

Overview: Drug and Gene Delivery

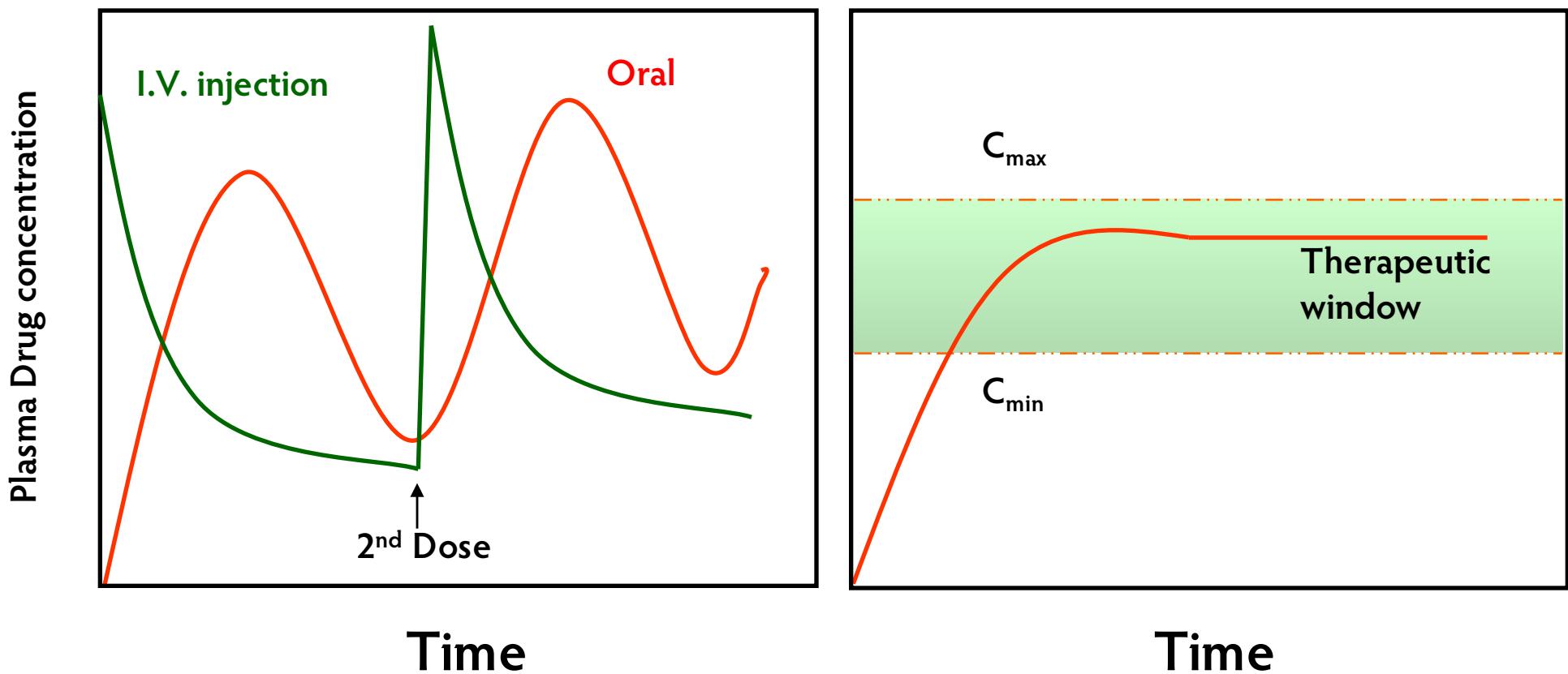
Lecture on Drug and Gene Delivery

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Columbia University

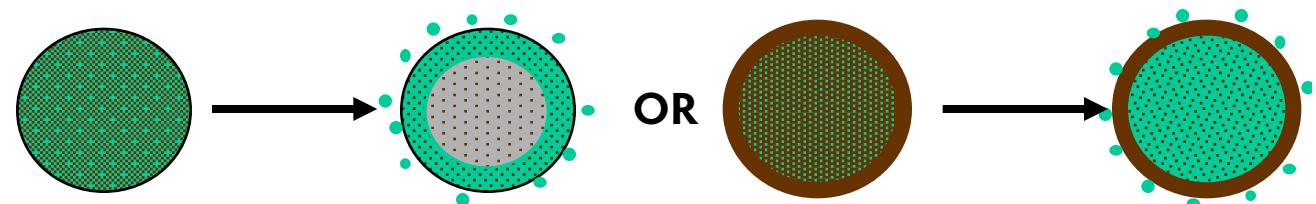
Fall Semester, 2016

Controlled Drug Delivery to Optimize Efficacy

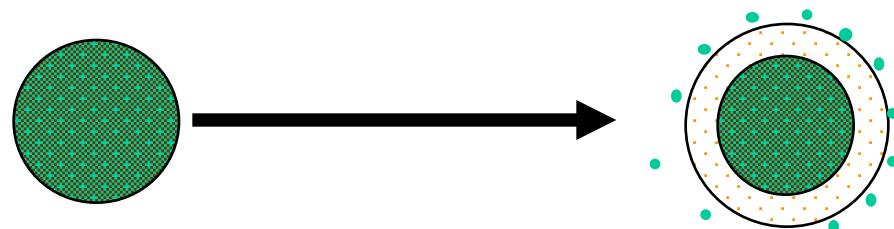


Drug Release From Polymeric Matrix

Diffusion-Controlled



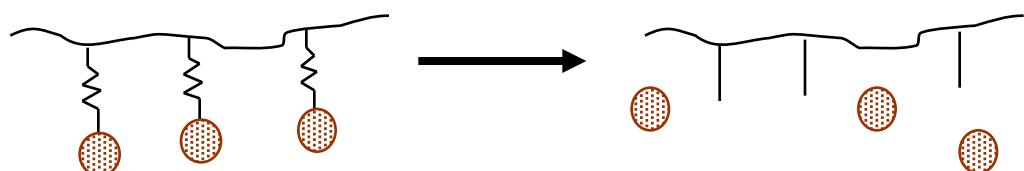
Matrix Swelling-Controlled



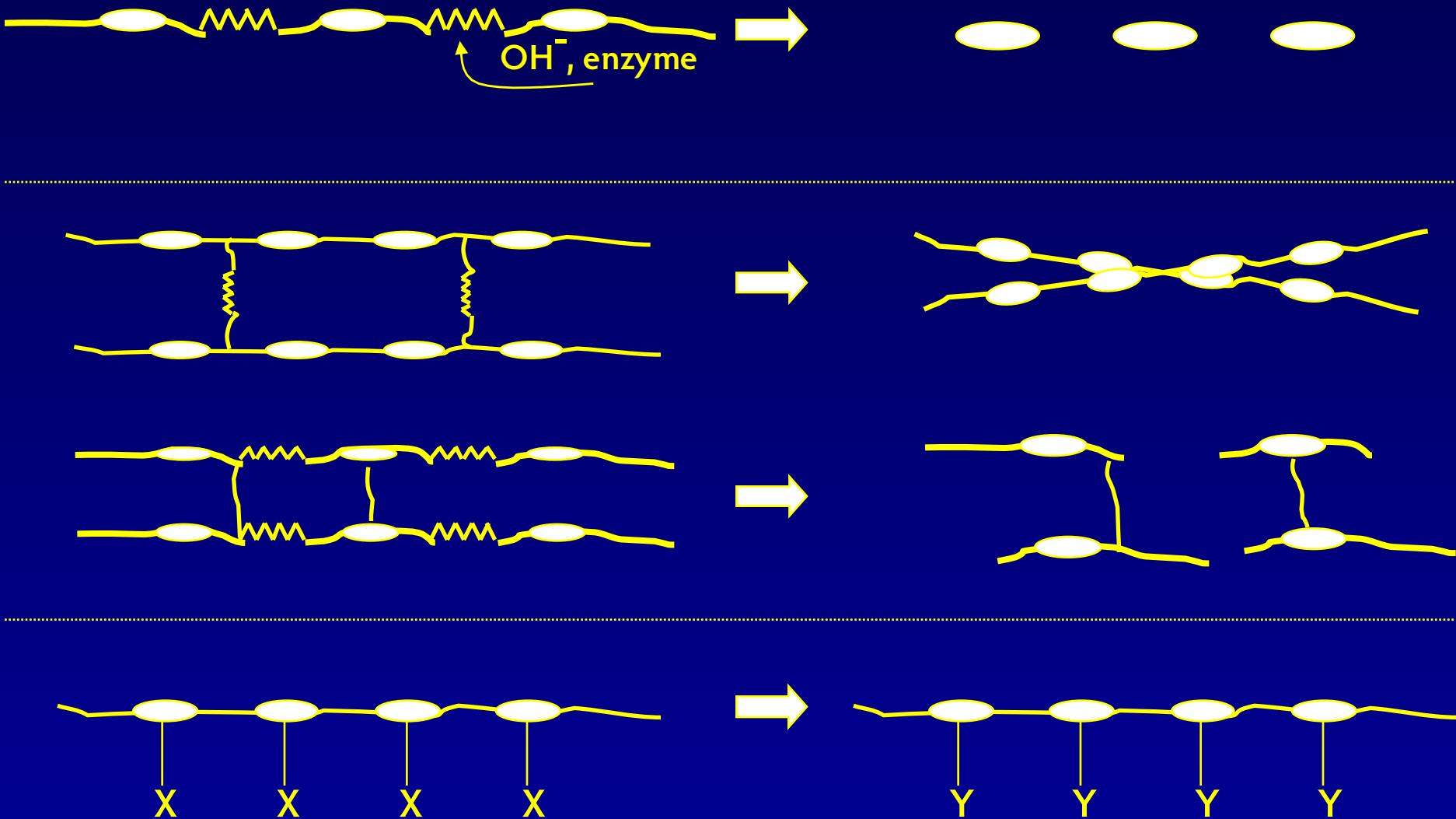
Matrix Degradation-Controlled



Pendant Cleavage-Controlled



Mechanisms of Biodegradation and Bioerosion



Surface versus Bulk Degradation

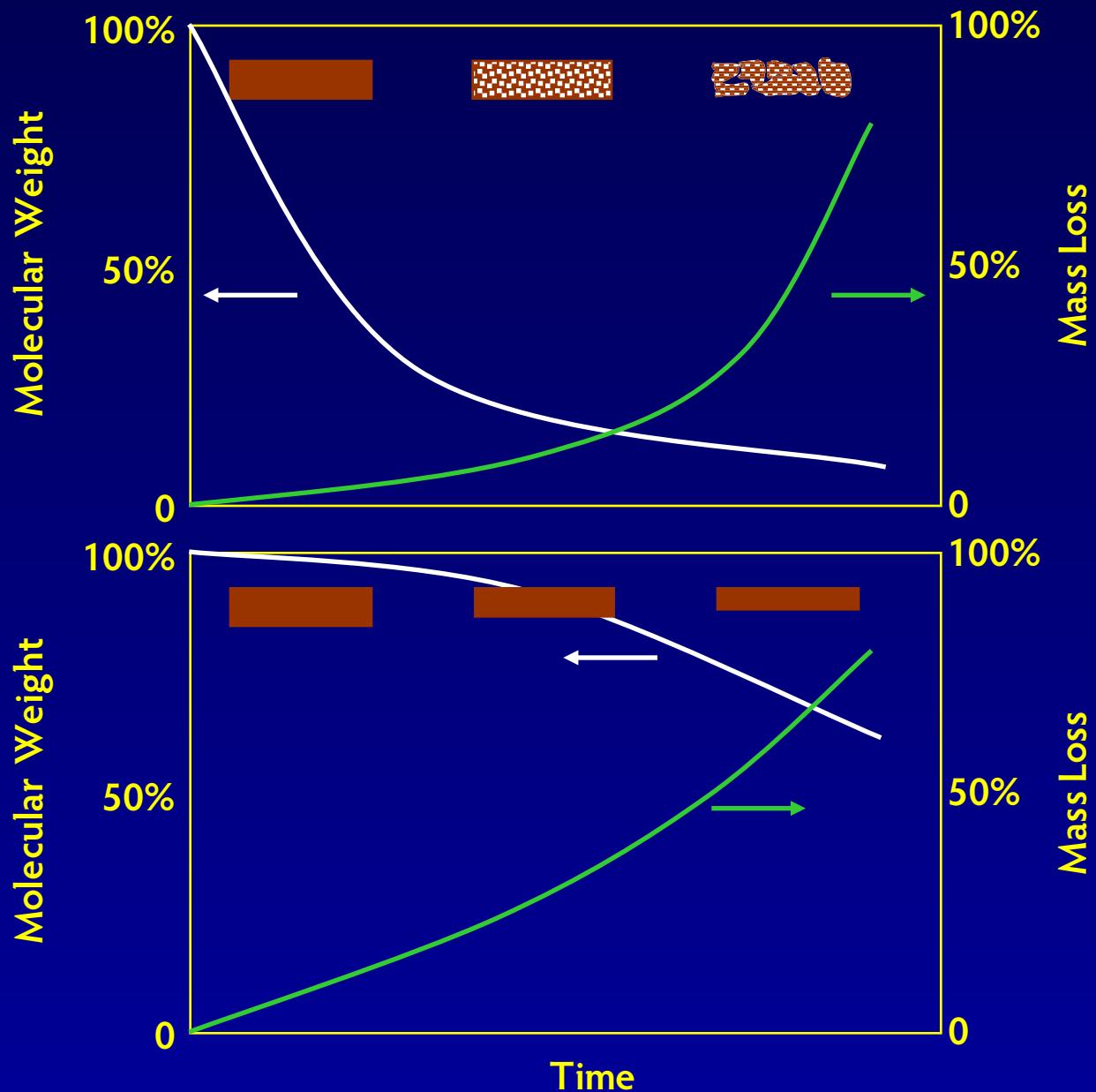
Bulk Degradation

$$R_{H_2O \text{ penetration}} \gg R_{\text{cleavage}}$$

Surface Degradation

$$R_{H_2O \text{ penetration}} \ll R_{\text{cleavage}}$$

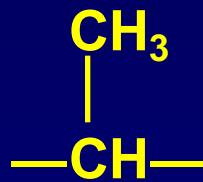
Geometry Dependent





Poly(glycolic acid)

