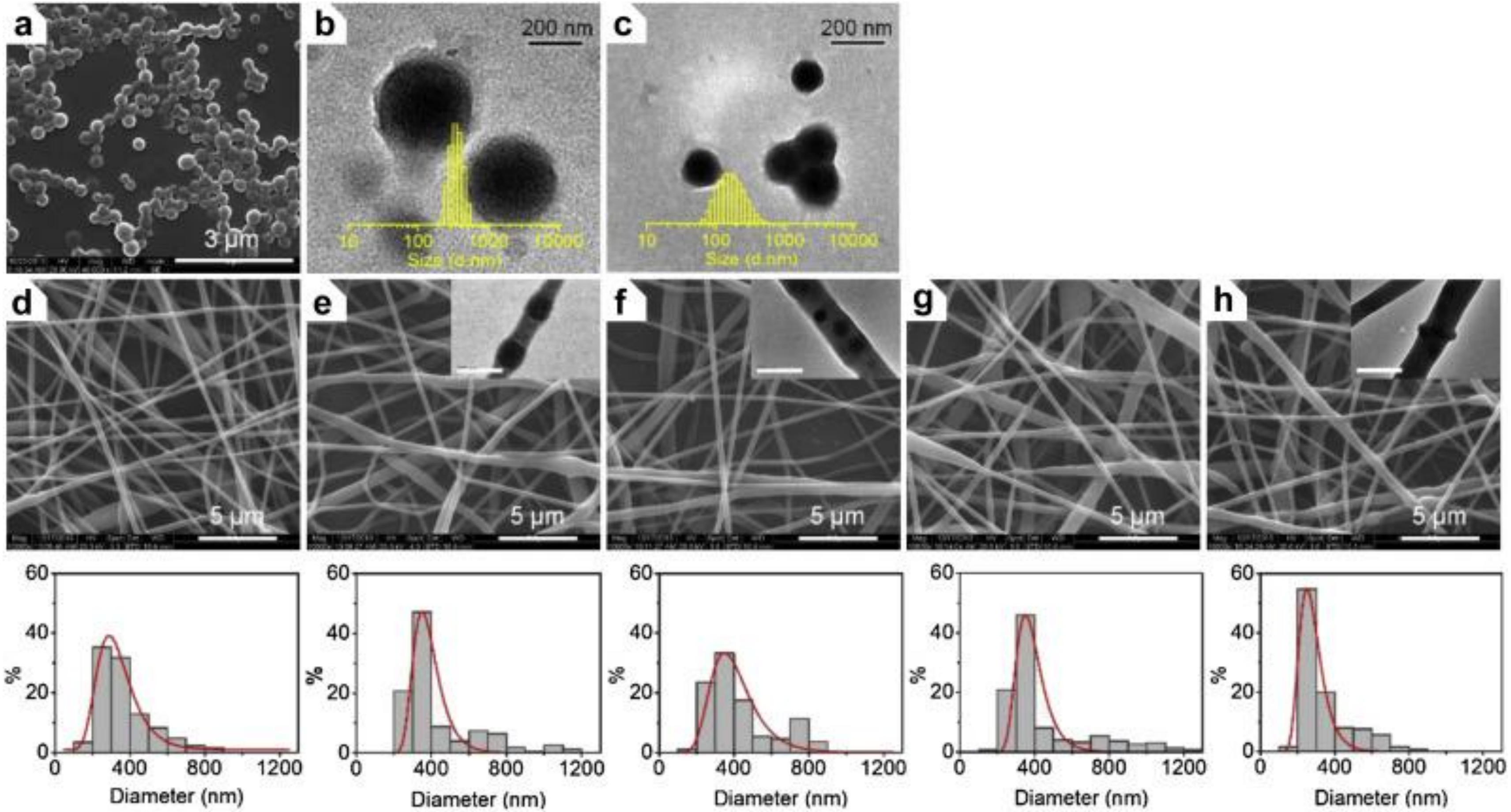
The scaffold was achieved by (1) the encapsulation of BMP-2 into bovine serum albumin (BSA) nanoparticles to maintain the bioactivity of BMP-2 and (2) the co-electrospinning of the blending solution composed of the BSA nanoparticles, DEX and the poly(ε-caprolactone)-co-poly(ethylene glycol) (PCE) copolymer.

http://dx.doi.org/10.1016/j.biomaterials.2014.10.015

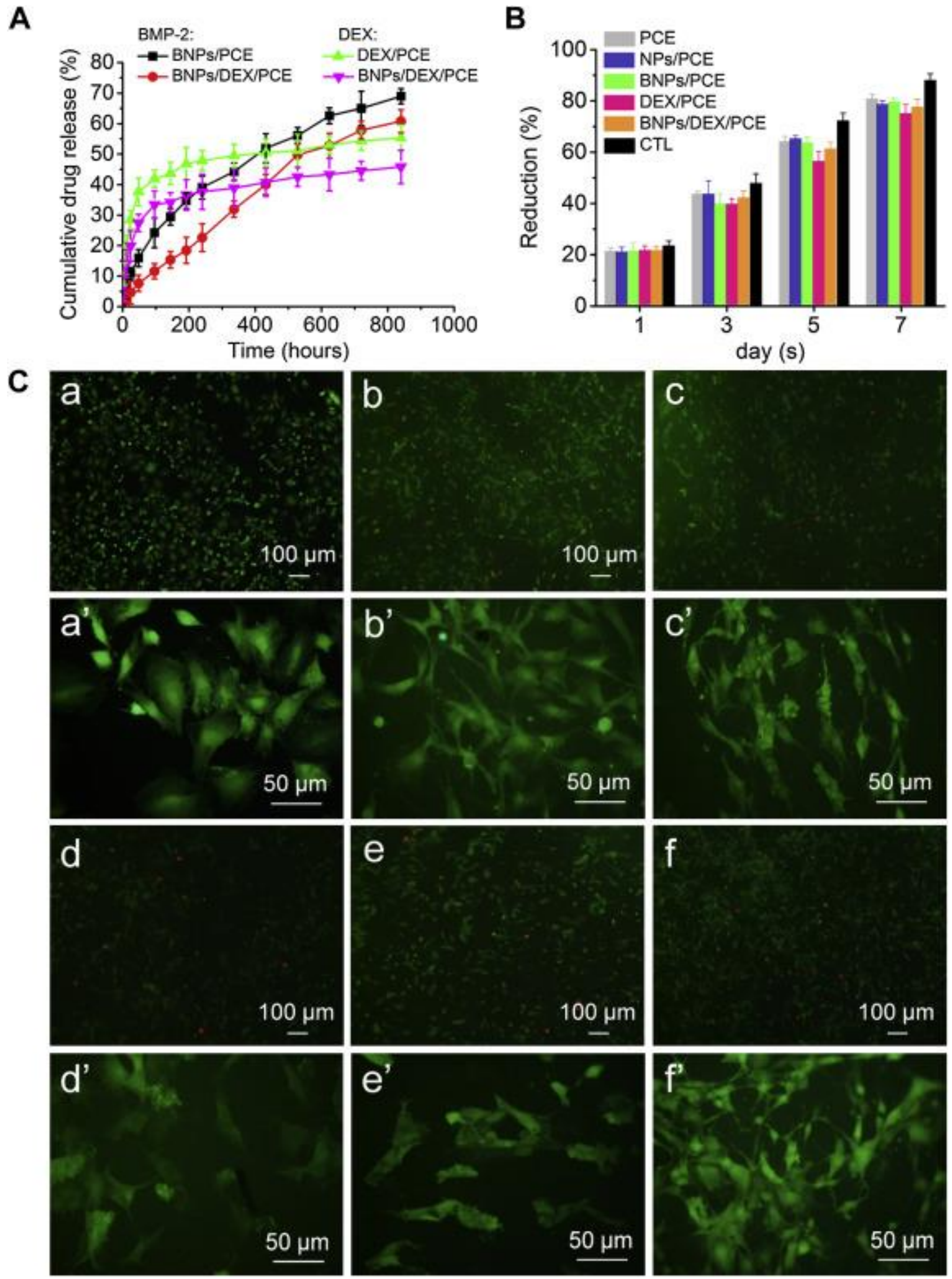
Nanoparticle-embedded electrospun nanofiber scaffold for bone tissue engineering

Morphology characterization of nanoparticles and electrospun nanofibers.

