Lecture 15: 'Stealth' particles

Last time: Nano- and micro-particle carriers

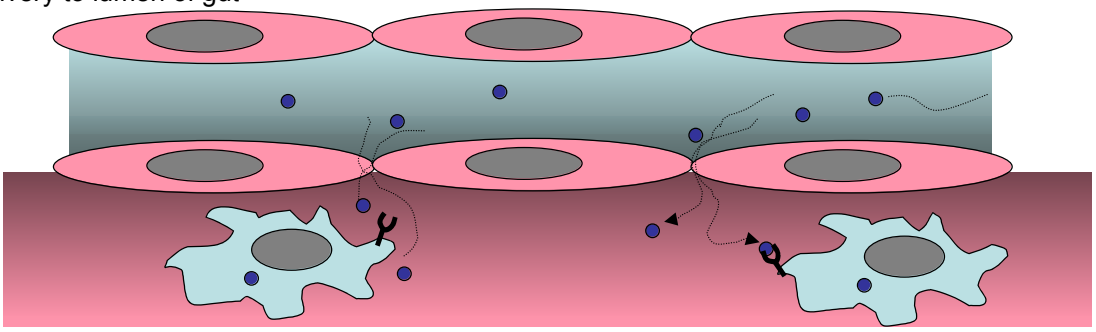
Today: Delivery of drugs to tissue from circulation
'stealth' particles theory and function

Reading: S. Stolnik et al. 'Long circulating microparticulate drug carriers,' *Adv. Drug. Deliv. Rev*. **16**, 195 (1995)

Delivery of drugs to tissues via systemic circulation

Avenues of systemic molecule delivery

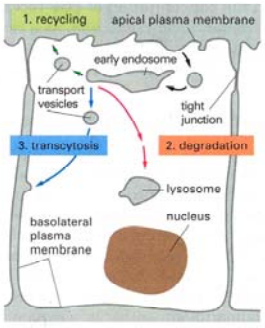
1. Intravenous injection
2. oral delivery to lumen of gut



Transcytosis:

vary according to the specific type of receptor. (1) Most receptors return same plasma membrane domain from which they came; (2) some receptogress to lysosomes, where they are degraded; and (3) some receptors d to a different domain of the plasma membrane, thereby mediating a s called *transcytosis* (Figure 13–32).

Figure 13–32 Possible fates for transmembrane receptor proteins that have been endocytosed. Three pathways from the endosomal compartment in an epithelial cell are shown. Receptors that are not specifically retrieved from endosomes follow the pathway from the endosomal compartment to lysosomes, where they are degraded. Retrieved receptors are returned either to the same plasma membrane domain from which they came (*recycling*) or to a different domain of the plasma membrane (*transcytosis*). If the ligand that is endocytosed with its receptor stays bound to the receptor in the acidic environment of the endosome, it will follow the same pathway as the receptor; otherwise it will be delivered to lysosomes.



objectives for systemic delivery to tissues/organs

- avoid premature elimination by kidneys
  - kidneys filter out molecules smaller than **X nm**
- Avoiding reticuloendothelial system (RES)
  - Particles larger than **200 nm** screened by monocytes and macrophages in liver and spleen (*Biochem. Biophys. Res. Commun*. **177**, 861 (1991))
    - Kupffer cells – macrophages in liver
  - Particulate removal aided by process of ***opsonization***
    - Process of 'tagging' foreign particles for efficient removal by macrophages

        - Particles tagged by different opsonins removed by cells in different organs e.g. liver vs. spleen
    - Components:
        - Complement proteins, particularly C3 and C5
        - Immunoglobulins
        - Other proteins known to facilitate particle uptake:
            - Fibronectin
            - C-reactive protein
            - Tuftsin
    - Protein adsorption to particles is key
        - Hydrophobic particles quickly removed from circulation *in vivo* (*Intl. J. Pharm*. **29**, 53 (1986))
- Penetration through capillary walls into tissues
    - Passive delivery due to 'leakiness' of vessels at sites of inflammation or tumor vasulature abnormalities[1]
- Avoid induction of antibodies against molecules/particles