- FDA approval

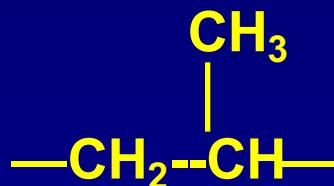


Poly(lactic acid)

- History of *in vivo* application
 - Suture materials
 - Drug carriers



Poly(ϵ -caprolactone)



Poly(β -hydroxybutyrate)

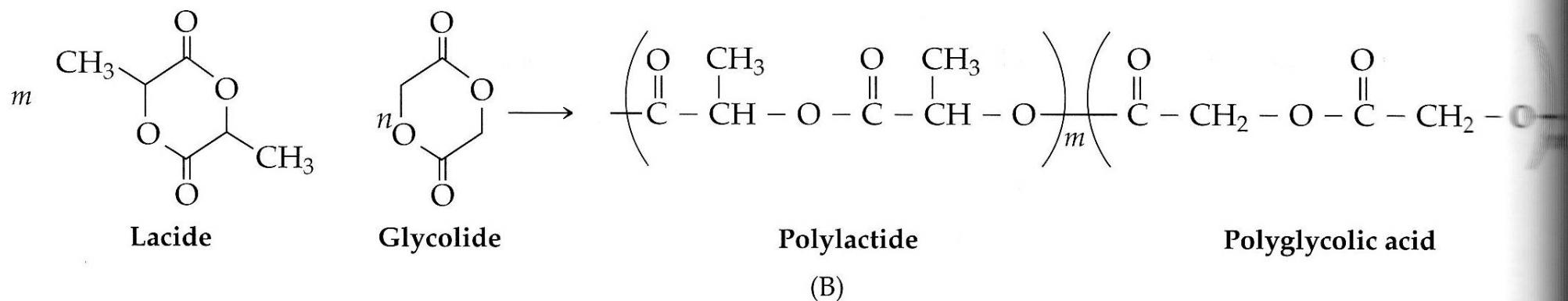
- Sudden “autocatalytic” degradation
- Slow degradation



Poly(propylene fumarate) and crosslinked network

Polyesters

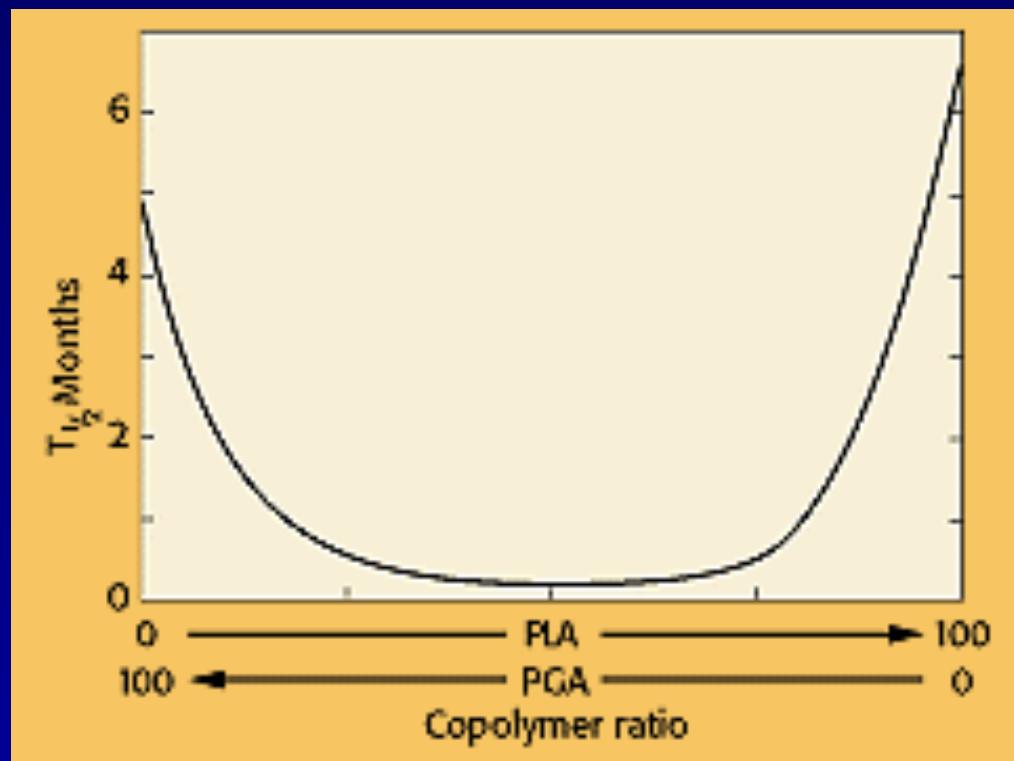
- PGA:
 - simplest linear aliphatic polyester
 - Highly crystalline (45–55%)
 - Fast degradation rate
 - sutures of PGA lose 50% of strength after 2 weeks, 100% at 4 weeks and completely absorbed in 4–6 months.
 - Very stiff, therefore, copolymerize with other polymers to decrease stiffness



Polyesters

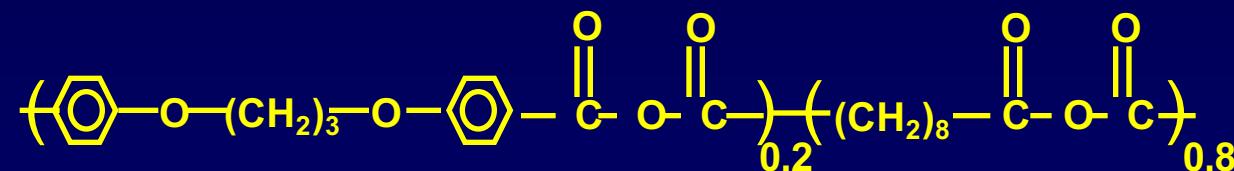
- Commonly used polyesters:
 - Poly(glycolic acid), PGA
 - Poly(lactic acid), PLA
 - Copolymer, PLGA or PLA_xGA_y , ($x + y = 100\%$)
 - $\text{PLA}_{25}\text{GA}_{75}$; $\text{PLA}_{50}\text{GA}_{50}$
 - Poly(ϵ -caprolactone), PCL

Copolymers amorphous because disruption of regularity of polymer chain by other monomer

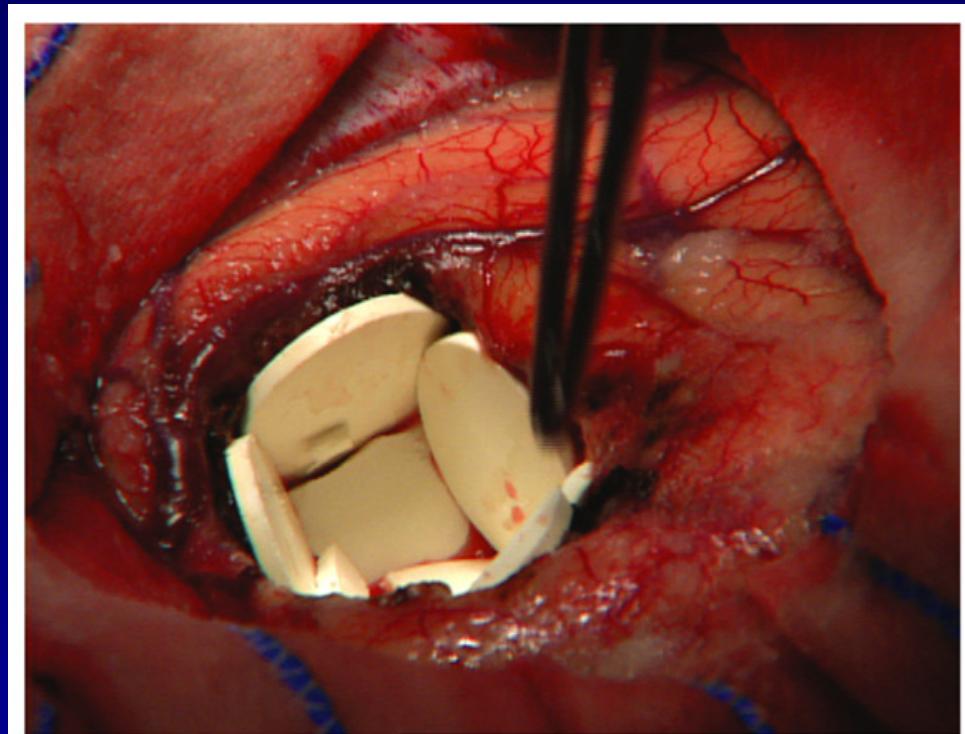


Factors that influence biodegradation and bioerosion

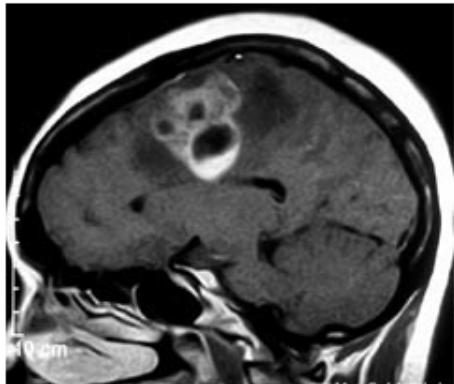
- Chemical structure
 - Hydrolytic stability / hydrophilicity / Mw
- Morphology
 - Crystallinity / Tg / Tm
- Geometry
 - Solid / microspheres/ film/ coating / fiber/ porous scaffolds
- Processing conditions
 - Fabrication process/ Additives/ Plasticizers



Gliadel: BCNU



Biodegradable Polyanhydride (Glidel) as a Carrier for Chemotherapeutics and Radio Seed



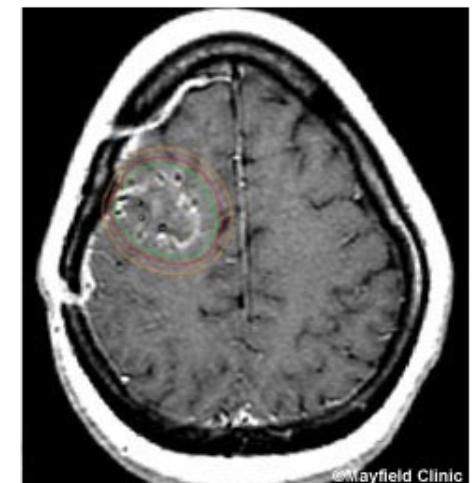
Patient with a glioblastoma tumor in the frontal lobe.



©Mayfield Clinic
Next, chemotherapy wafers are placed in the tumor bed. Then the dura and craniotomy are closed in standard fashion.

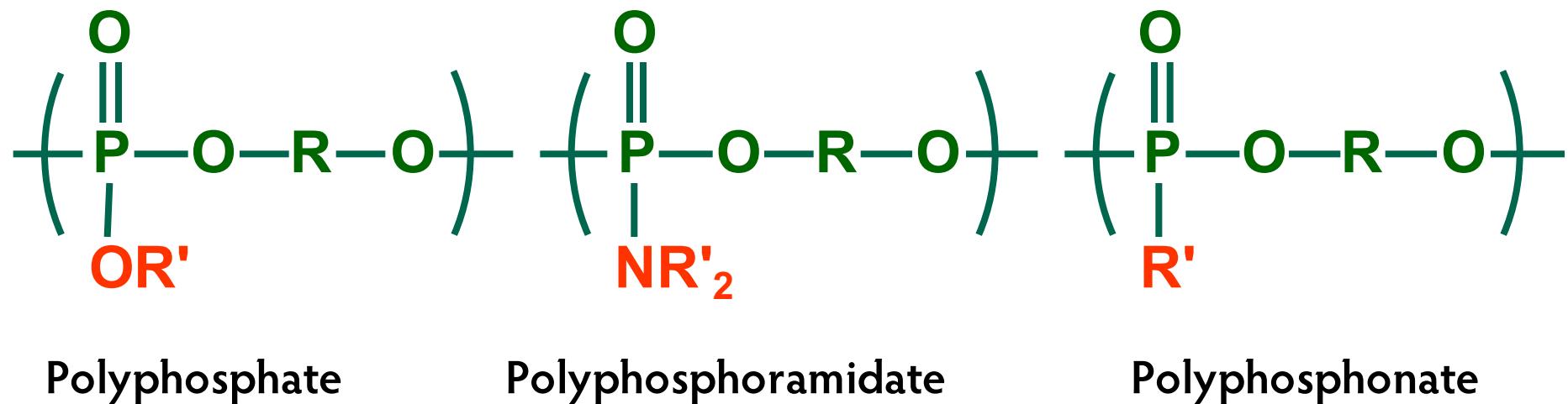


©Mayfield Clinic
After tumor is removed, radioactive I-125 seeds are placed in the tumor bed.