SEM images of NPs (**a**), TEM image of NPs (**b**) and BNPs (**c**), the insets are their size distribution determined by DLS; SEM images of electrospun nanofibers: PCE (**d**), NPs/PCE (**e**), BNPs/PCE (**f**), DEX/PCE (**g**), BNPs/DEX/PCE (**h**), the inserts are TEM images of the corresponding nanoparticles-embedded nanofibers (Scale bar: 500 nm). The histograms are the diameter distributions of the nanofibers from corresponding SEM images.



http://dx.doi.org/10.1016/j.biomaterials.2014.10.015

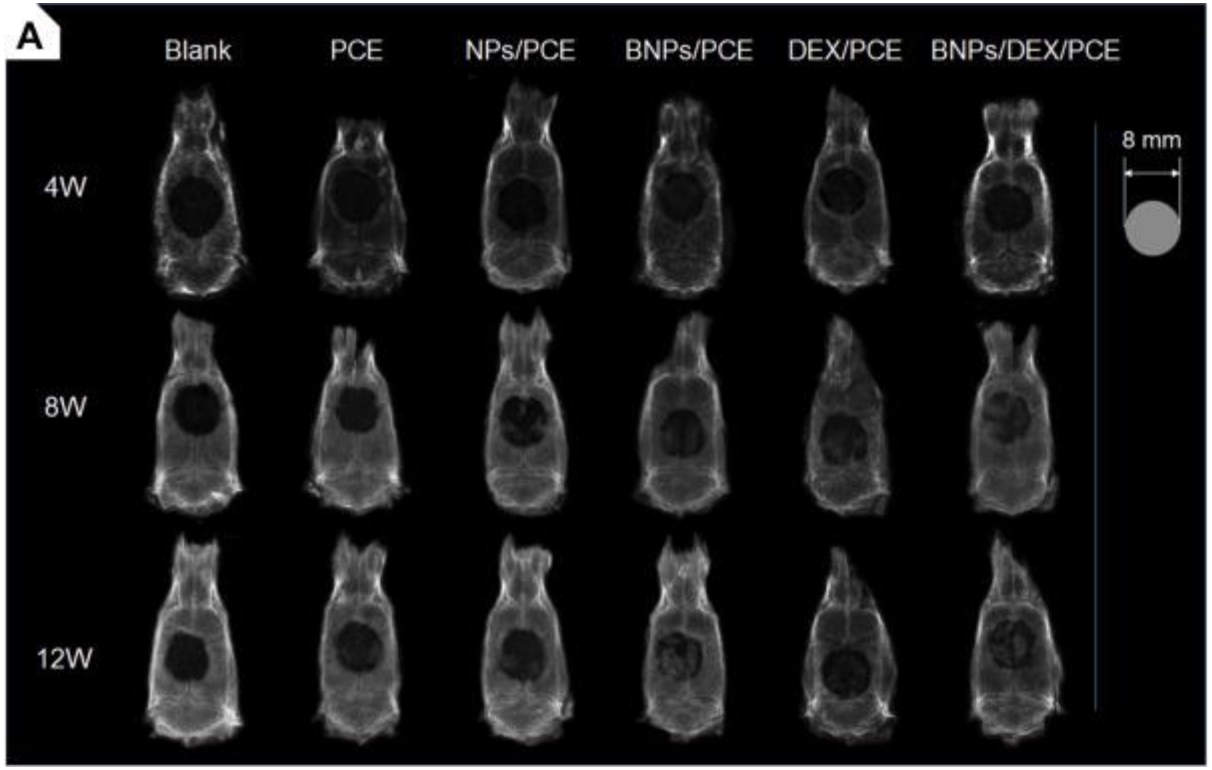


- *In vitro* cumulative drug release profiles of different drugs (**A**), among which, the black and red colors are the **BMP-2 release profiles** from nanoparticle-embedded nanofibers, while the green and pink colors are the **DEX release profiles** of single-DEX-loaded and nanoparticle-embedded nanofibers.
- *In vitro* cell viability of MSCs seeded on different electrospun scaffolds (**B**).
- Fluorescent images of MSCs on day 3 (**C**): Control (CTL) (a, a′), PCE (b, b′), NPs/PCE (c, c′), BNPs/PCE (d, d′), DEX/PCE (e, e′), **BNPs/DEX/PCE (f, f′),** in which a′, b′, c′, d′, e′ and f′ are the high magnification images of a, b, c, d, e and f respectively.
- Among these, the live cells were stained green, while dead cells were stained red.

This slide is not required.

http://dx.doi.org/10.1016/j.biomaterials.2014.10.015

This slide is not required.



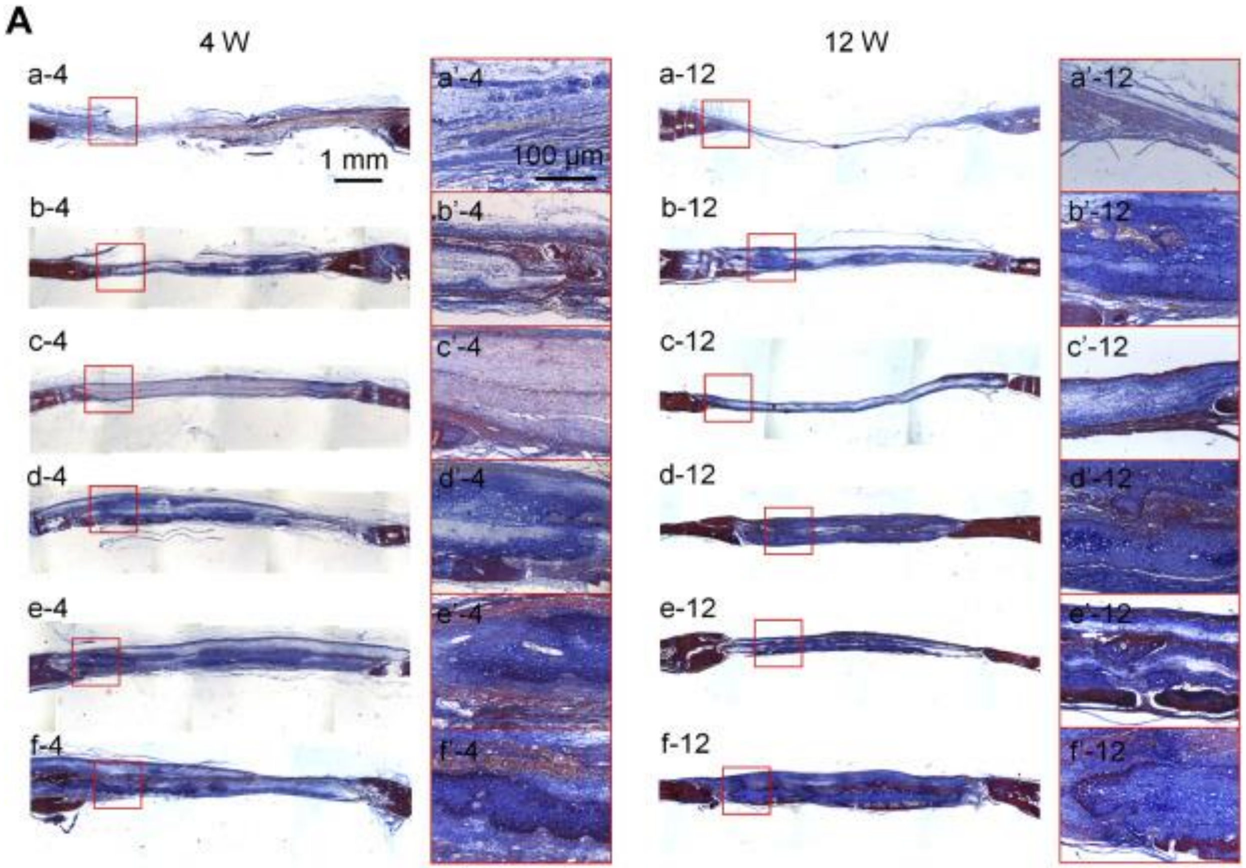
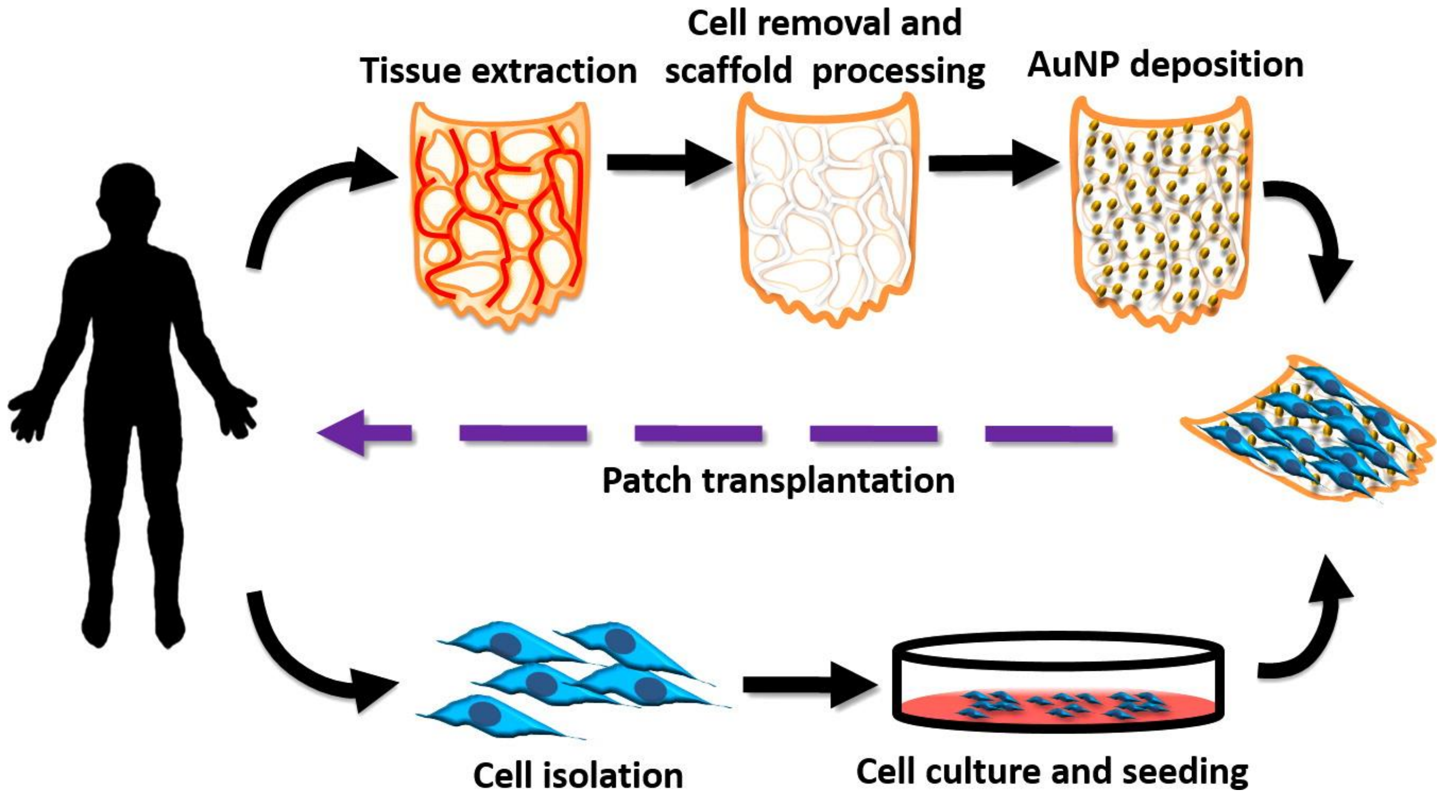