- FUTURE OBJECTIVE:
    - Pre-targeting drugs that trigger permeability of vasculature at desired sites to allow tissue access – a needed advance to make tissue-specific targeting truly work[1]

Stealth particles[2,3]

- How to avoid uptake by scavenger cells?
    - Van Oss showed in 1978 (*Ann. Rev. Microbiol*. **32**, 19 (1978)) that many bacteria have a highly hydrophilic hydrated surface layer of protein, polysaccharide, and glycoprotein that reduces interactions with blood components and inhibits phagocytosis
    - Davis at about the same time (*J. Biol. Chem.* **252**, 3578 (1977)) showed that PEGylated proteins are non-immunogenic and have greatly increased half-lives in vivo

Concept[4,5]

- Similar to design of protein adsorption-resistant surfaces, provide entropic barrier
    - Eliminate protein binding to particle/molecule
    - Enhanced solubility (proteins)/stability (particles) in water
    - Functionalization of particles with PEG increases *in vivo* circulation time
        - Reduced protein adsorption to particle/molecule surface
        - Receptors of macrophages unable to bind particle/molecule
- Same polymers employed as in the design of protein-resistant surfaces
    - Most studied: poly(ethylene glycol)
    - Others: dextran
- PEGylation applied to all forms of molecular, nano-, and micro-particulate carriers

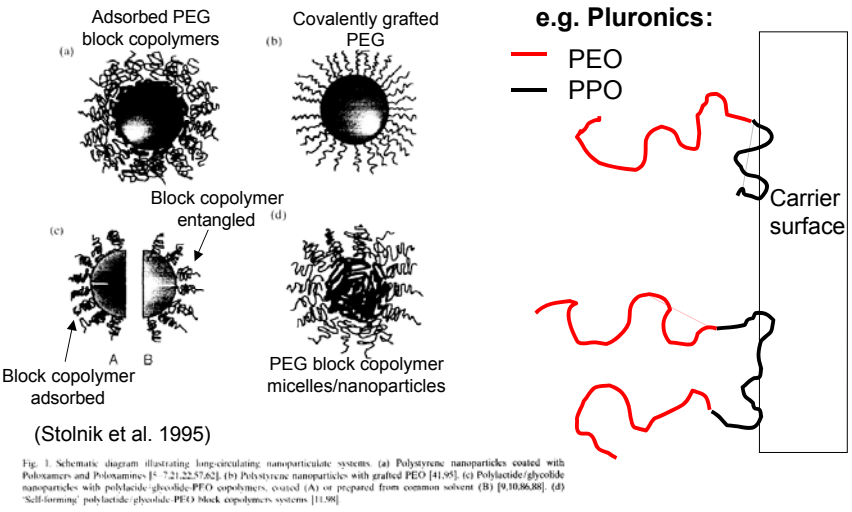
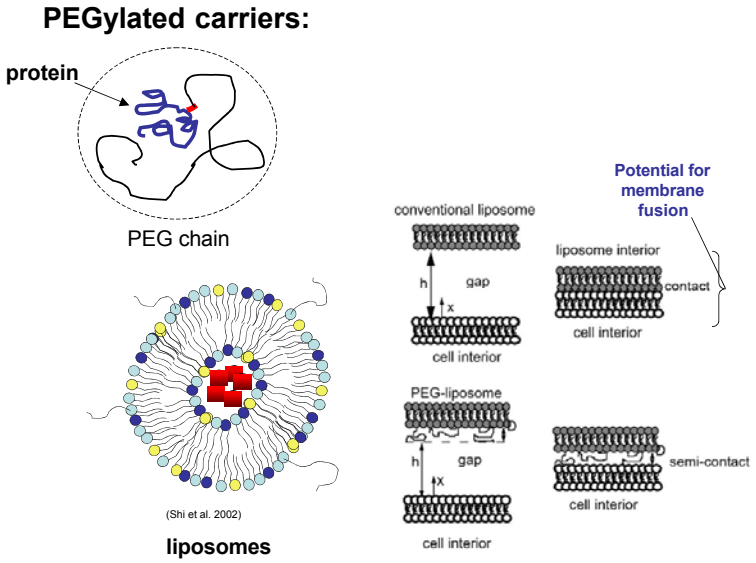