©Mayfield Clinic
Two weeks after placement of the seeds and wafers, radiation therapy is delivered for 6 weeks.

Polyphosphoester Drug Delivery System



Polyphosphate

Polyphosphoramidate

Polyphosphonate

- ◆ **Structure Versatility**
- ◆ **Favorable Physico-Chemical Properties**
- ◆ **Biocompatibility**
- ◆ **Pendant Chain Functionality**

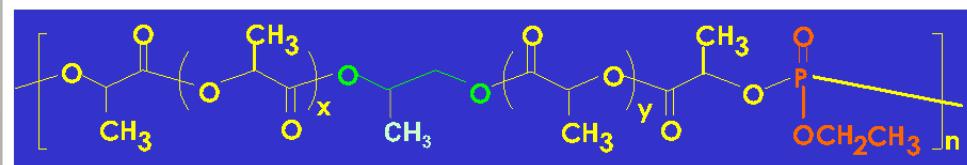
Polyphosphoester Drug Delivery System



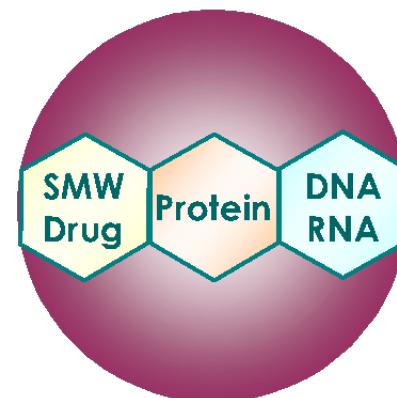
PACLIMER® Microspheres

LIDOMER™ Microspheres

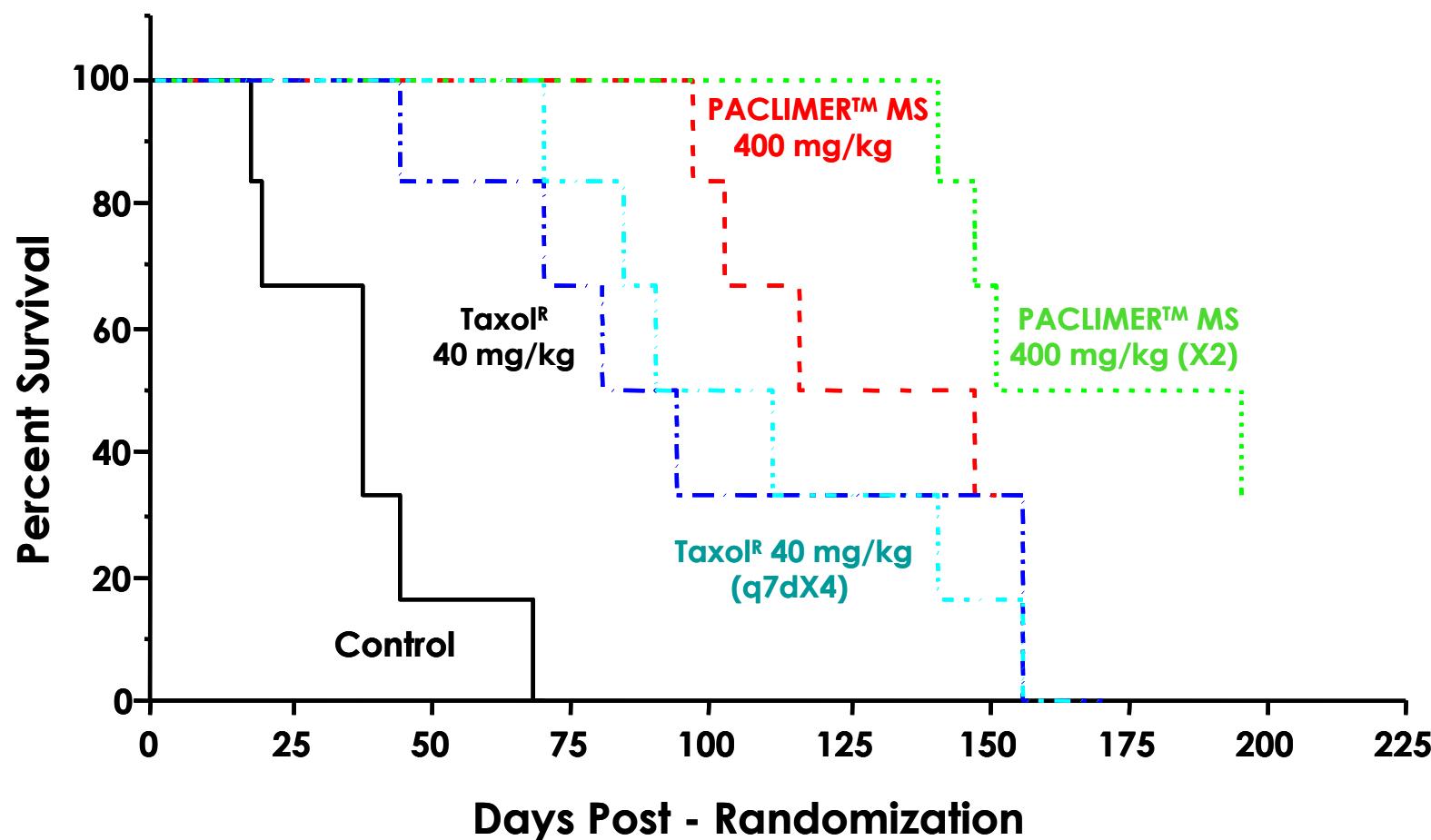
GUILFORD
PHARMACEUTICALS



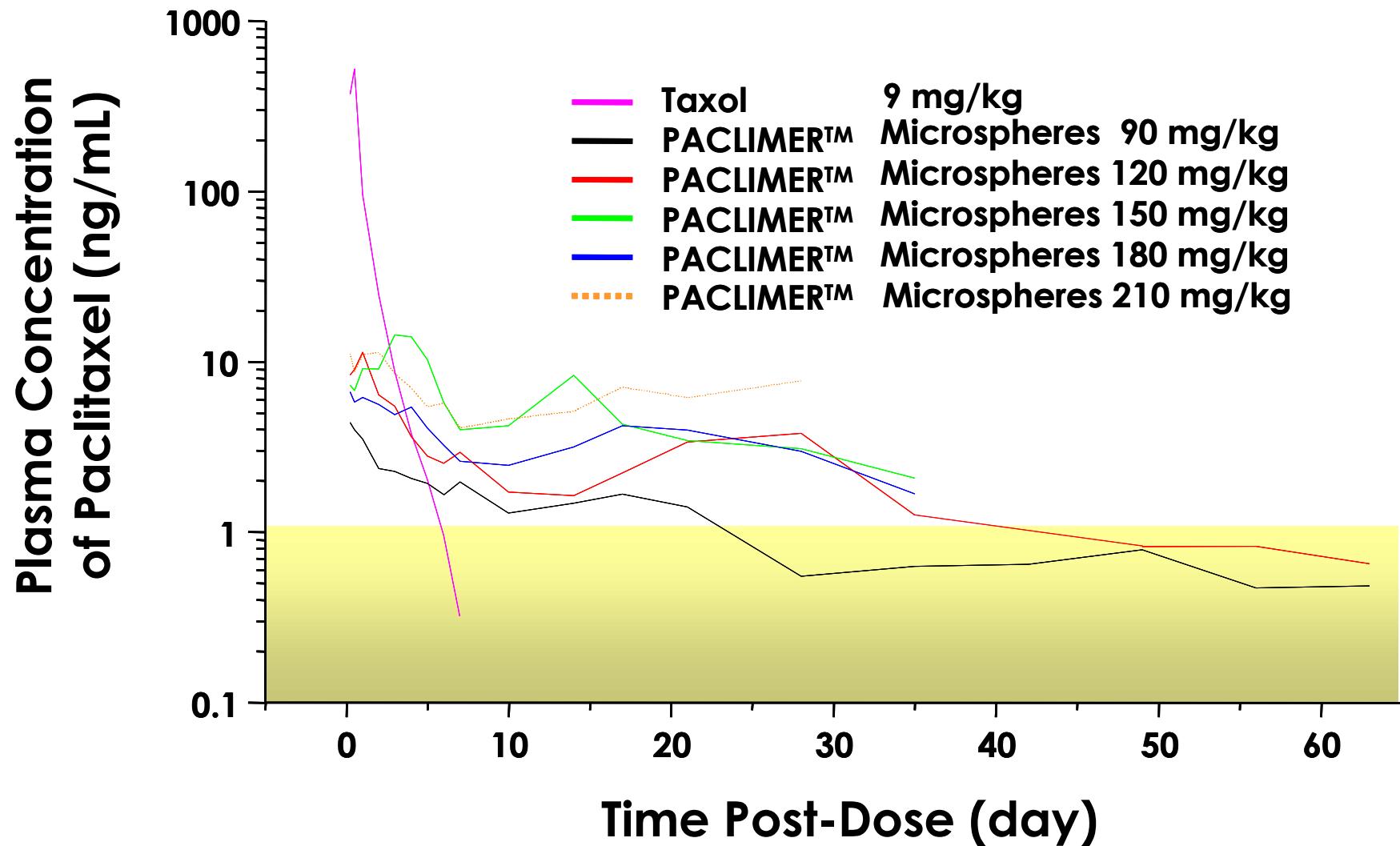
PACLIMER™ Delivery System:
Paclitaxel-encapsulating microspheres for
the treatment of ovarian and lung cancers,
and for pain management.



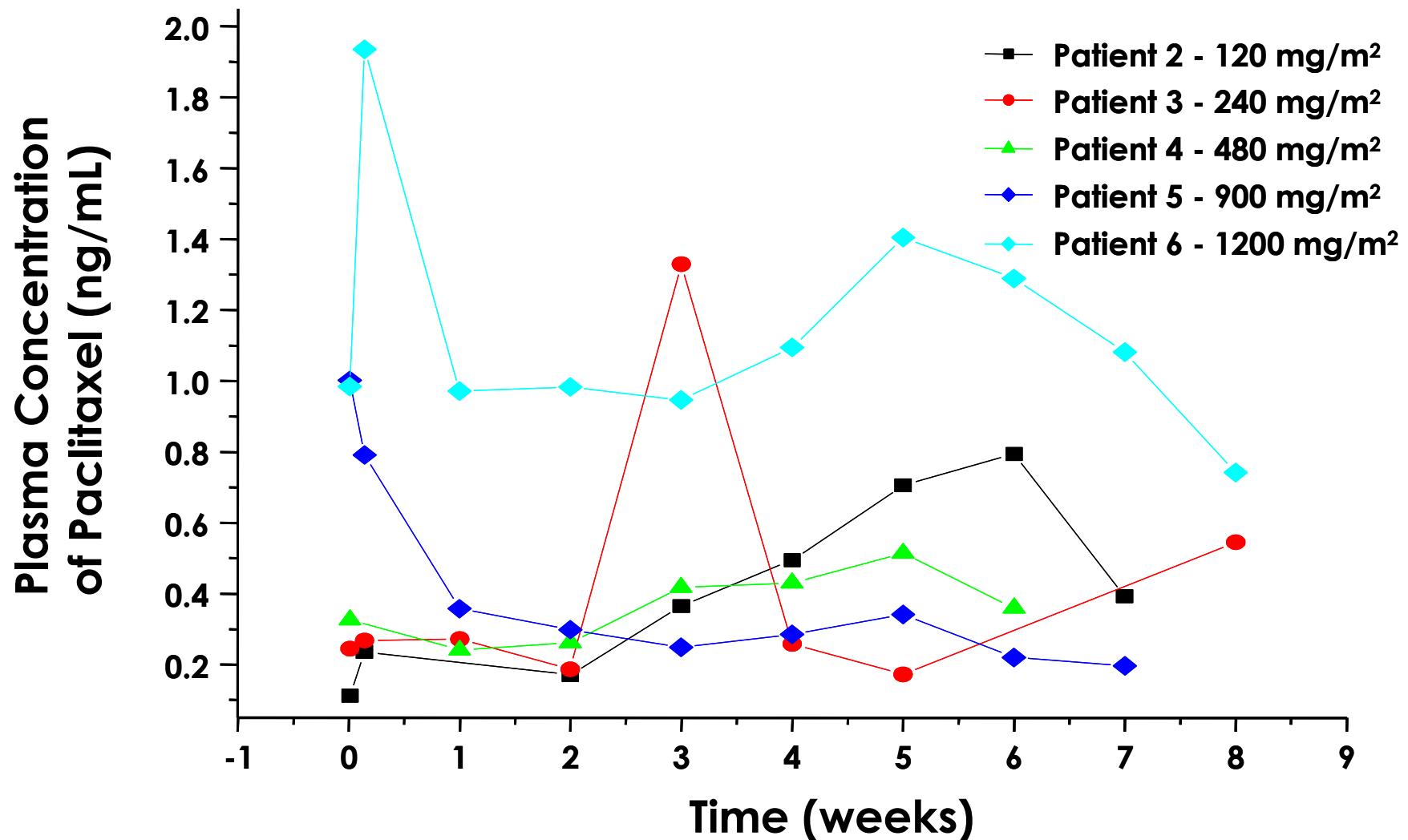
Sustained Delivery of Paclitaxel by PACLIMER® Microspheres Prolonged Survival of OVCAR-3- Bearing Rats