Fig. 5. Radiograms of the X-ray detection after implanted *in vivo* for 4, 8, 12 weeks respectively (**A**), and the rightmost disk is a calvarial defect template with a diameter of 8 mm. The statistical evaluations of apparent repair area (**B**), mean gray value (**C**) and bone repair level (**D**) were calculated from the radiograms (n = 3) for Control group without material (a), PCE (b), NPs/PCE (c), BNPs/PCE (d), DEX/PCE (e), **BNPs/DEX/PCE (f)**. The normal calvaria mean gray value was calculated from an undamaged calvarial bone. Asterisk mark represents the significant difference between each other, n = 3 for each group, $p < 0.05$.

Enhancement of electrical properties

Gold Nanoparticle‑Decellularized Matrix Hybrids for Cardiac Tissue Engineering

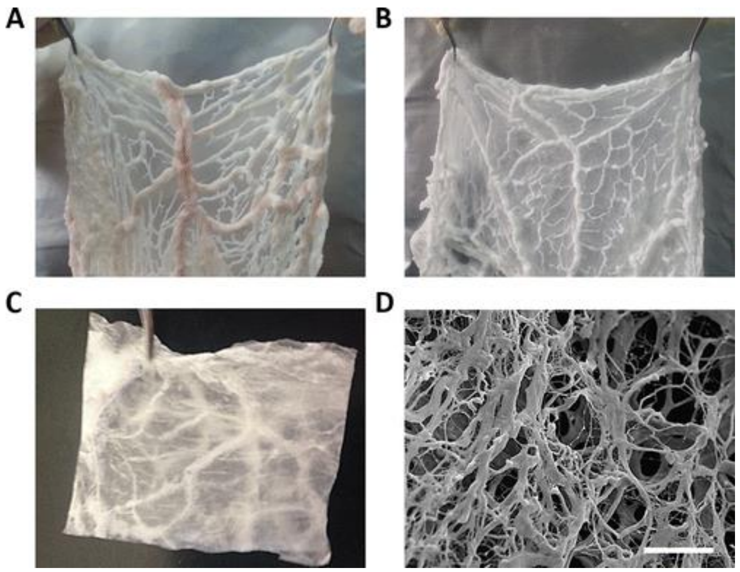
- **lack of quick and efficient electrical coupling** between adjacent cells may jeopardize the success of the treatment
- After cell isolation and seeding within the scaffolds, the cells become rounded, and their **gap junction proteins such as connexin 43** are internalized or lost, disrupting proper anisotropic transfer of the electrical signal
- deposited gold nanoparticles on fibrous decellularized omental matrices

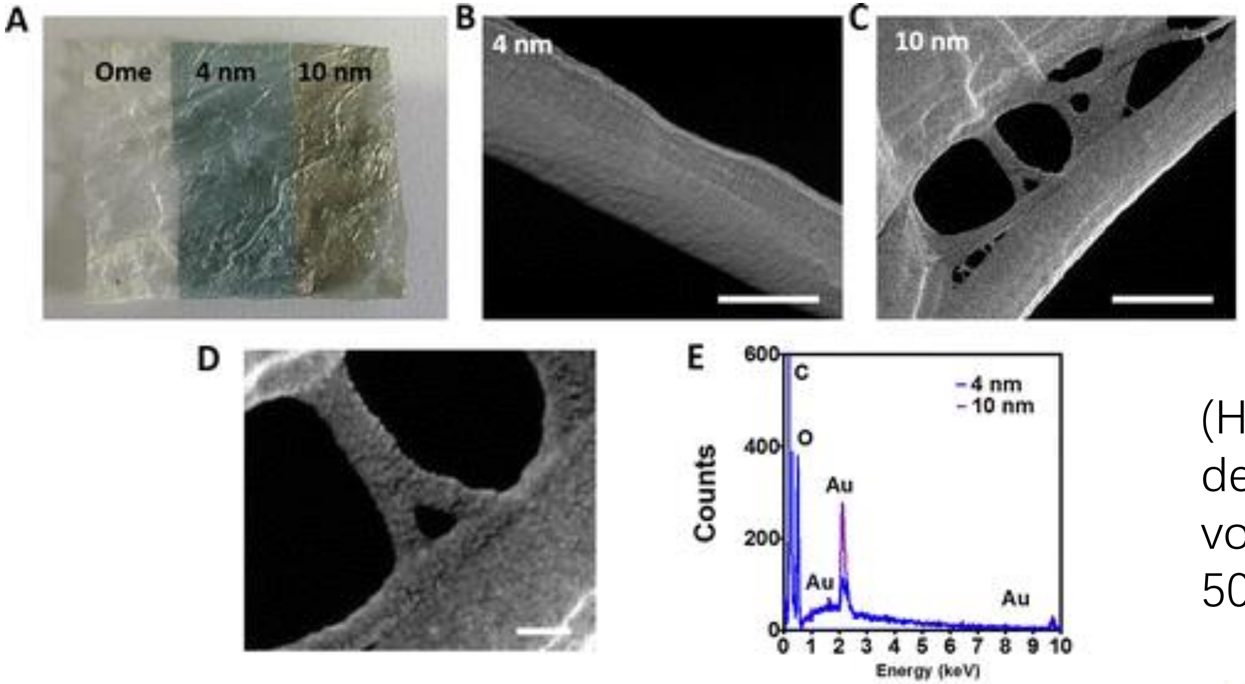


The decellularized scaffold is decorated with conductive motifs using an e-beam evaporator to produce a hybrid scaffold. Cells are isolated from the same patient, cultured *in vitro*, and seeded on the hybrid scaffold to produce a personalized cardiac patch.