Fig. 1. Schematic diagram illustrating long-circulating nanoparticulate systems. (a) Polystyrene nanoparticles coated with Poloxamers and Poloxamines [e.g. 7,21,22,57,62]. (b) Polystyrene nanoparticles with grafted PEO [41,95]. (c) Polylactide/glycolide nanoparticles with polylactide/glycolide-PEO copolymers, coated (A) or prepared from common solvent (B) [9,10,86,88]. (d) 'Self-forming' polylactide/glycolide-PEO block copolymers systems [11,98].



- PEG binds 2-3 water molecules per repeat unit[5]
  - Causes PEGylated compounds to function as though they are 5-10 times larger than their true molar mass
    - Observed in SEC and gel electrophoresis experiments[4]
- PEG/water 'shield' can reduce activity of protein, but generally the increased circulation time makes up for this

Theory of stealth particle repulsion of protein binding to carriers[6,7]

- Theory of Halperin[7], building on previous analyses of Alexander/de Gennes and Szleifer

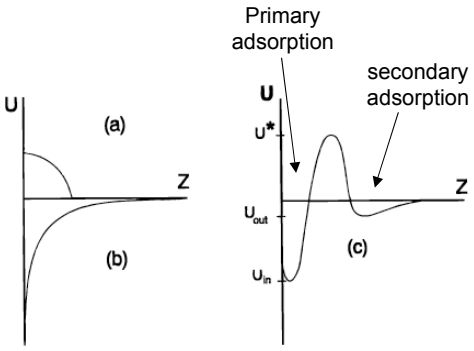


Figure 1. The effective potential U_{eff} experienced by a protein approaching a brush-coated surface (c), which is the result of the superposition of two contributions: (b) the purely attractive interaction potential between the bare surface and the protein U_{bare} and (a) the purely repulsive interaction between the protein and the swollen brush U_{brush}.

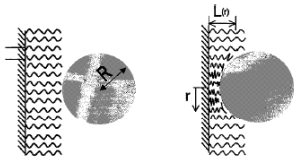


Figure 2. Large proteins can approach the surface only by compressing the brush. The free energy penalty associated with the compression mechanism favors secondary adsorption at the outer edge of the brush.

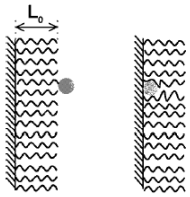


Figure 3. Small proteins, $R < L_0$, can penetrate the brush with little effect on its overall concentration profile. This insertion mechanism favors primary adsorption at the surface.

(Halperin 1999)

- Experimental measurements and comparison with theory
  - Measurements made using surface plasmon resonance[6]
  - Experimental details:
    - σ = on graphs! [area/chain]
    - PEG MW = 2000 g/mole (N = 45)
    - Layer thicknesses L_0 = 40-60 Å
    - Sizes of tested proteins:
      - BPTI (bovine pancreatic trypsin inhibitor) : MW = 6000- g/mole; R ~ 21x21x30 Å
      - HSA (human serum albumin): MW = 66,200 g/mole; R = 38x38x150 Å
      - FBN (Fibrinogen): MW = 340,000 g/mole; R ~ 55x55x460 Å rod-link protein