Plasma Concentration of Paclitaxel in Dog Following Intraperitoneal Administration

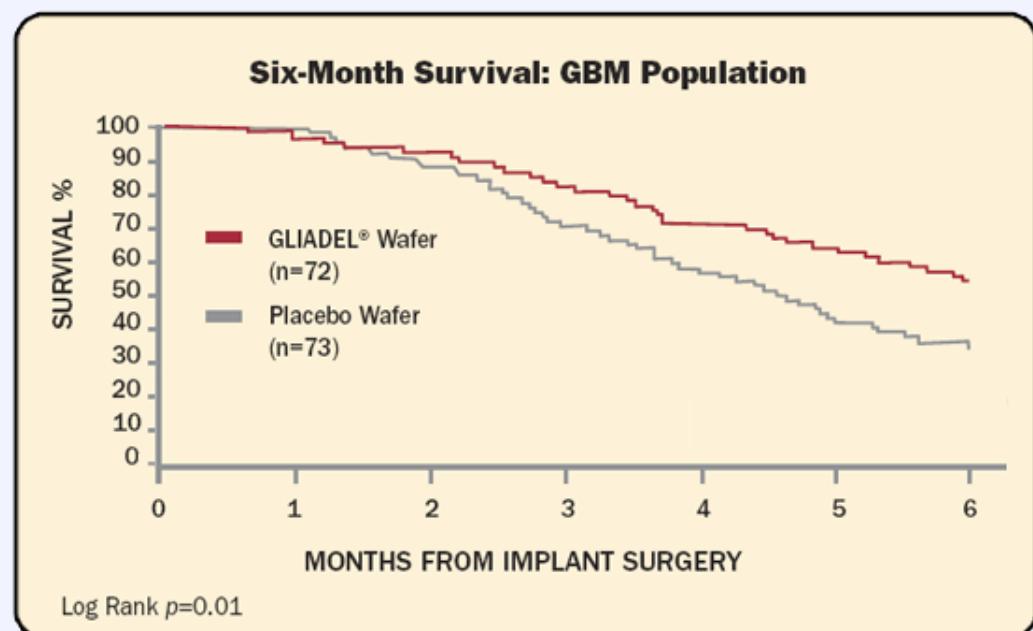


Plasma Concentration of Paclitaxel Following Intraperitoneal Administration of PACLIMER™ Microspheres to Patients with Ovarian Cancer

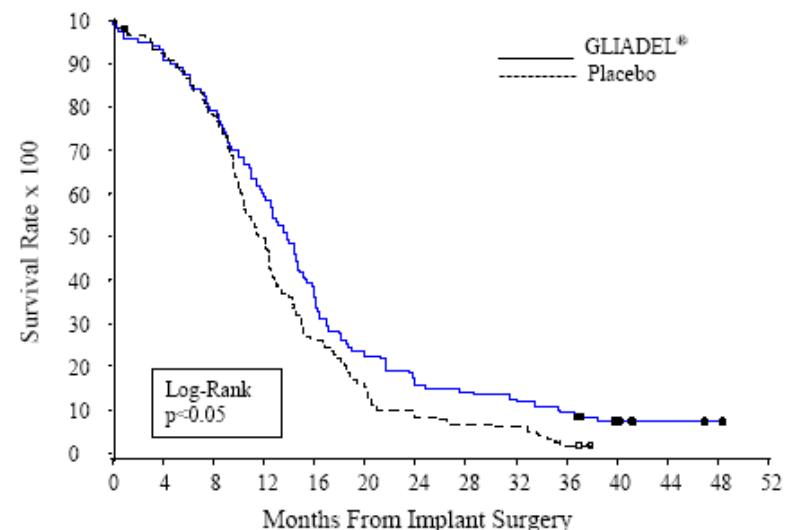


Prolongation of Survival of Patients by Gliadel Treatment

Recurrent Glioblastoma Multiforme¹



Phase 3 randomized, double-blind, multicenter, study of the safety and efficacy of GLIADEL® Wafer vs placebo in surgery involving 222 patients with recurrent malignant glioma who had failed initial surgery and radiation therapy. Chemotherapy was withheld at least 4 weeks (6 weeks for nitrosoureas) prior to and 2 weeks after surgery.



Routes of Administration

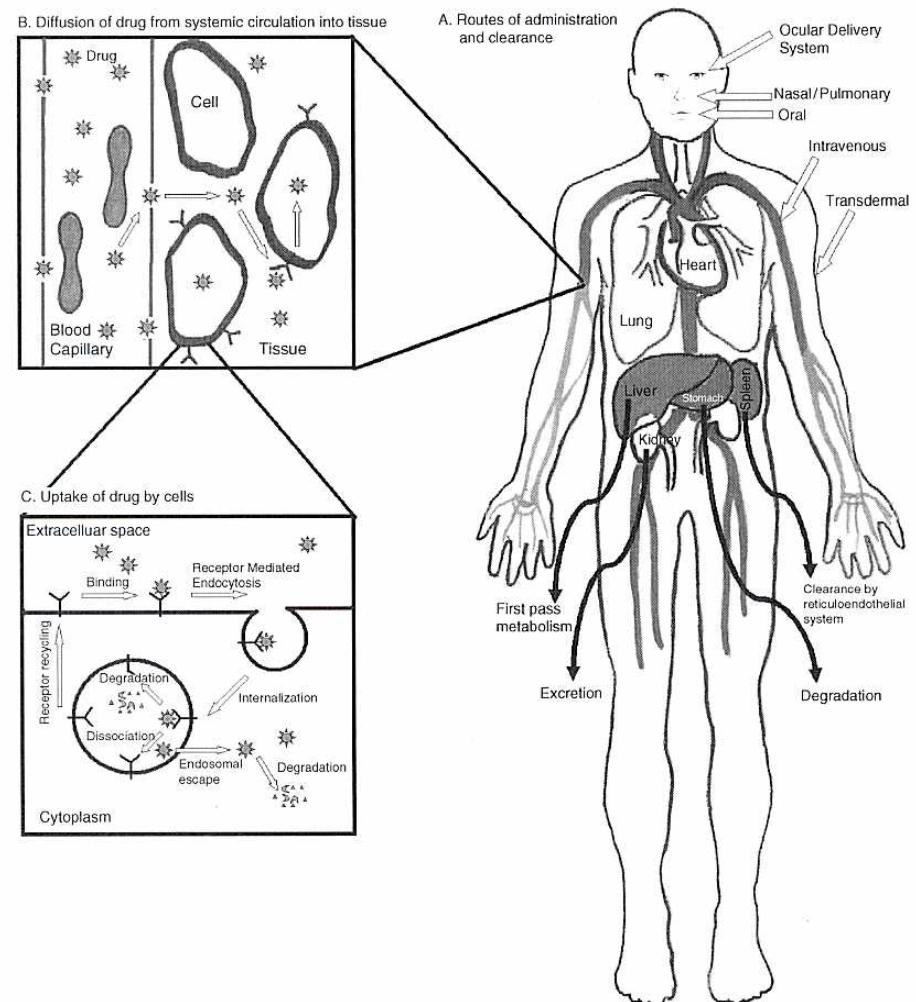
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An Introduction to Biomaterials

TABLE 19.2

Drug Delivery Routes Corresponding to Regions of the Body that Come into Contact with the External Environment

Physiological Barrier	Location in the Body	Mode of Delivery
Mucosal tissue	GI tract	Oral formulations, sublingual formulations, enemas
	Nasal passages	Nasal sprays, inhalants
	Lung	Pulmonary delivery systems
	Uterus	Intrauterine delivery systems
	Vagina	Intravaginal delivery systems
	Skin	Topical creams, transdermal patches
	Eyes	Ocular delivery systems



Transdermal Delivery Device

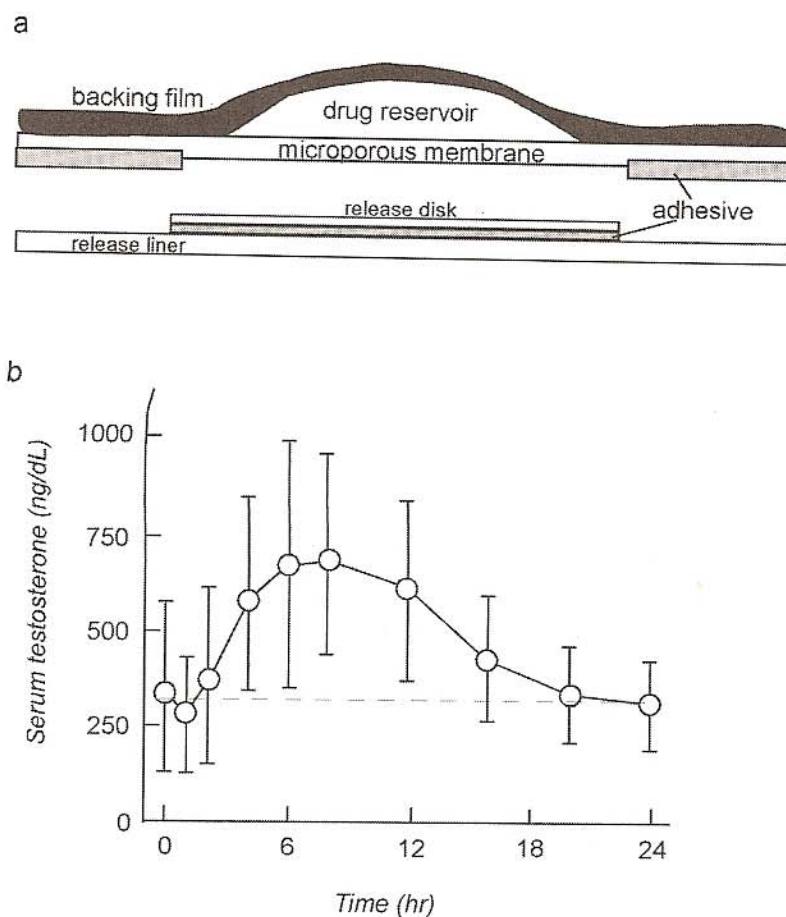
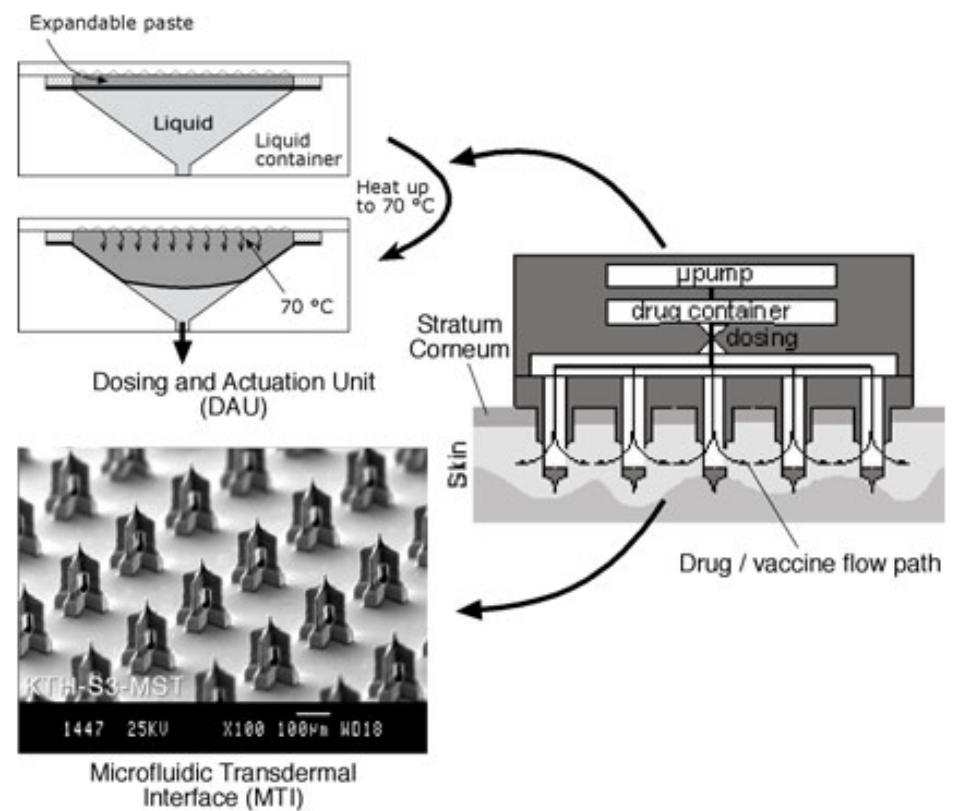
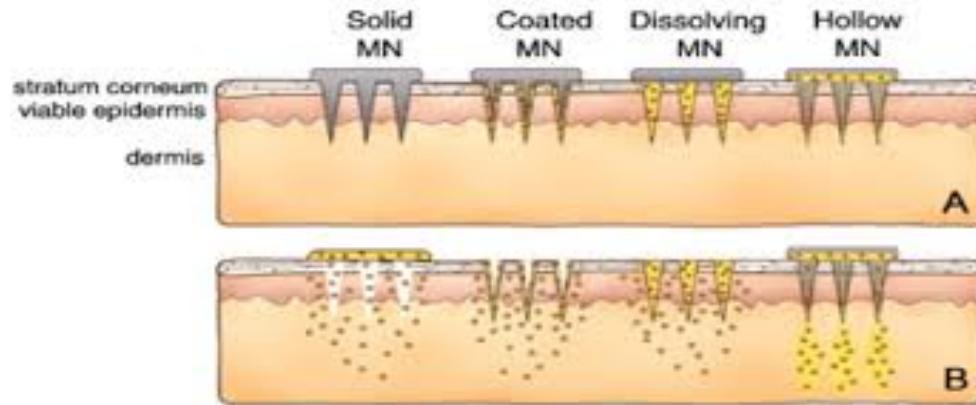