Nano Lett. 2014 Oct 8;14(10):5792-6. doi: 10.1021/nl502673m

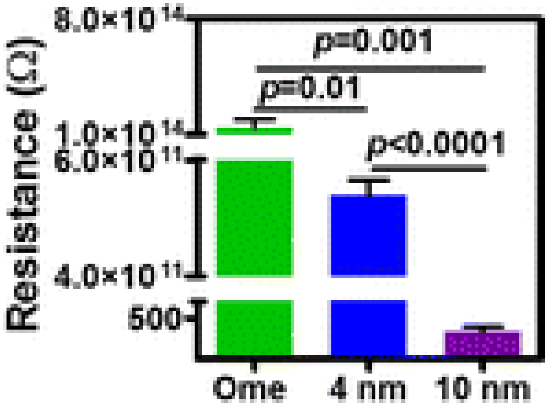
Enhancement of electrical properties





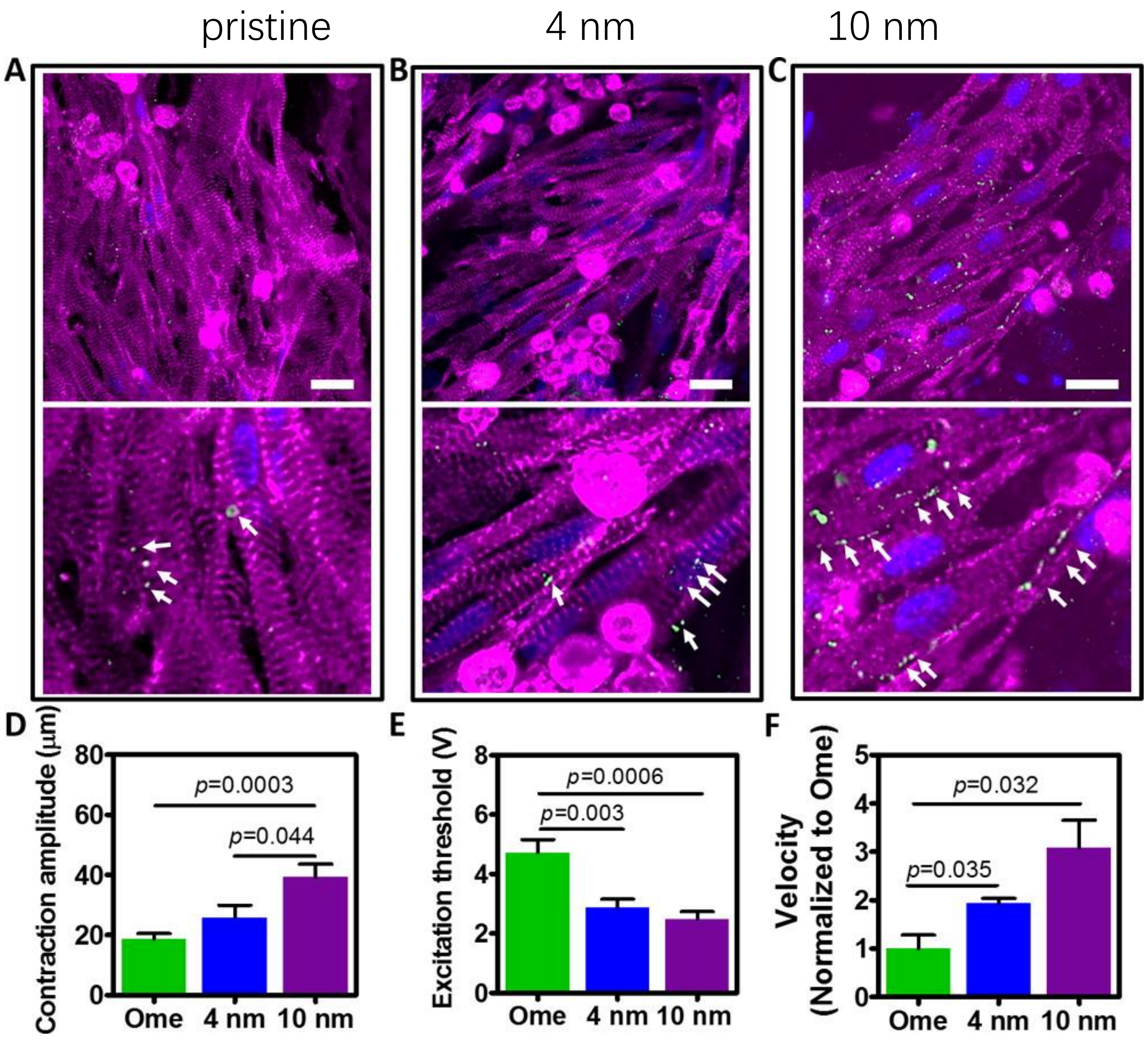
AuNP scaffolds. (A) AuNPs were deposited onto the scaffolds using an e-beam evaporator. The color of the scaffolds has changed due to the difference in NP dimensions. (B, C) ESEM images of Au-deposited scaffolds. Homogeneously deposited AuNPs were visualized on the 4 nm (B) and 10 nm (C). (D) Higher magnification of C. (E) Energy-dispersive X-ray spectroscopy of the fibers.

(H) Resistance calculation derived from current–voltage curves. Bar: B, C = 500 nm, D = 100 nm.



Nano Lett. 2014 Oct 8;14(10):5792-6. doi: 10.1021/nl502673m

Enhancement of electrical properties



Cardiac cell organization and engineered tissue function on day 5.

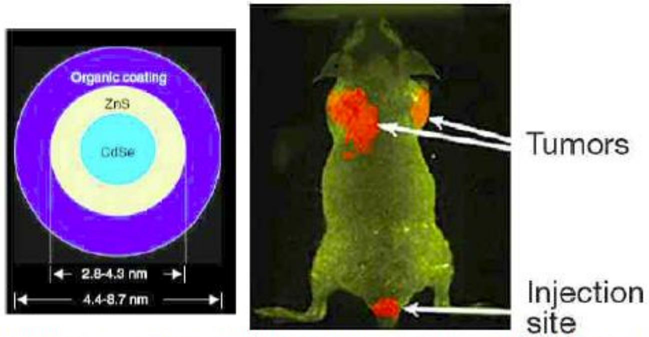
(A–C) Immunostaining of cardiac α sarcomeric actinin (pink), **connexin 43 (green)**, and nuclei (blue) of cardiac cells within the pristine (A), 4 nm (B), and 10 nm (C) scaffolds. Lower panels are higher magnifications. **White arrows indicate the location of connexin 43 molecules.**

(D) Spontaneous contraction amplitude. (E) Excitation threshold. (F) Velocity of calcium transients during spontaneous contractions. The velocity is normalized to tissues engineered in pristine scaffolds. Bar = 20 μm.

Nano-biomaterials for drug/diagnostics delivery

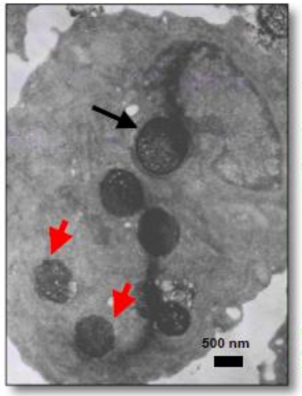
Nanomaterials as vectors for multiple systemic treatments and diagnostics:

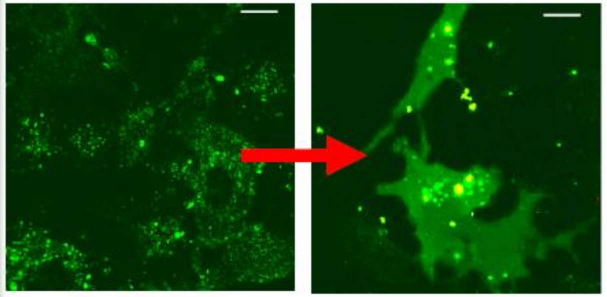
in vivo imaging/diagnostics



(Choi, Bawendi, Frangioni et al. Nat. Biotech. 25 1165- 1170 (2007); Gao, Nie et al. Nat. Biotech. 22 969-976 (2004))