Comparison of theory with experiment

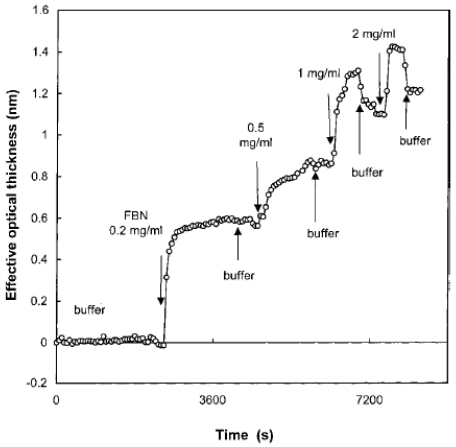


FIGURE 7: Characteristic time course for fibrinogen adsorption onto a supported DSPE monolayer measured by SPR.

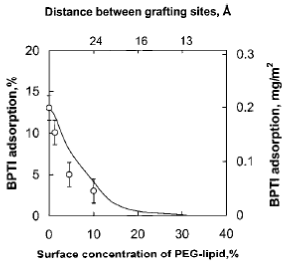


FIGURE 9: Adsorption of BPTI as a function of the SAPDS-PEG surface concentration. The solid line corresponds to the theoretically predicted dependence of the adsorption on the mole fraction of polymer−lipid in the monolayer.

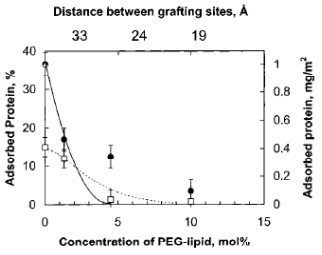


FIGURE 10: Adsorption of HSA (□) and FBN (●) as a function of the PEG-lipid surface concentration. The solid and dashed lines correspond to the theoretically predicted dependence of the protein adsorption on the surface polymer−lipid concentration.

(Efremova et al. 2000)

Function of stealth particles

- ***PEGylated molecules***
  - PEGylated IFN-α2a[4]
    - Treatment of Hepatitis C- has antiviral activity (induces macrophages to kill virus)
  - PEGylated interleukin-6
    - 100-fold increase in blood half life with pegylation
    - thrombopoietic potency increased 500-fold

Table 1 | **Influence of pegylation on pharmacokinetics and pharmacodynamics***

Pharmacokinetic effect	Pharmacodynamic effect
Interferon-α2a	
Sustained absorption	*In vivo* antiviral activity increased 12- to 135-times
Increased half-life (from 3–8 h to 65 h)	Antitumour activity increased 18-fold
Decreased volume of distribution (from 31–73 l to 8–12 l)	Improved sustained response to chronic hepatitis C
Decreased systemic clearance (from 6.6–29.2 to 0.06–0.10 l/h)	
Interleukin-6	
Increased half-life (from 2.1–206 min)	Thrombopoietic potency increased 500-times
Tumour necrosis factor	
Increased half-life (from 3 to 45–136 min)	Antitumour potency increased 4-to 100-times

*Influence of pegylation on pharmacokinetics and pharmacodynamics of some therapeutic proteins, compared with corresponding native proteins (adapted from REF. 18).