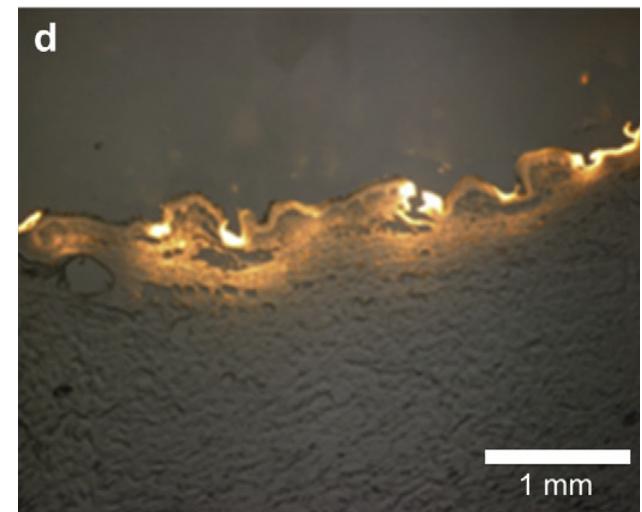
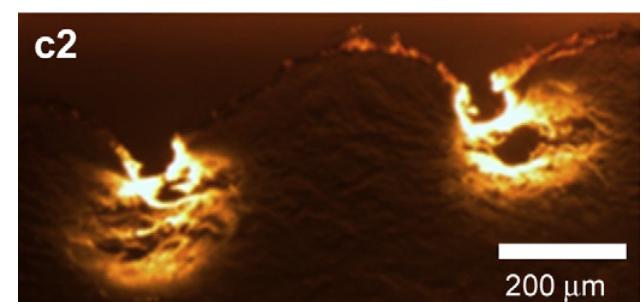
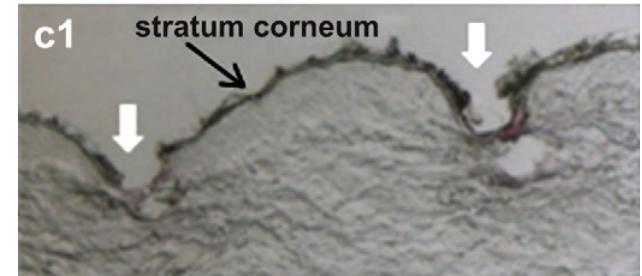
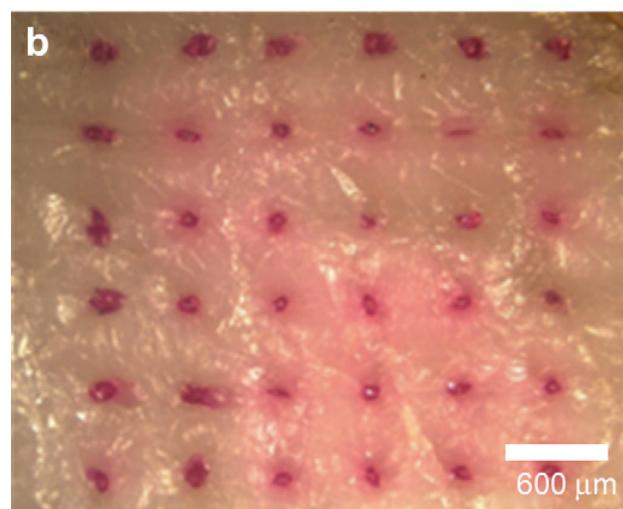
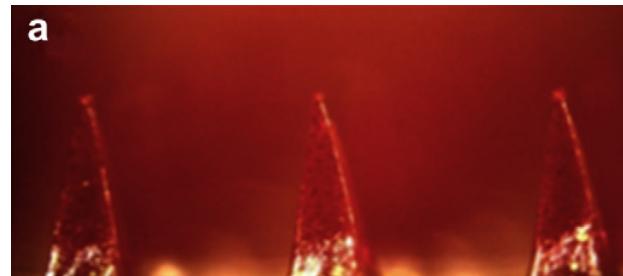
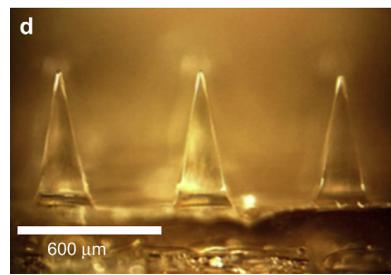
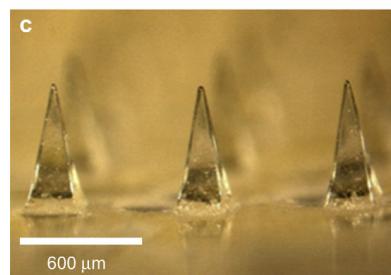
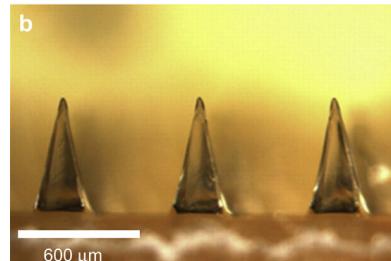
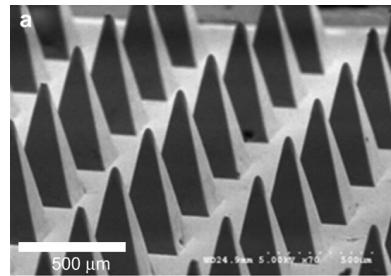
Figure 9.4 Serum concentrations after placement of a transdermal device. (a) Schematic diagram of the transdermal testosterone-releasing system. To initiate therapy, a protective backing layer (composed of the release liner and disk) is removed and the device is applied to the skin with the microporous membrane contacting the external surface of the skin. (b) A transdermal patch that releases testosterone was applied and serum concentrations were monitored over the subsequent 24 h. Serum concentration of testosterone increases during the first few hours and then remains nearly constant for the remainder of the 24-h measurement period. Data from [4] for Androderm® (SmithKline Beecham).

Transdermal Drug Delivery Systems

- Dosing and actuator unit
- Liquid through outermost skin using hollow, side-opened microneedles
- Leakage can be an issue



Microfabricated (dissolving) Microneedles for Transdermal Drug Delivery

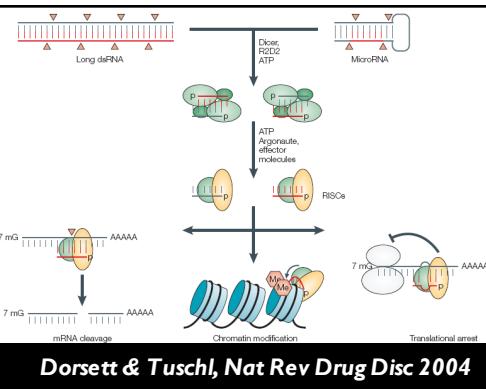
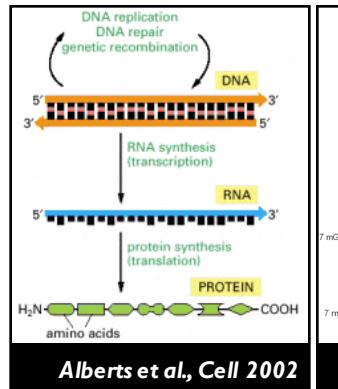


Potential of Nucleic Acid-Based Therapeutics



- Human genome map
- Bioinformatics
- Systems biology

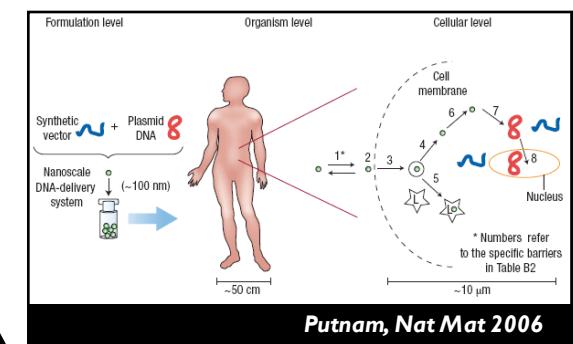
Nucleic Acid Therapeutics



- DNA (plasmid)
- RNA (siRNA, miRNA, ribozyme)
- Antisense ODN
- Aptamer
- PNA

Delivery Systems

- Direct (chemical modification or conjugation)
- Viral vectors
- Nonviral vectors (polyplex, lipoplex, liposome, micelles)



Therapeutic potential depends on achieving safe and efficient delivery to target cells

Pros and Cons of Drug vs. Gene Delivery

Drug

Type of Compound

No limitation

Delivery Profile and Bioactivity

More predictable temporal and spatial profiles

Ease of Use

Formulation required

Gene

Limited to proteins

Control at the molecular level and applicable to non-soluble factors

More difficult to control (rate, level, duration)

Bioactive; may act in paracrine manner

Simple; convenient in evaluating new factors

Viral vs. Non-Viral Carriers



High

Delivery efficiency

Low

Poor

Targeting efficiency

Poor

Limited packaging capacity

DNA size

High

Relatively complicated

Scale-up production

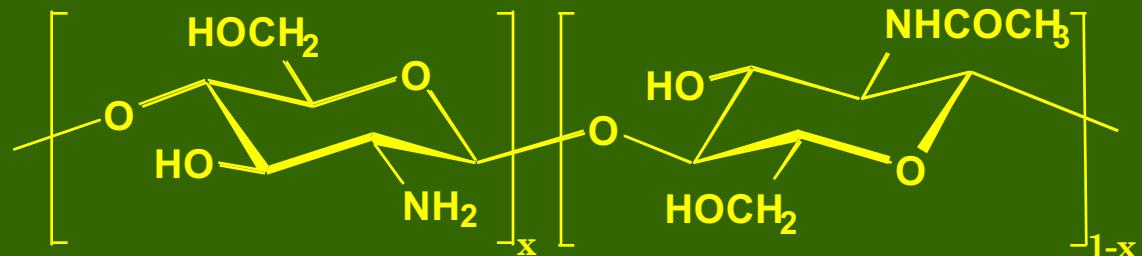
Manageable

Long-term concern

Toxicity/Immunogenicity

*Should be
manageable*

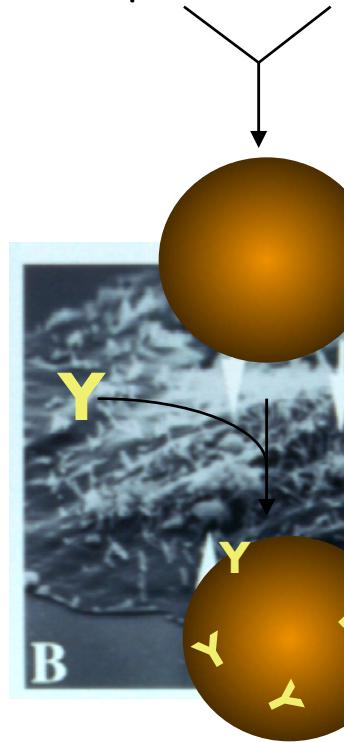
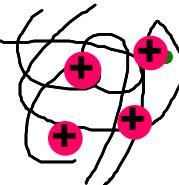
Chitosan



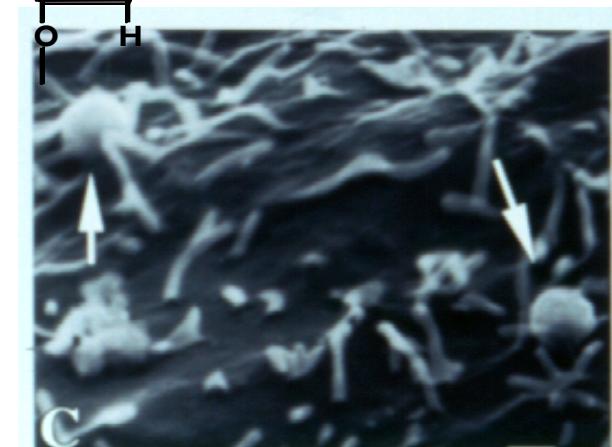
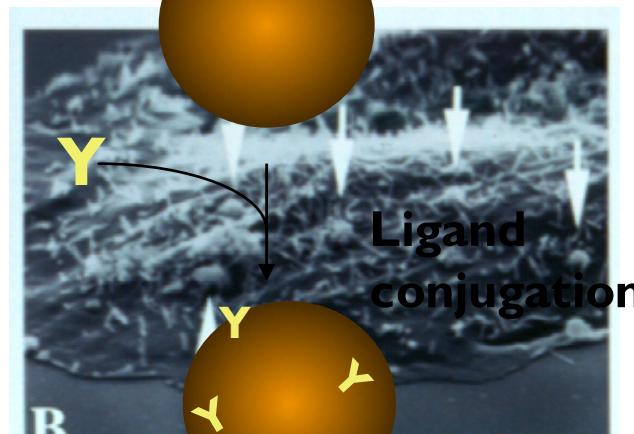
Cationic chitosan