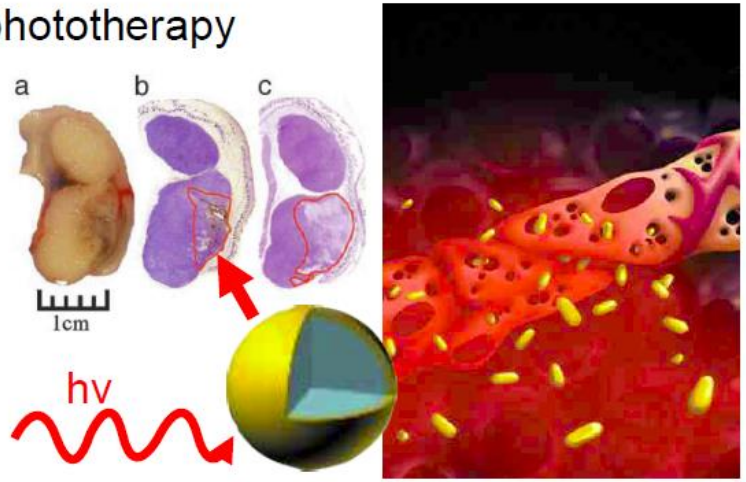
drug delivery





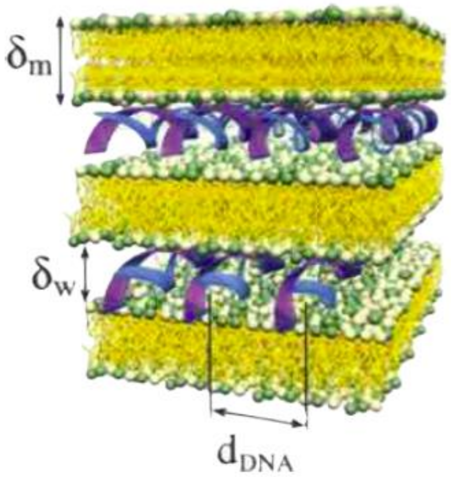
(Hu, Irvine et al. Nano Lett. 7 3056-3064 (2007))

phototherapy



JL West and N Halas et al., PNAS 100 13549-13554 (2003); Annu. Rev. Biomed. Eng. 2003. 5:285–92

Gene delivery

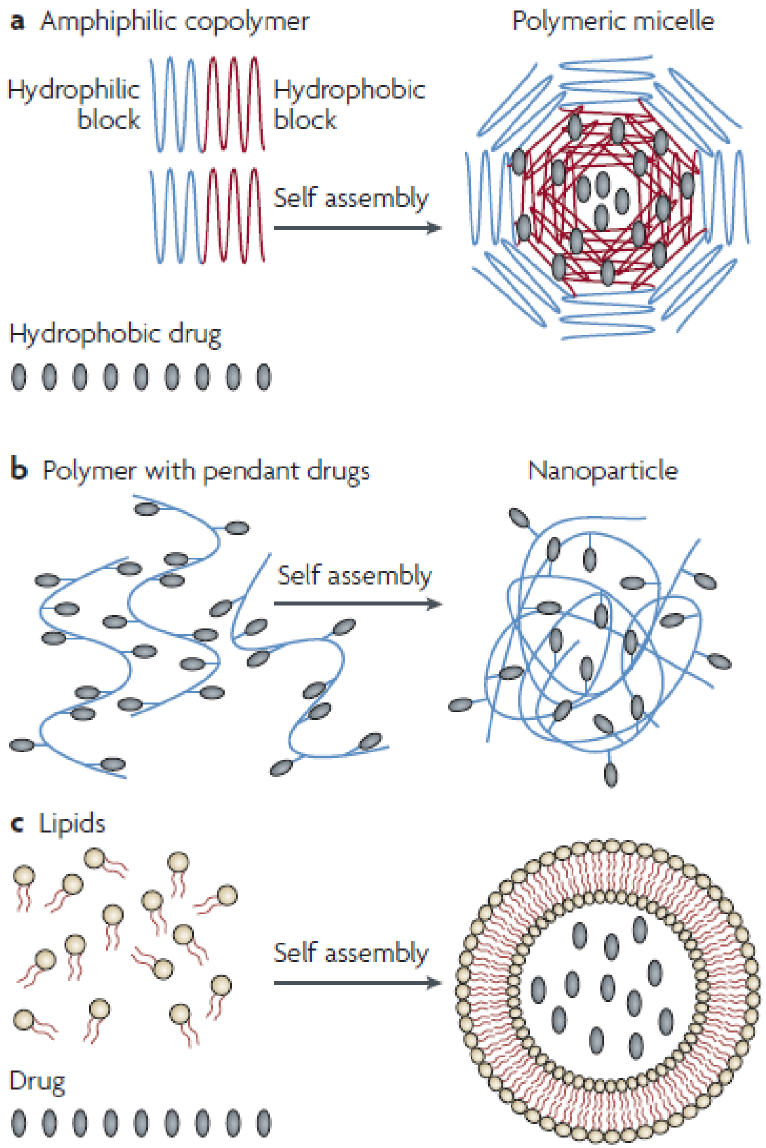


Koltover, Safinya et al., Science. 281 78-81 (1998)

Motivations for developing drug delivery system

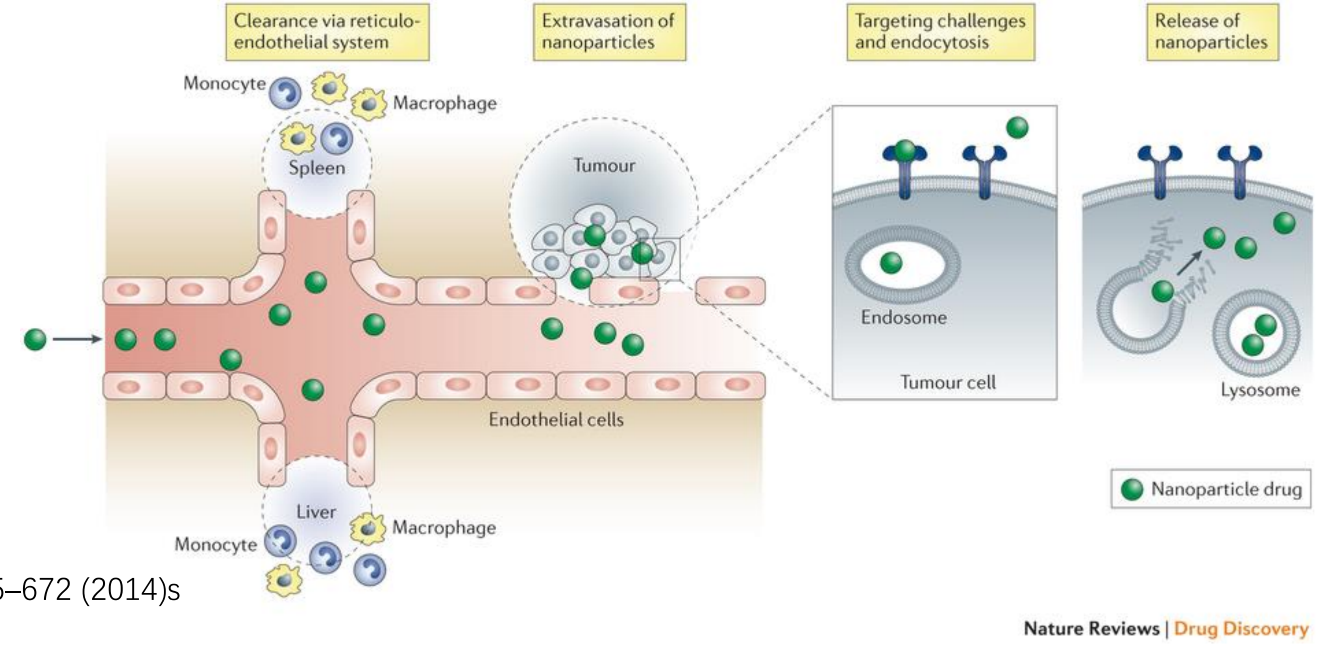
- Poor solubility of many drugs
- Rapid clearance
- Susceptibility to enzyme degradation
- Poor availability to tissues of interest
- Systemic toxicity and side effect

Nanoparticle synthesis as drug carriers

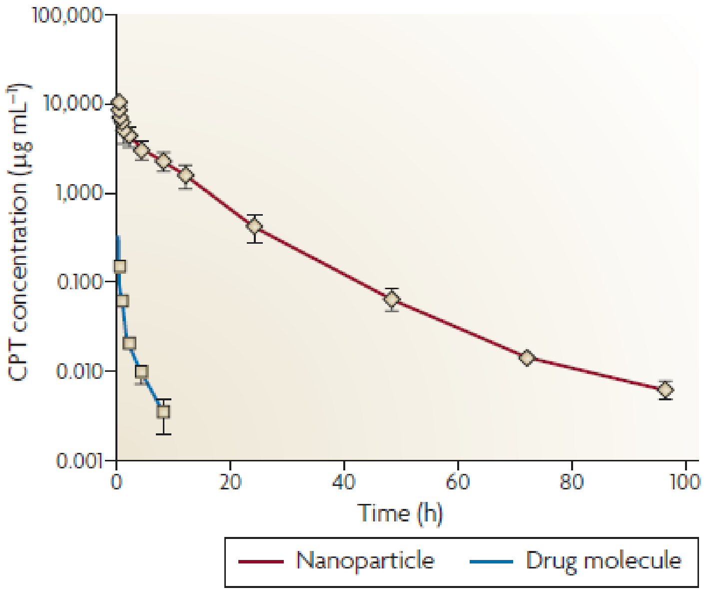


Nat. Rev. Drug Discov. **7**, 771-782 (2008).