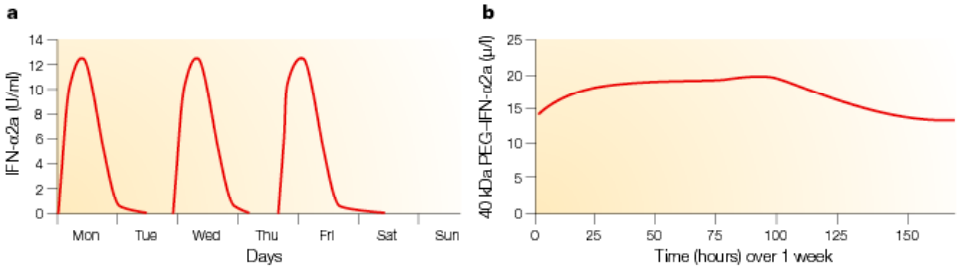


Figure 4 | **Pharmacokinetic profiles for interferon (IFN)-α2a and 40 kDa polyethylene glycol (PEG)–IFN-α2a.** These graphs represent blood levels in humans resulting from subcutaneous injection of **a** | IFN-α2a and **b** | branched PEG 40 (kDa) IFN-α2a. IFN-α2a is injected every other day and its short lifetime in circulation leads to pulsed blood concentrations levels which cycle below efficacious levels. The branched PEG 40 (kDa) IFN-α2a has a long circulating lifetime due to the presence of the PEG, and the once-weekly injection leads to near constant blood concentrations above the therapeutic level over the one-week period.

(Harris, 2003)

- ***PEGylated microspheres:***
  - Li et al. showed significant increases in circulation time by preparing controlled release microparticles using PEG-PLGA block copolymers[2]
    - Formation of microspheres by double emulsion process
      - Block copolymer self-emulsifies and PEG coats both internal and external aqueous interfaces
        - Benefit of improved protein stability within microspheres?

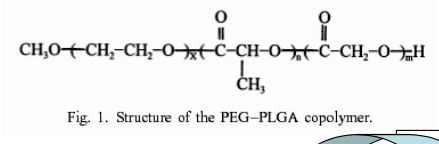
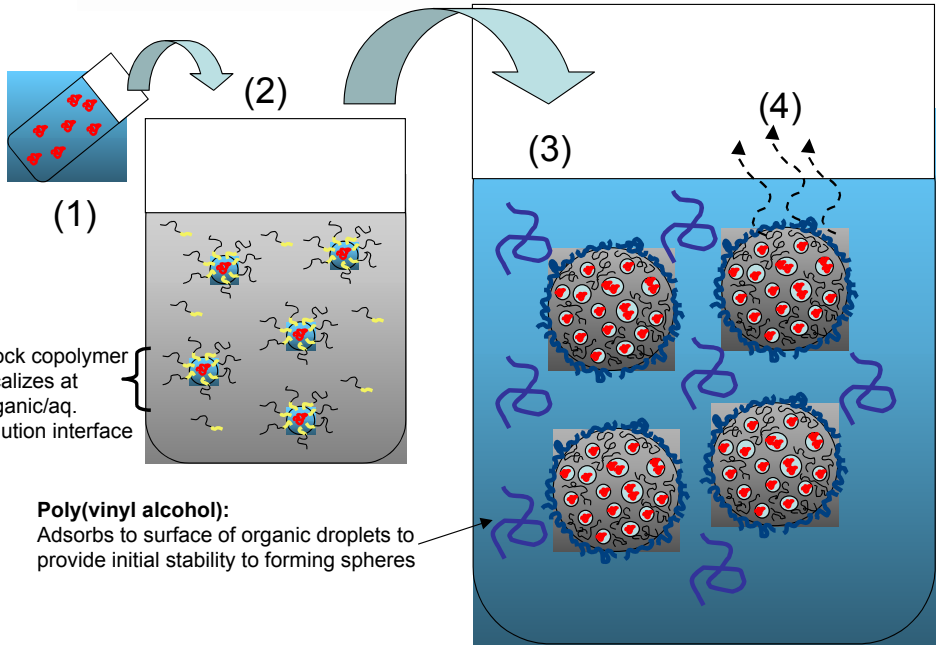
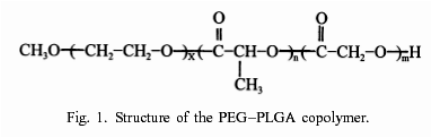


Fig. 1. Structure of the PEG–PLGA copolymer.