- **Mucoadhesive property**

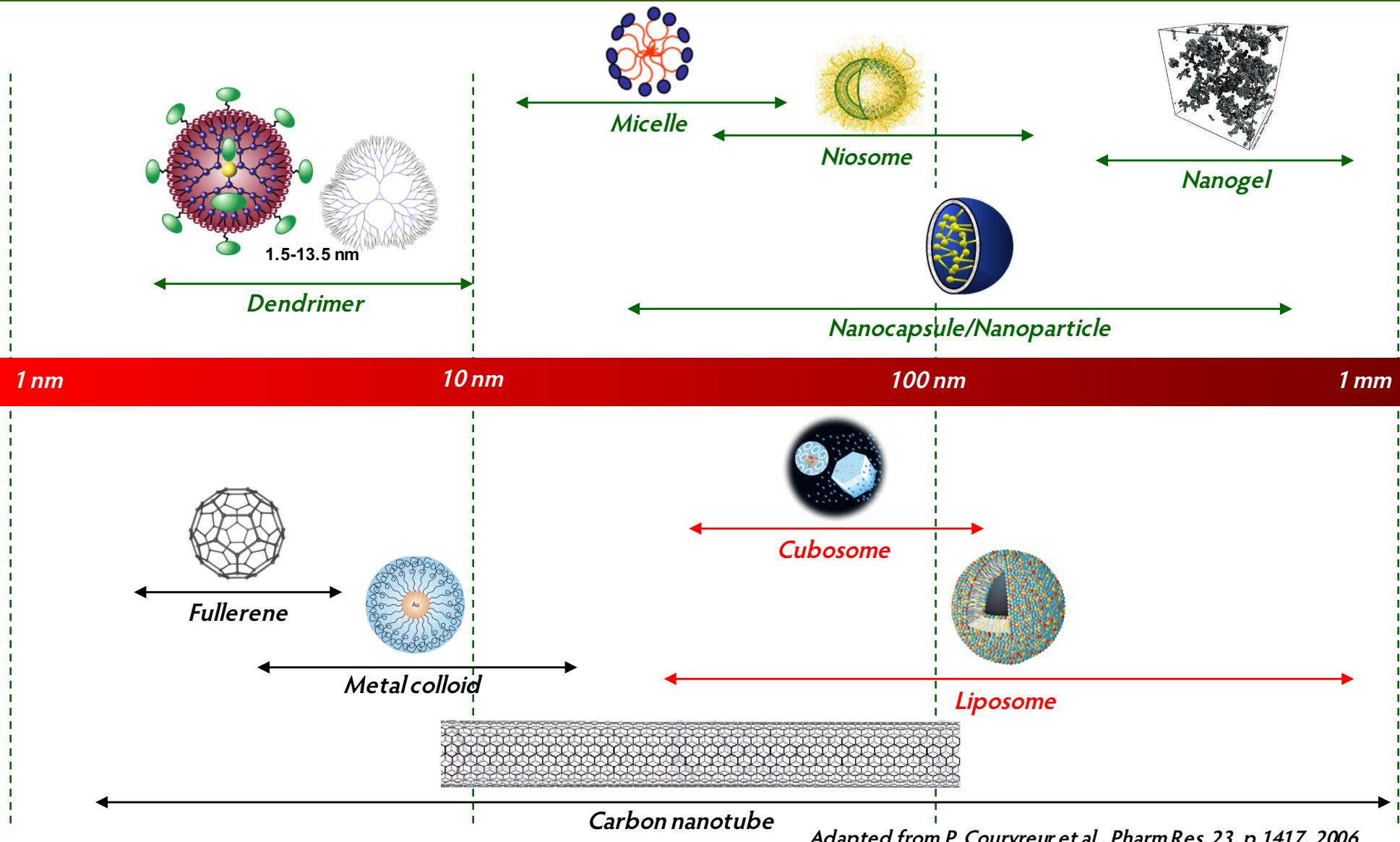
Increases trans and para-cellular transport across intestinal epithelium



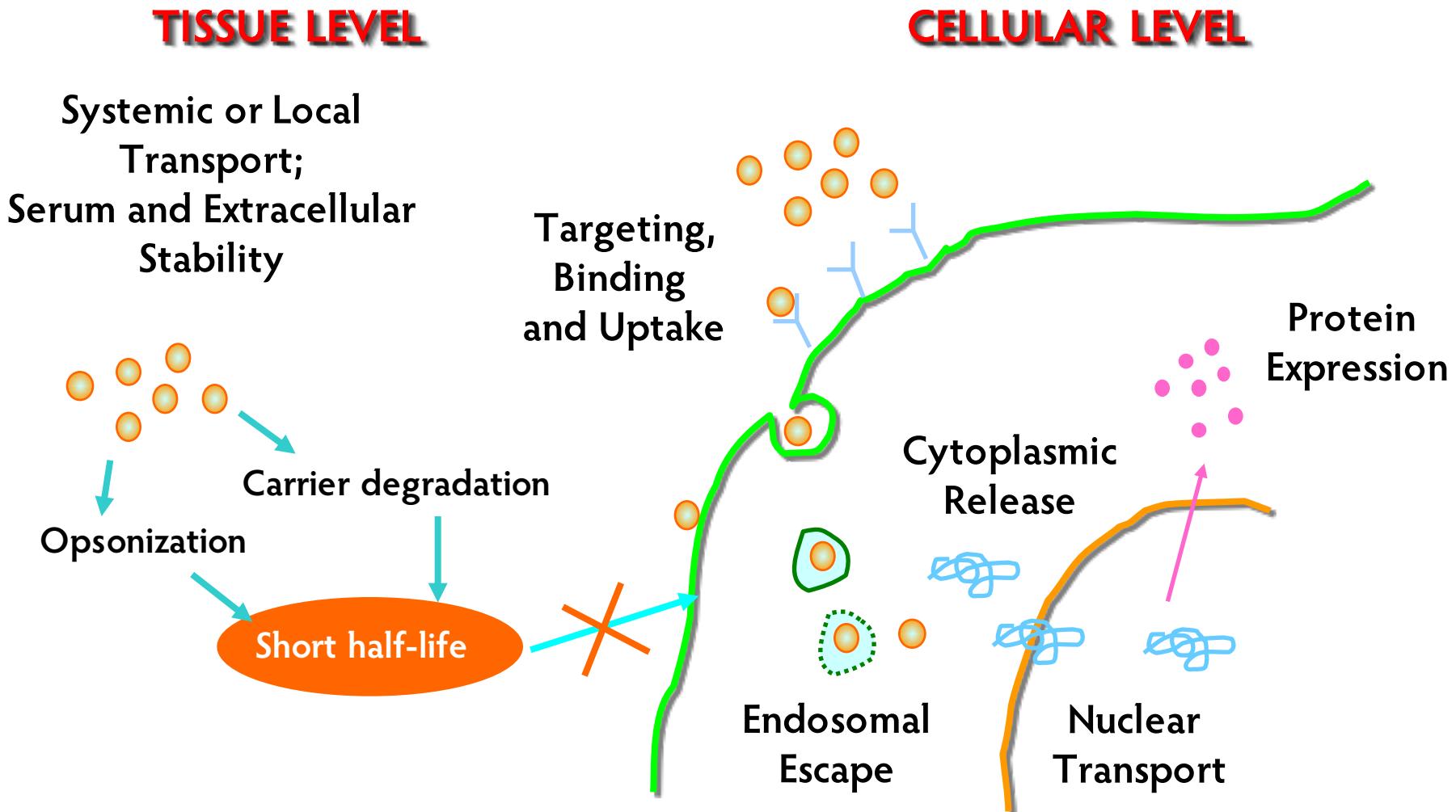
Scale Bar = 210 nm



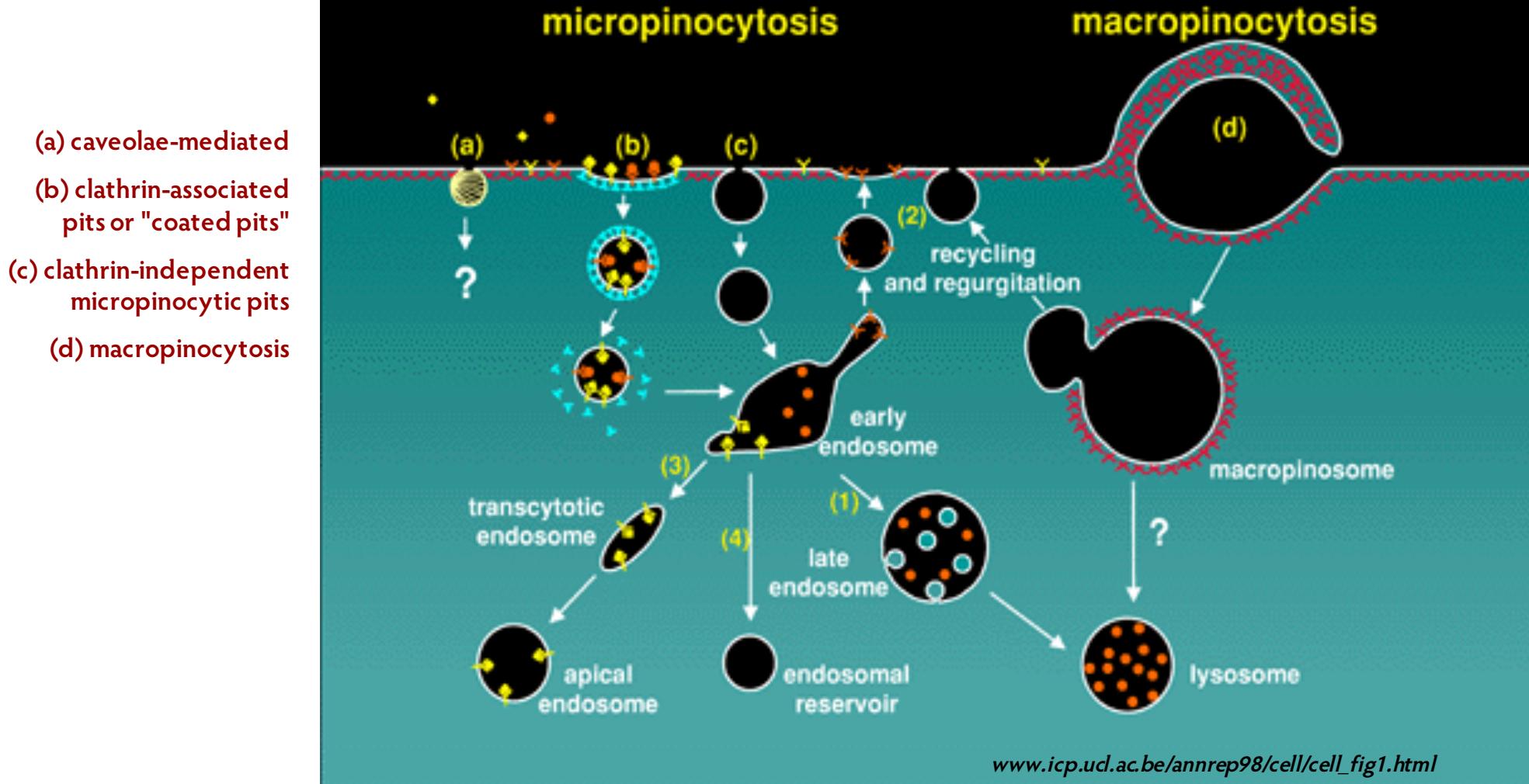
Nanomaterials Applied to Delivery of Drug and Gene in a Targeted and Sustained Manner