Clearance of nanoparticles and alteration of pharmacokinetics

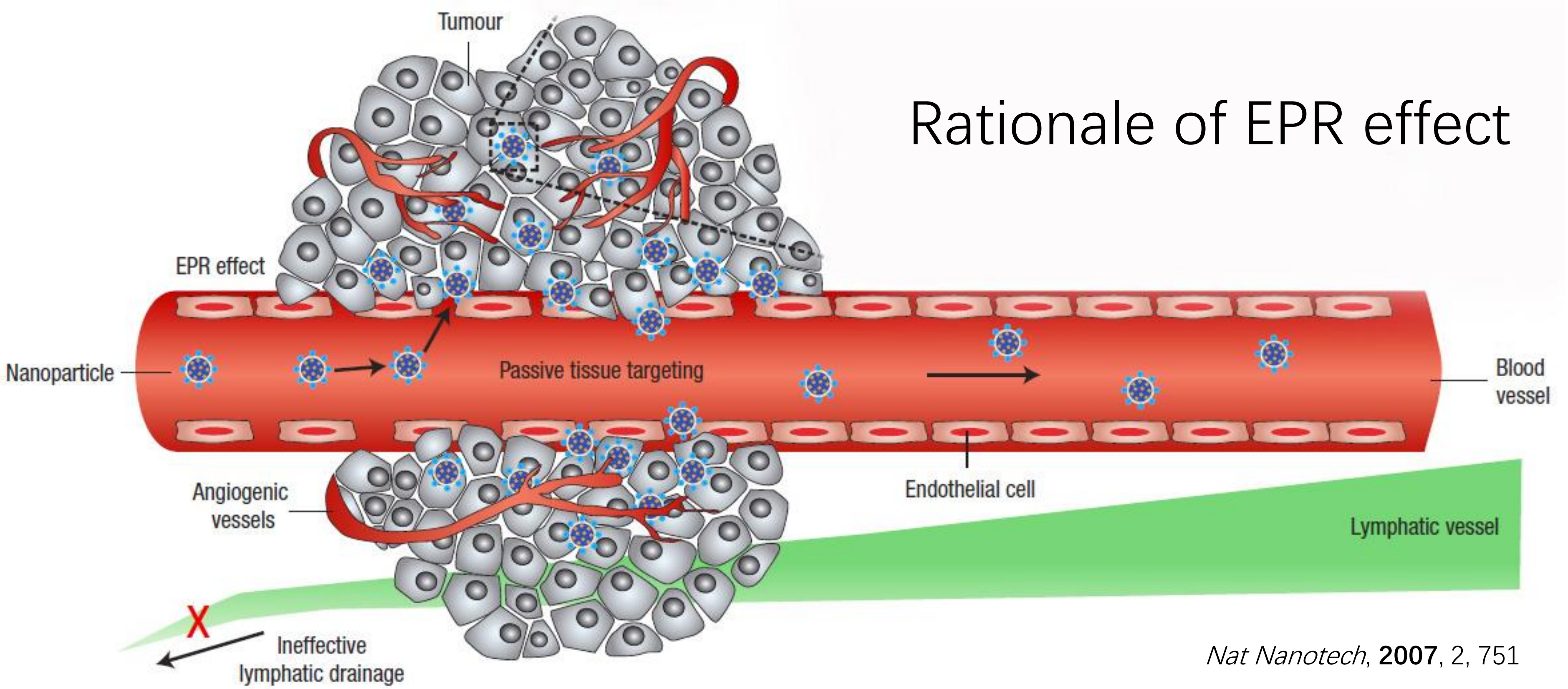


Nature Reviews Drug Discovery 13, 655–672 (2014)s



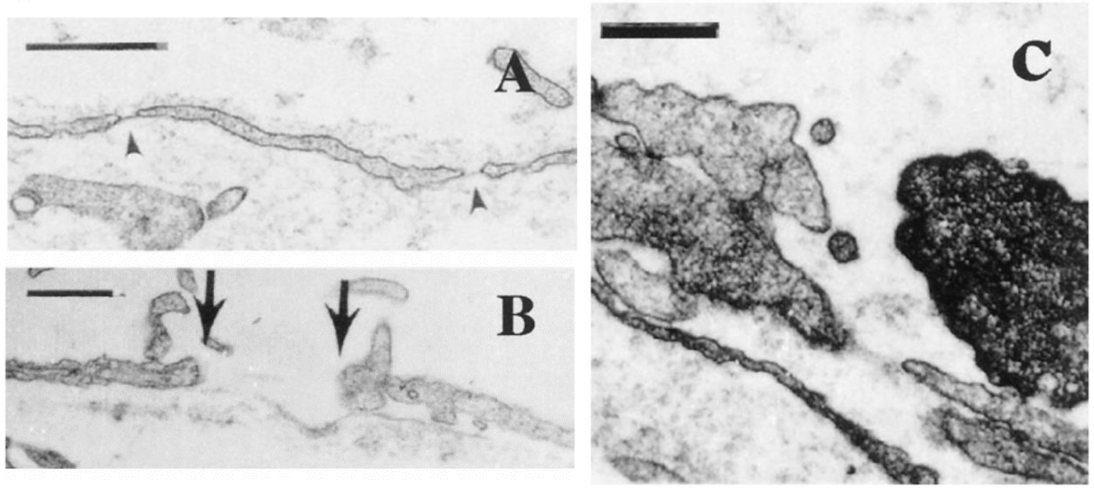
Nat. Rev. Drug Discov. **7**, 771-782 (2008).