PEG = 5KDa, PLGA = 40 KDa



Block copolymer localizes at aqueous/polymer interfaces



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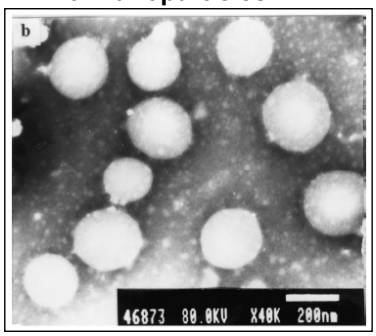
- Results:
  - Unmodified PLGA particles $t_{1/2}$ = 13.6 min.
  - PEG block copolymer particles $t_{1/2}$ = 270 min (4.5 hr)
  - Altered biodistribution: high blood availability and reduced spleen/liver uptake
    - No PLGA particles remaining in blood after 12 hrs

TEM of nanoparticles



Release properties of diblock particles

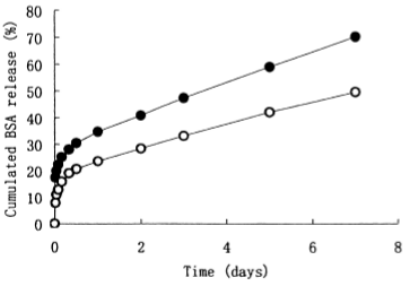


Fig. 6. Release profiles of BSA from PLGA (○) and PEG–PLGA (●) nanoparticles.

Increased $t_{1/2}$ in blood:

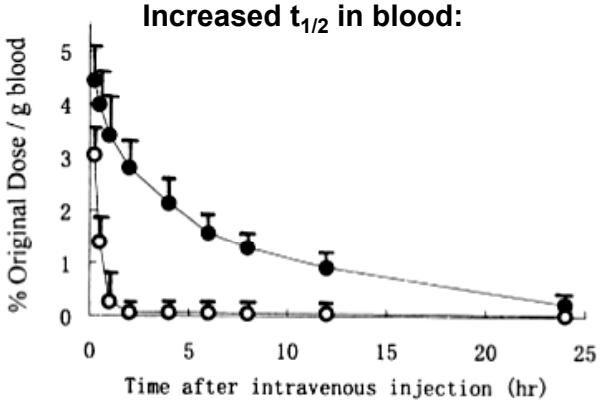
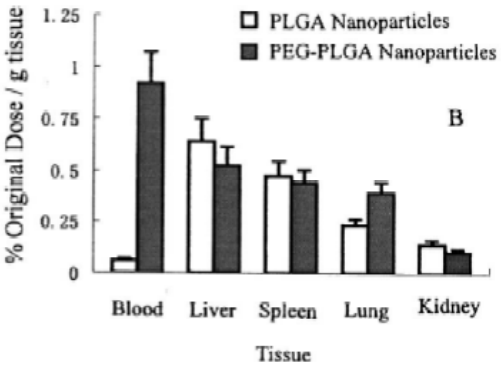


Fig. 7. Blood clearance curves of [^{125}I]BSA in PLGA (○) and PEG–PLGA (●) nanoparticles.

Altered biodistribution:



(Li et al., 2001)

uncloaking PEGylated carriers

- Use of cleavable PEG-carrier linkages to 'unmask' carrier at selected site/time
- Allow full drug activity at site of action
- Example:

'Stealth' Carriers in Clinical Use[4,5]

- Pegademase (Adagen)
  - Pegylated adenosine deaminase (enzyme)
  - Treatment of severe combined immunodeficiency (SCID)- hereditary lack of adenosine deaminase
- Pegaspargase (Oncaspar)
  - Pegylated asparaginase (enzyme)
  - Treatment of leukemia
    - Leukaemic cells cannot synthesize asparagines; asparaginase kills cells by depleting extracellular sources of this amino acid
- Pegylated IFN-α2a (Pegasys)
  - Treamtent of hepatitis C
- Doxil (Alza)
  - Pegylated liposomes carrying anti-cancer drug doxorubicin
  - Improves treatment from daily 30min injections for 5 days every 3 weeks to once-a-month single injections
  - Approved for treatment of Karposi's sarcoma

References

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