Nanoparticle-mediated Delivery of Nucleic Acid into Cell Nucleus

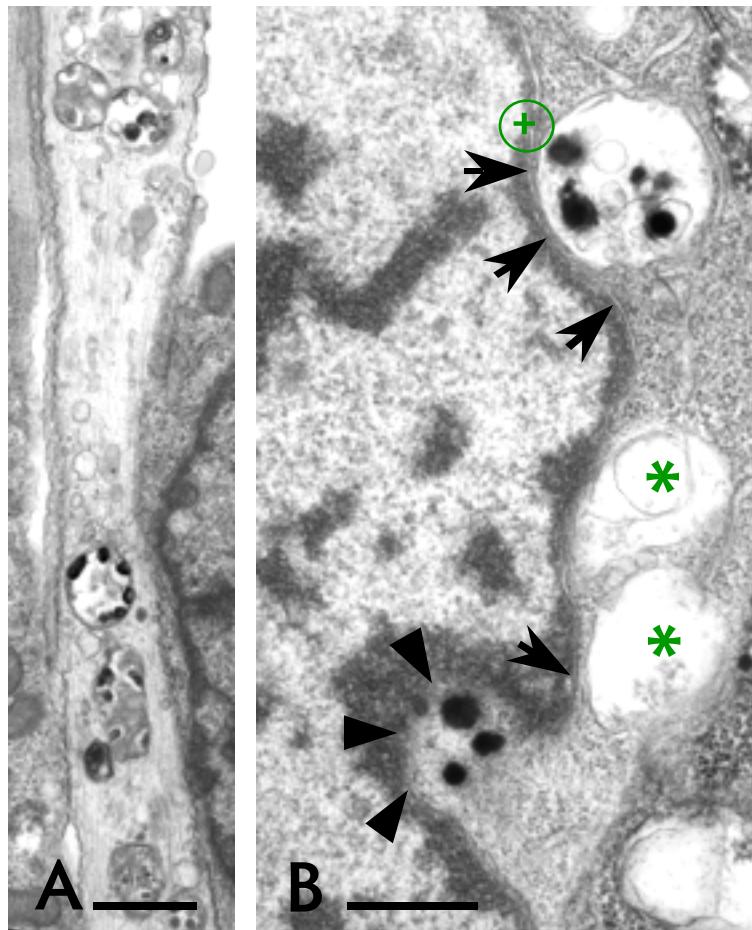


Routes of Intracellular Uptake

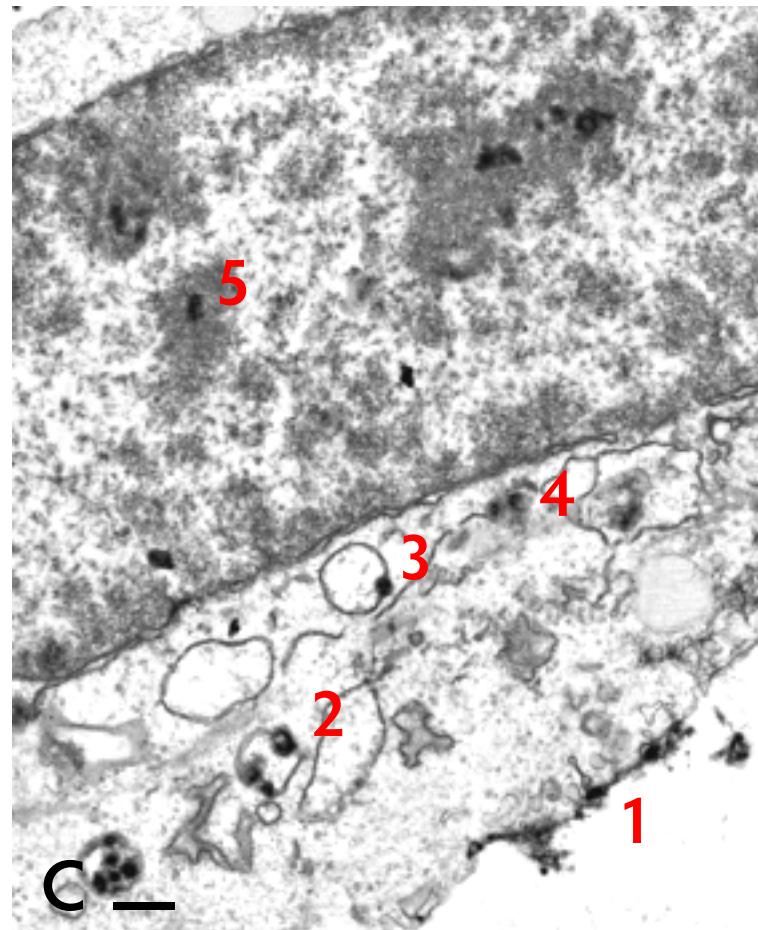


A General Pathway for Complex Internalization and Trafficking to the Nucleus

Axonal transport *Invagination against the nuclear envelop*



PEI in Schwann Cell



* Empty vesicle
+ Nuclear pore complex

Scale bar = 500 nm

- 1) binding and internalization
- 2) localization to endosome
- 3) interactions of complexes with the vesicle membrane
- 4) free complexes in the cytoplasm
- 5) complexes in the nucleus

Non-viral Gene Delivery



Low expression level

Transient gene expression



requires

Repeat Administration



Non-invasive

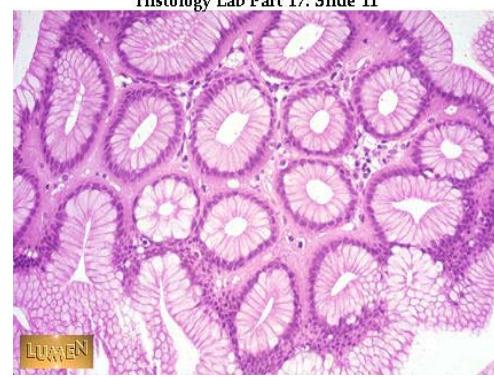
Potential Mechanism of Oral Vaccination for Allergy



1. Oral DNA delivery
(Immunization)



Generation of IgG2a
or Th₁ type
response



Suppression of IgE
(Th₂) synthesis

No anaphylaxis
upon sensitization
and challenge

2. Transfection of Epithelial Cells or immune Cells in the Intestine
3. Antigen production and presentation in Peyer's patch
4. Antibody and CTL generation in lymphoid tissue locally and systemically

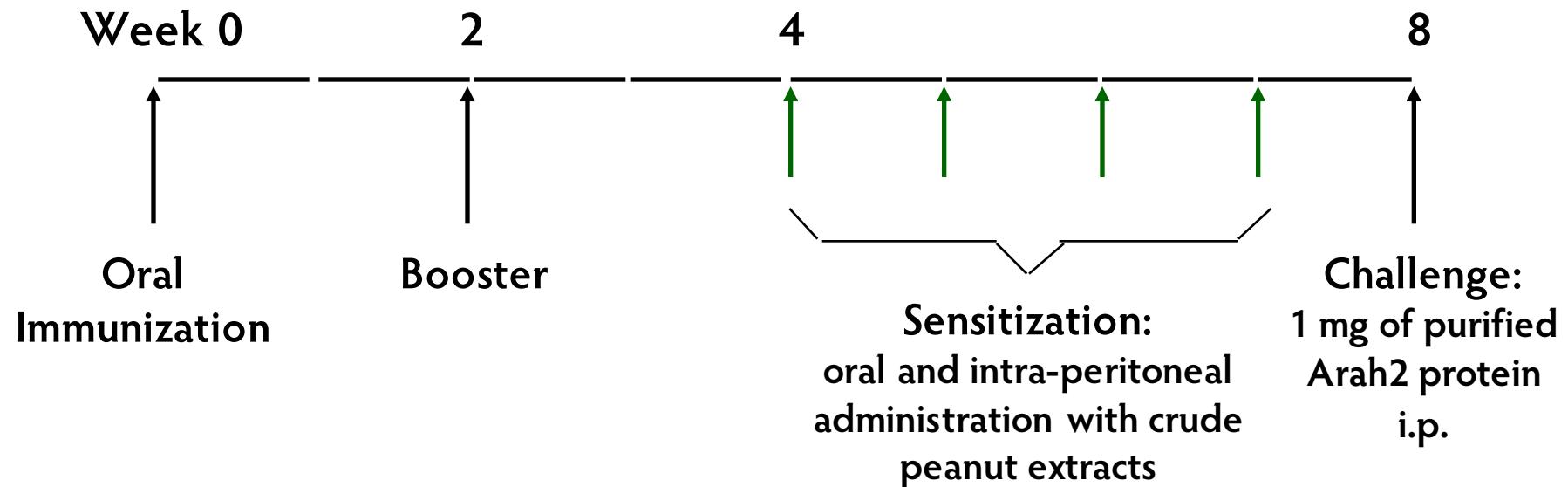
Features of DNA Vaccination

- Introduce only the antigenic protein without the unwanted side effects associated with bacterial and viral vaccines
- Express the antigen in its native form for optimal processing and presentation
 - *gain access to class I MHC pathways of antigen presentation for more effective generation of CTL response than protein antigens*
- Generate both humoral and T-cell mediated protective immunity

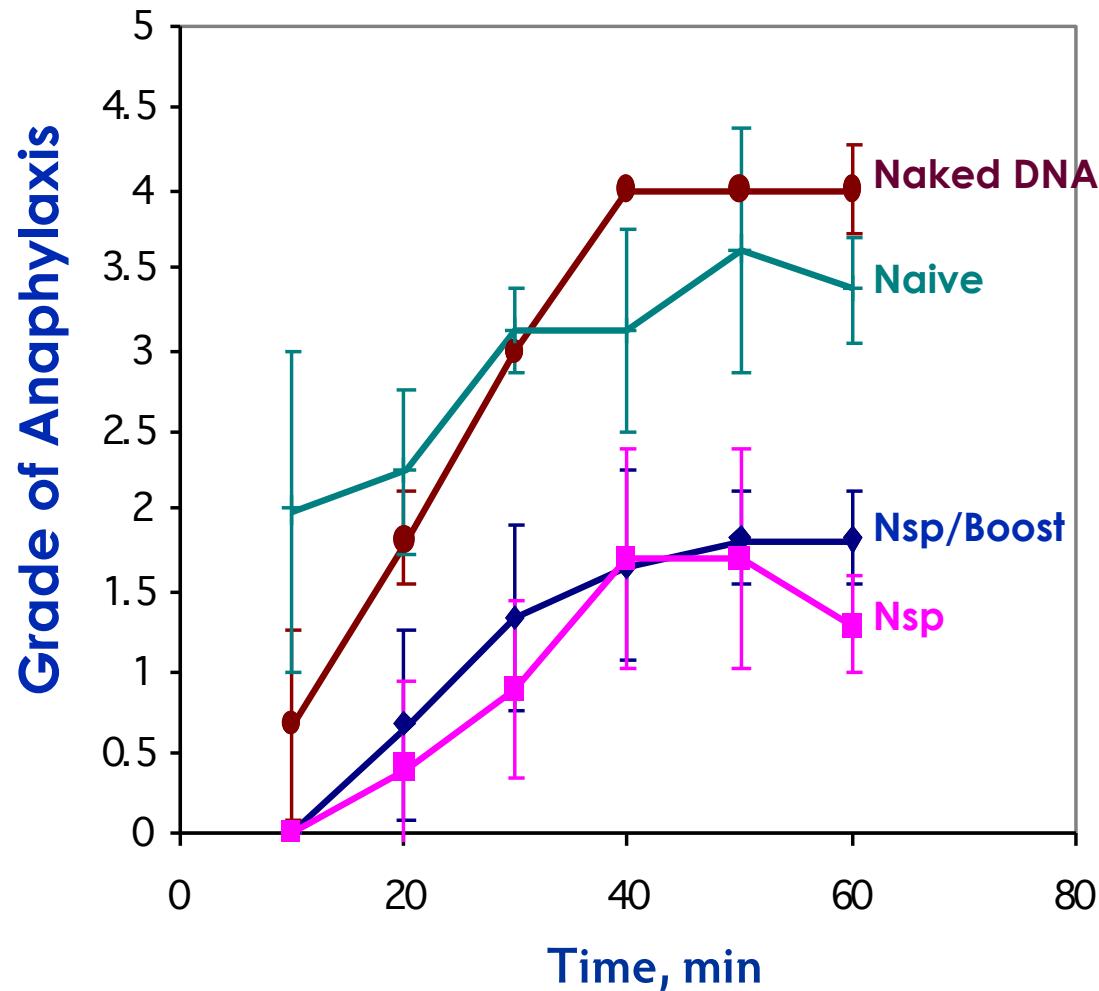
DNA Vaccine Against Peanut Allergy

Deliver orally pCR3Arah2, a gene encoding a major protein fraction in peanut, to which over 95% of peanut allergy patients are sensitive, by chitosan-DNA nanospheres

- Characterize immune response in AKR/J mice vaccinated with Arah2-DNA-chitosan nanospheres with or without booster shots