Enhanced permeation and retention effect in tumors



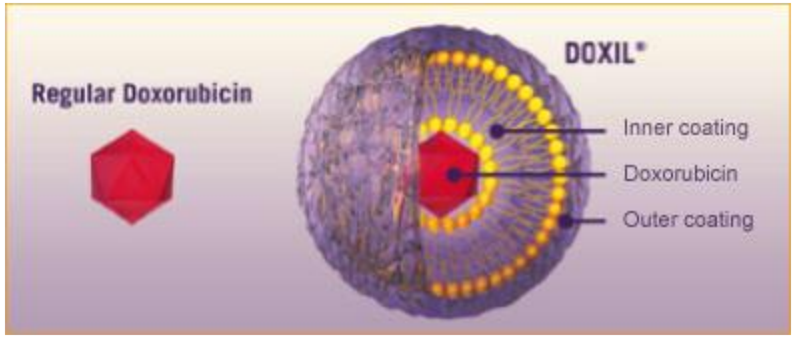
Nat Nanotech, **2007**, 2, 751

Pore size: 380-780 nm



PNAS, ***1998***, *95, 4607*

EPR effect for human patients



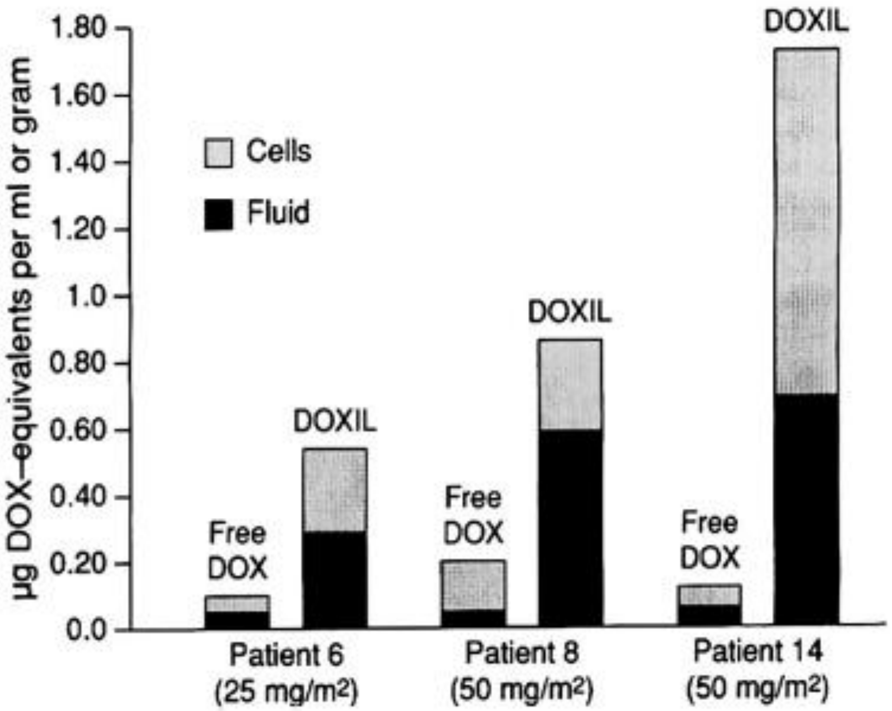
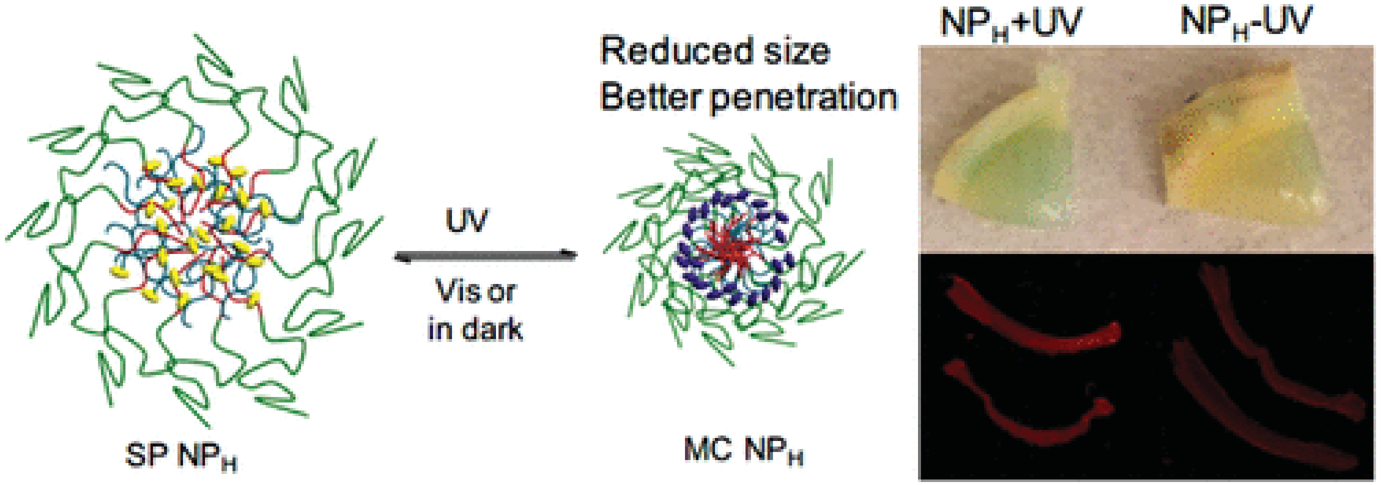
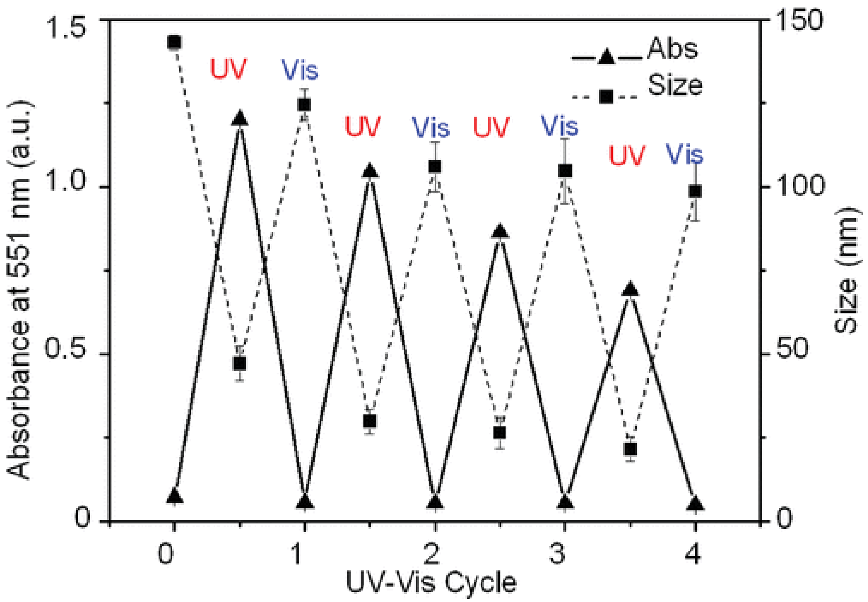


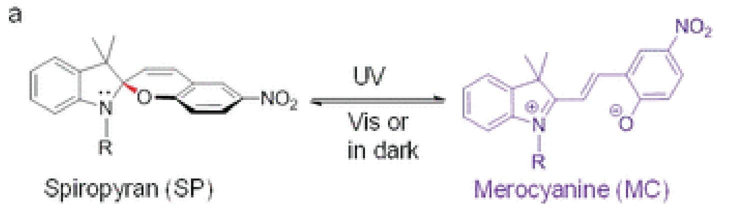
Figure 1. Doxorubicin levels in patients' tumor biopsies, comparing free DOX and DOXIL. Reprinted with permission from ref 26. Copyright 2012 Elsevier Ltd.

Chem. Rev., **2015**, *115* (19), pp 11147–11190

Responsive nanoparticles for drug delivery

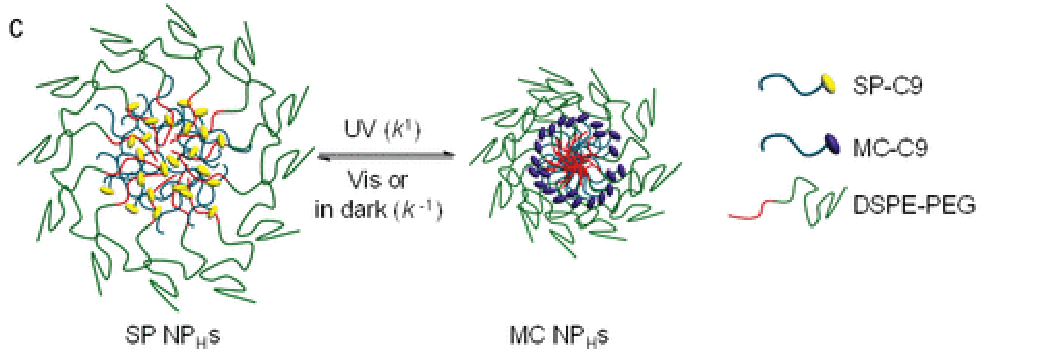


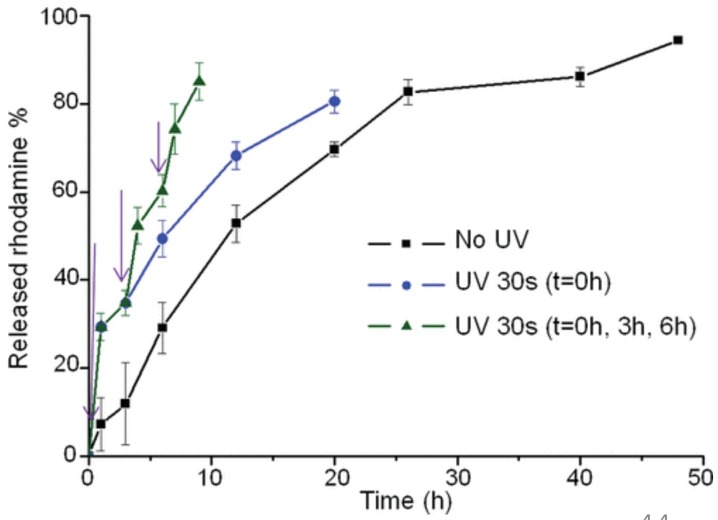




b

R	SP form	MC form
C_7H_{15}	SP-C7	MC-C7
C_9H_{19}	SP-C9	MC-C9
$C_{18}H_{37}$	SP-C18	MC-C18



