Lecture 4: Degradable Materials with Biological Recognition (part II)

Last time:	Biological recognition *in vivo* Engineering biological recognition of biomaterials: adhesion/migration peptides
Today:	Engineering biological recognition of biomaterials: enzymatic recognition and cytokine signaling
Reading:	S.E. Sakiyama-Elbert and J.A. Hubbell, 'Functional Biomaterials: Design of Novel biomaterials,' *Annu. Rev. Mater. Sci*. **31**, 183-201 (2001) J.C. Schense et al., 'Enzymatic incorporation of bioactive peptides into fibrin matrices enhances neurite extension,' *Nat. Biotech*. **18**, 415-419 (2000)
Supplementary Reading:	'The Extracellular Matrix,' pp. 1124-1150, *Molecular Biology of the Cell*, Lodish et al.

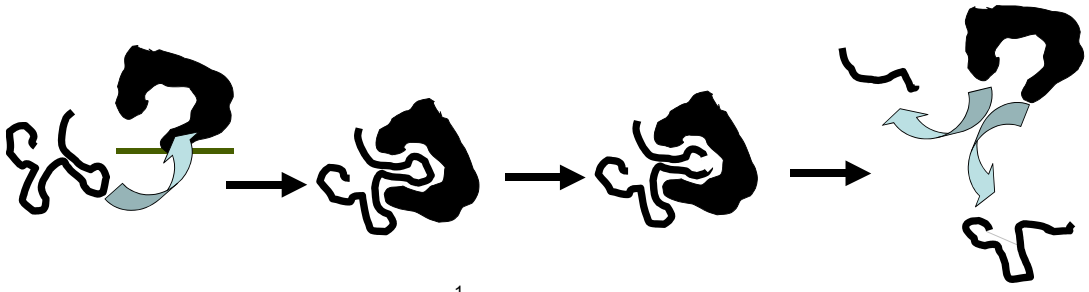
Recognition of Biomaterials by Proteases: Engineering Enzyme-mediated degradation of polymers

Basic concept: include peptide sequences in the polymer chain which are cleaved by enzymatic activity of serum proteins/cellular secreted products (active breakdown) [hydrolysis active but slow...]

- Amide bond -(NH)-(CO)- provides natural hydrolytic mechanism for degradation, but breaks down very slowly in physiological conditions

Remodeling enzymes *in vivo*:

1) binding of target by enzyme
2) specific site cleavage

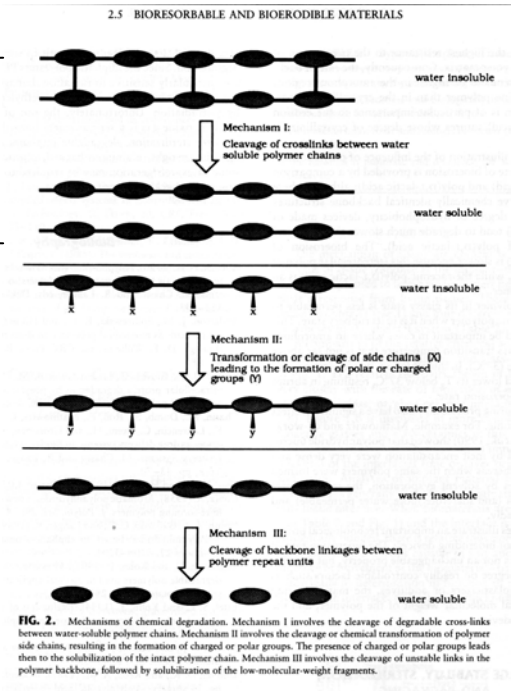


- What is enzymatic cleavage used for *in vivo*?[1] (Reading Ch.1 4 Voet and Voet "Enzymatic catalysis")
  - Remodling ECM, migration of cells through matrix
  - Removing functional groups from signaling molecules (phosphorylation/dephosphorylation)
- Utility in the design of biomaterials:
  - Enzymatic cleavage can breakdown polymers more quickly than hydrolysis
  - Polymers can be designed to be surface-eroding easily (enzyme can't access interior)
  - Degradation could be localized to tissue where enzyme is produced

Reminder of breakdown mechanisms:

Used for biodegradable hydrogels

Main mechanism exploited in solid polymers



(Ratner, *Biomaterials Science*)

(SLIDE)

Cleavage of synthetic polymers by enzymes

Cell source	Enzyme	Native function	Acts on	Degradation Mechanism	Result
Various bacteria	lipases	protease	Polyesters, polyesteramides	III	Monomers or dimers
Tritirachium album (mold)	Proteinase K	Protease	Poly(lactide)	III	Monomers or dimers
Mammalian cells	esterases	protease	Poly(alkyl cyanoacrylates)	II	Water-soluble polymers
Mammalian cells	Papain, pepsin	proteases	polyesteramides[2]	III	Untested
Mammalian cells	α-chymotrypsin	Serine protease	Aromatic peptides in polyesteramides[3] (e.g. Ala, Val, Leu)	III	Untested
Mammalian cells	elastase	protease	Polyesteramides	III	untested

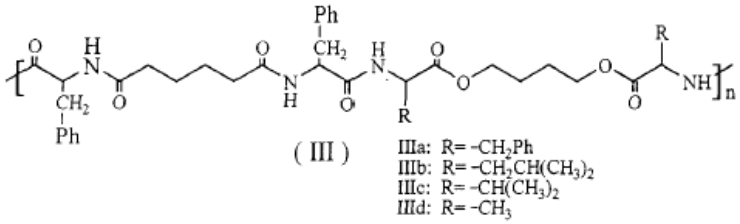
- Pepsin: protease from papaya
- Papain: main protease in gastric juice of stomach
- Chymotrypsin: digestive enzyme

- Mold and bacterial proteases not relevant *in vivo*, but make these polymers also of significant interest for environmentally-friendly packaging

- Of interest in the use of biodegradable materials in environmentally-friendly packaging, but not a concern for in vivo applications

- Data comparing *in vitro* and *in vivo* degradation rates indicates that enzymatic cleavage of most synthetic polymers is negligible
- Polyesteramides have been synthesized with enzymatic recognition:

Enzymatic attack on polyesteramides
(SLIDE)



- Breakdown by mechanism III
- Enzymatic breakdown of polymers is fast relative to simple hydrolysis:

Polyesteramide breakdown by papain:

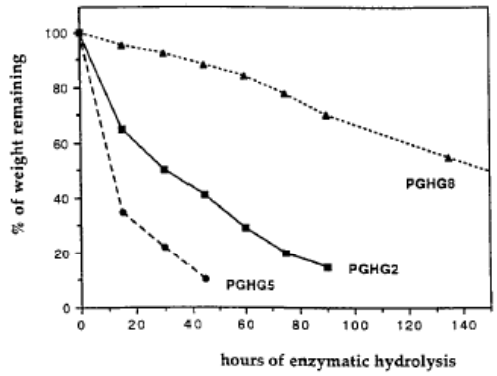
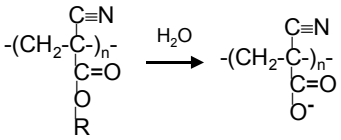


Figure 10. Weight changes of three representative polymers during *in vitro* enzymatic degradation with papain in a 0.05*M* phosphate buffer. PGHG5 corresponds to the polymer of the studied poly(ester amide) series that has a faster degradation rate.

- What does papain do in vivo?

Esterase action on poly(alkyl cyanoacrylates[4]):



Poly(akyl cyanoacrylates): formation of poly(2-cyanoacrylic acid)

- Breakdown by mechanism II

Mechanism:

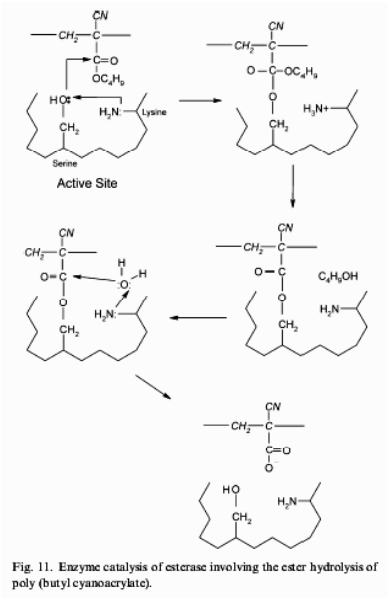


Fig. 11. Enzyme catalysis of esterase involving the ester hydrolysis of poly (butyl cyanoacrylate).