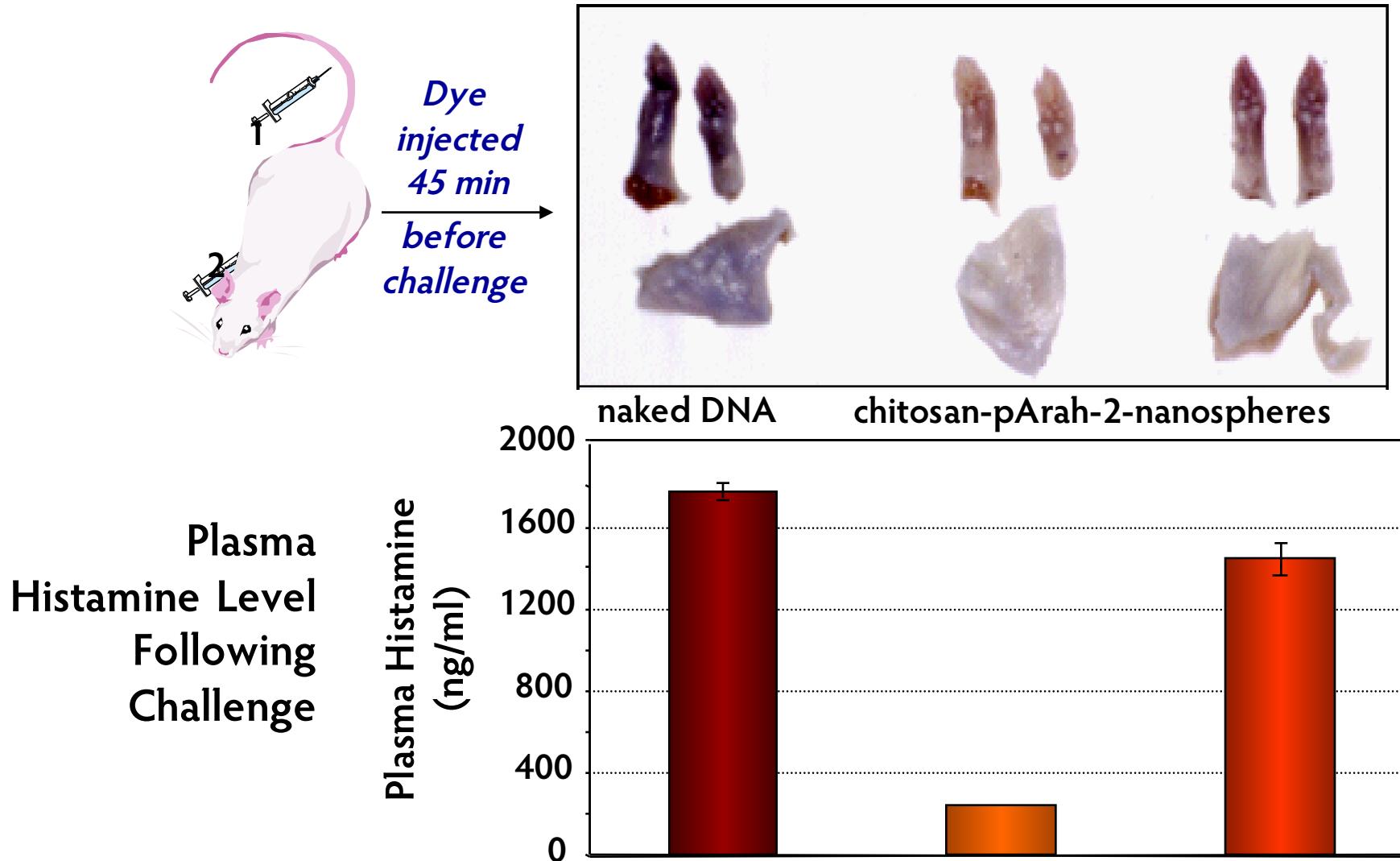
Oral Immunization with Chitosan-DNA Nanoparticle Protects Mice From Peanut Allergen Challenge



- 0: No sign of reaction
- 1: scratching and rubbing around the nose and head
- 2: decreased activity with an increasing respiratory rate, *pilar erecti* and/or puffing around the eyes
- 3: labored respirations, cyanosis around the mouth and tail
- 4: slight or no activity after prodding or tremors
- 5: death

Roy K, Mao HQ, Huang S, Leong KW. Oral gene delivery with chitosan-DNA nanoparticles generates immunologic protection in a murine model of peanut allergy. *Nature Medicine*, 1999. 5(4): p. 387-391.

Immunization with Chitosan-DNA Nanoparticle Reduces Vascular Leakage Following Challenge, and Lower the Plasma Histamine Level



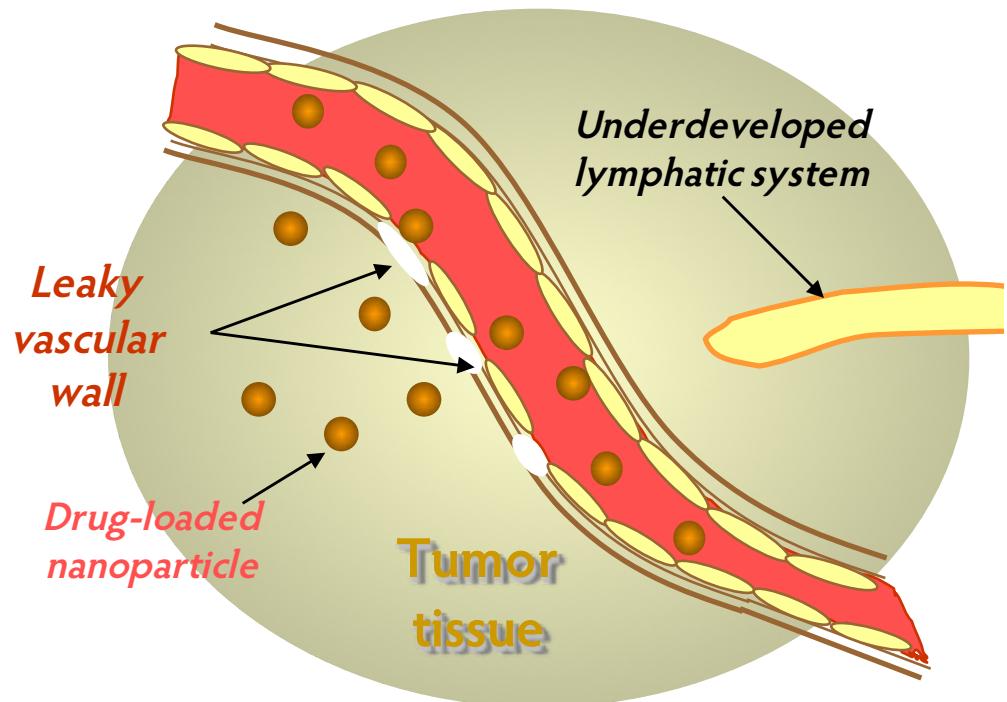
Roy K, Mao HQ, Huang S, Leong KW. Oral gene delivery with chitosan-DNA nanoparticles generates immunologic protection in a murine model of peanut allergy. *Nature Medicine*, 1999. 5(4): p. 387-391.

Nanoparticle Technology to Enhance Efficacy of Drug Therapy

- Abnormal Tumor Vasculature
- Extensive angiogenesis and high vascular density (hypervasculature)
- Lack of smooth-muscle layer; more leakage at tumor vessels under hypertensive state
- Defective vascular architecture; irregular vasculature networks, large openings, fenestration
- Whimsical blood flow; no constant blood flow and direction
- Meager lymphatic clearance that leads to enhanced retention of macromolecular drugs and lipidic particles in the interstitium of tumors
- Slow venous return that leads to accumulation of macromolecular drugs and lipidic particles from the interstitium of tumor

Improved Delivery of Chemotherapeutics to Tumor Tissue

Enhanced Permeation and Retention (**EPR**) Effect



Nanotherapeutics: Unique Opportunities of Applying Nanostructures to Medicine

Drug therapy

Nanoparticles to facilitate site-specific, targeted, image-guided, catheter-based delivery

- *Improve efficacy of cancer therapy*
- *Achieve site-specific delivery of potent but unstable drug*

Gene therapy

Nanoparticles to deliver DNA and siRNA

- *Achieve safe and efficient delivery of DNA therapeutics*

Immunotherapy

Nanoparticles to co-deliver antigen and immunoadjuvant

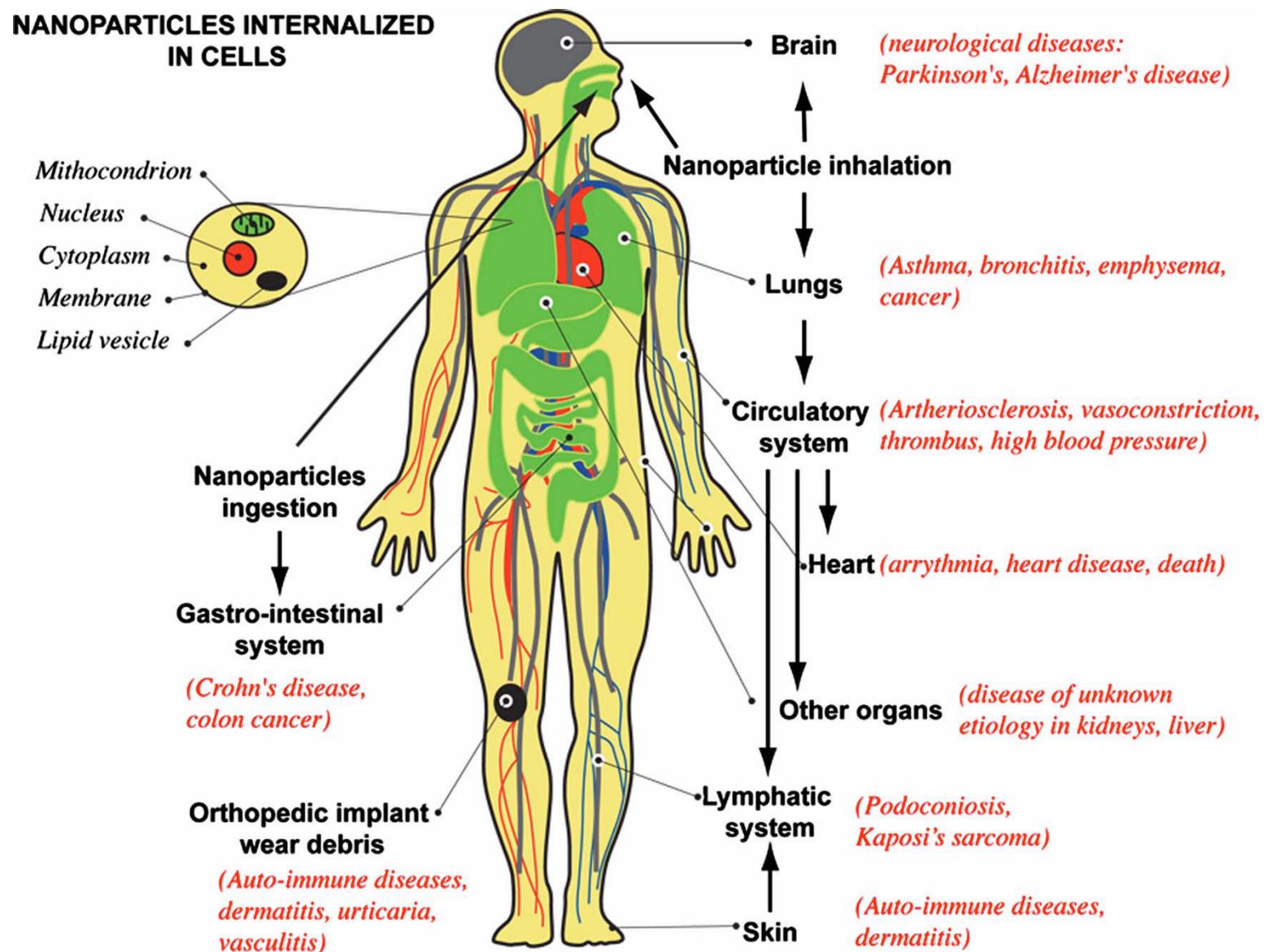
- *Achieve efficacious genetic immunization for infectious disease and cancer*

Cell therapy

Nanofibers and nanopatterns to mimic the extracellular matrix for manipulation of cellular response in vitro and in vivo

- *Create biomimetic scaffold to expand and control differentiation of stem cells*
- *Control tissue response to implantable medical devices in vivo*

Pathways of Exposure to Nanoparticles, Affected Organs, and Associated Diseases



Co-Delivery of Therapeutic Cargoes to Enhance Therapeutic Efficacy

Therapeutics Drug/Drug	Application	Explanation of synergistic effect	Ref
Doxorubicin and Combrestatin	Cancer	Combrestatin is a vascular disrupting agent that targets the tumor blood vessels, causing tumor vasculature shutdown, trapping DOX in the tumor.	4
Doxorubicin and Verapamil	Cancer	Verapamil is a calcium channel antagonist which also acts as a P-gp inhibitor. This increases the cell sensitivity and DOX accumulation within the cell.	5
Heparin with Taurocholate (LHT7) and Suberoylanilide hydroxamic acid (SAHA)	Cancer	LHT7 is an anti-angiogenic drug. SAHA causes cell cycle arrest, angiogenesis. Together they inhibit angiogenesis and cell proliferation.	6
Fluoroorotic acid and Irinotecan	Cancer	Irinotecan inhibits DNA re-ligation by inhibiting topoisomerase 1, and fluoroorotic acid inhibits DNA synthesis.	7
Cyclophosphamide and Doxorubicin	Cancer	Cyclophosphamide increases permeability of tumor micro vessels, leading to increased DOX accumulation in the tumor.	8
Vincristine and Topotecan	Cancer	Topotecan acts in the S-phase or G2-M phase by converting DNA topoisomerase 1 to a cellular toxin, and vincristine leads to mitotic arrest by depolarizing the microtubules.	9
Doxorubicin and Paclitaxel	Cancer	DOX bound to DNA prevents formation of tubulin, and paclitaxel degrades existing microtubules.	10

Co-Delivery of Therapeutic Cargoes to Enhance Therapeutic Efficacy

Drug/Plasmid		
Doxorubicin and pORF-hTRAIL gene	Cancer	TRAIL causes apoptosis by transmitting apoptotic signals through an extrinsic pathway. DOX causes DNA damage through intrinsic pathway. 14
Doxorubicin and Survivin mutant gene	Cancer	Survivin leads to increased resistance against DOX. The plasmid is a strong negative mutant of survivin which aims to reduce the resistance. 15
Paclitaxel and pEGFP-hTRAIL gene	Cancer	TRAIL targets cancer cells over normal cells but glioma gains resistance very quickly. PTX makes the cells more sensitive to TRAIL-induced apoptosis due to crosstalk between intrinsic and extrinsic pathways. 16
Doxorubicin and p53 antitumor gene	Cancer	DOX is a chemotherapeutic and p53 enhances sensitivity of cells to the chemotherapeutics. 17