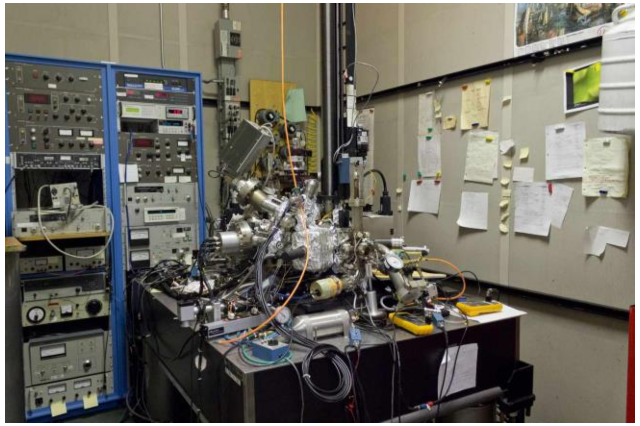


Photoswitchable Nanoparticles for Triggered Tissue Penetration and Drug Delivery | Journal of the American Chemical Society

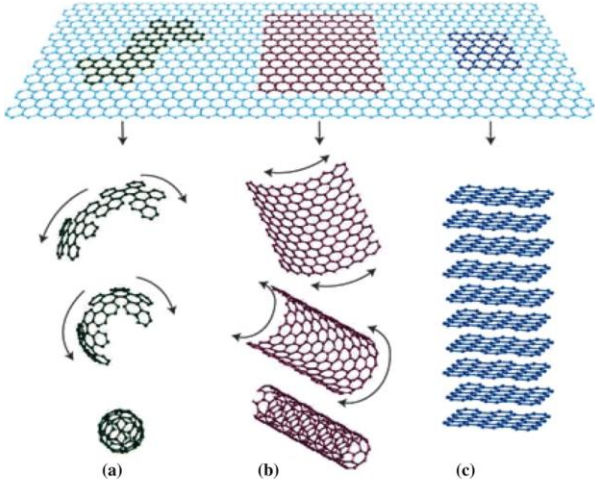
Which picture of the following represents a Nobel prize discover?

A

1986 Nobel Prize in Physics
Awardees: Gerd Binnig and Heinrich Rohrer
Topic: Invention of the scanning tunnelling microscope (STM), which enabled the first atomic-scale images of surfaces and paved the way for nanoscale research.



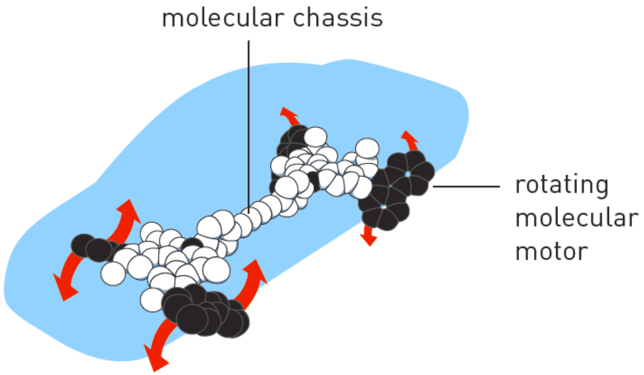
B



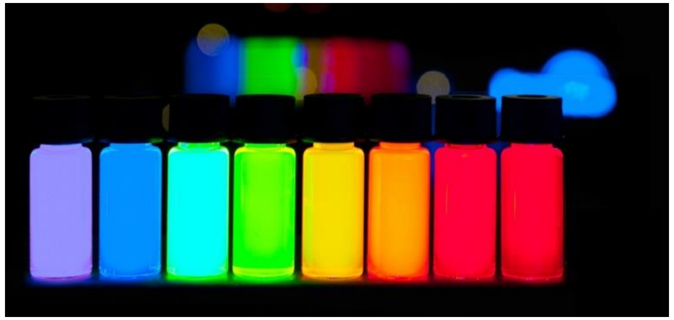
2010 Nobel Prize in Physics
Awardees: Andre Geim and Konstantin Novoselov
Topic: Groundbreaking experiments on graphene—a one-atom-thick, two-dimensional material whose unique properties arise from its nanoscale dimensions.

C

2016 Nobel Prize in Chemistry
Awardees: Jean-Pierre Sauvage, Fraser Stoddart, and Ben Feringa
Topic: Design and synthesis of molecular machines—nanoscale devices that can perform mechanical tasks, opening up new directions in nanotechnology and materials science.

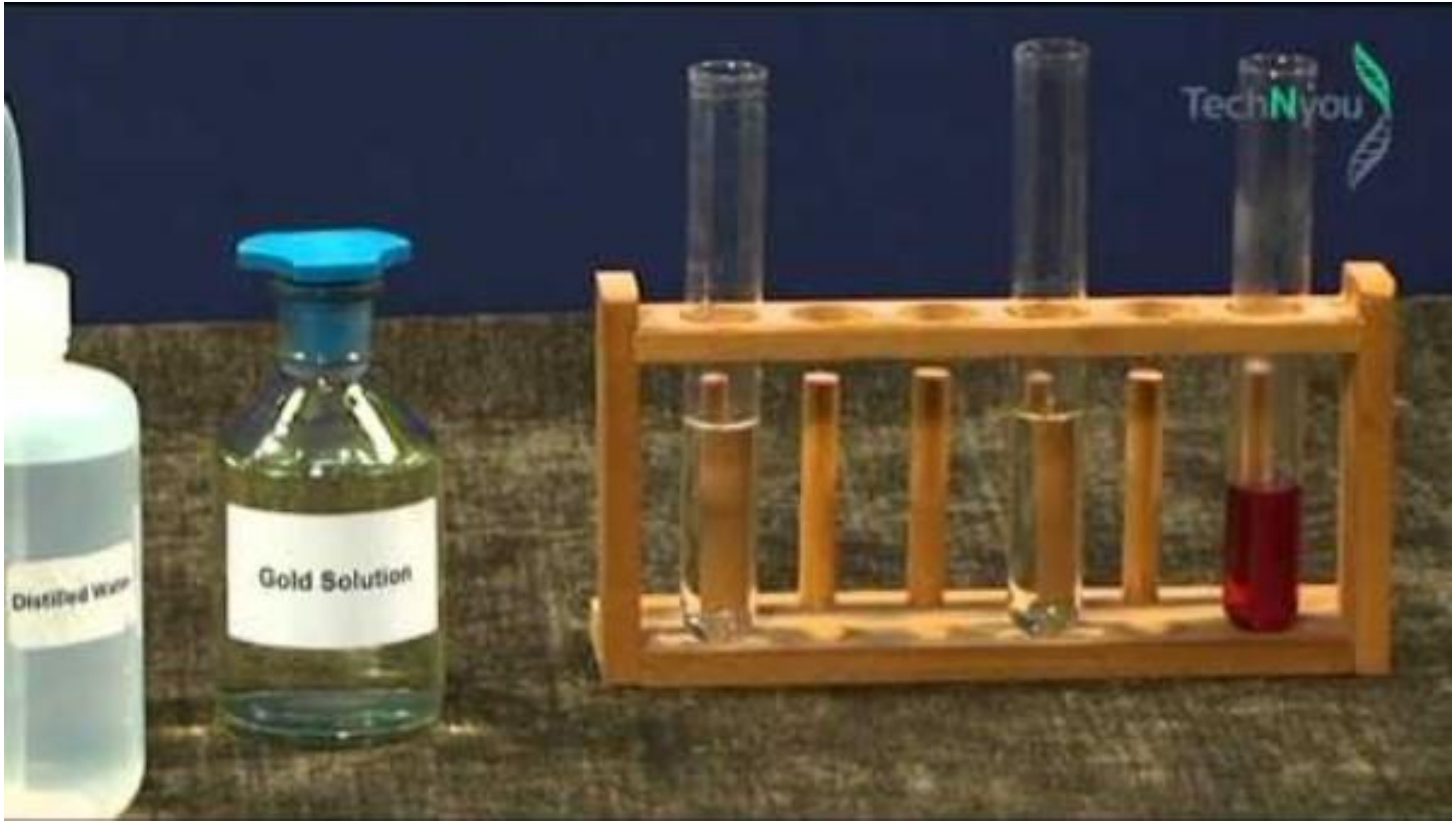


D



2023 Nobel Prize in Chemistry
Awardees: Moungi G. Bawendi, Louis E. Brus, and Alexey Ekimov
Topic: Discovery and synthesis of quantum dots—semiconductor nanocrystals whose optical properties (like emitted color) are determined by their tiny size.

How to make gold nanoparticles?



[MAKING GOLD NANOPARTICLES]