data on degradation of 250nm-diam. porous particles: ***(SLIDE)***

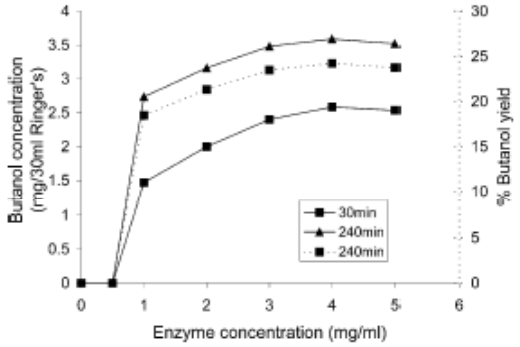


Fig. 2. Butanol production with variation in enzyme concentration (pH 7, 37 °C).

- What does esterase do in vivo?

Enzymatic activity in vivo on peptide sequences:[5,6]

Cleavage Enzyme	**Functions *in vivo***	**Target amino acid sequences**
Plasminogen activator (urokinase or tissue-type plasminogen activator) / plasminogen → plasmin	Degradation of fibrin matrices, angiogenesis, tumor progression; urokinase can bind to cell surface receptor	on fibrinogen: Arg_{104}-Asp_{105}, Arg_{110}-Val_{111}, Lys_{206}-Met207, Arg_{42}-Ala_{43}, Lys_{130}-Glu_{131}, Lys_{84}-Ser_{85}, Lys_{87}-Met_{88}
Matrix metalloproteinases (soluble and cell-surface): e.g. Fibroblast Collagenase (MMP I)	Facilitate cell migration	Type I collagen: Gly_{775}-Ile_{776} In smaller peptides: Gly-Leu or Gly Ile bonds
Elastase	Elastin remodeling	Poly(Ala) sequences

REFS for MMP: (REF J. Biol. Chem. 256, 9511 (1981); J. Biol. Chem. 264, 393 (1989); J. Biol.l Chem. 266, 6747 (1991))

Note that proteases often have complementary protease inhibitors:
e.g. plasminogen activators inhibited by plasminogen activator inhibitor type-1 (a serine protease inhibitor)

Examples of peptide sequences used to allow enzymatic cleavage in biomaterials

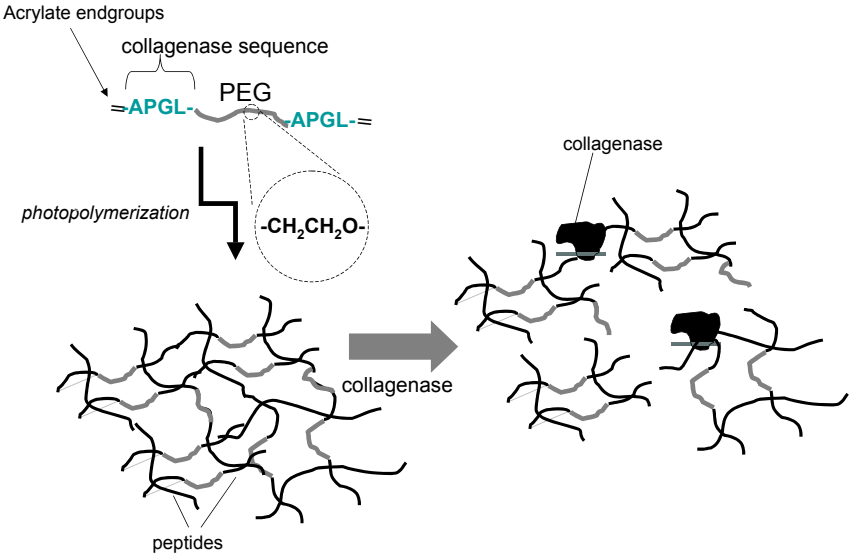
(Work led by J. West and J.A. Hubbell[7-9])

recognition by collagenase: -Ala-Pro-Gly-*/*-Leu-

recognition by plasmin: -Val-Ala-*/*-Asn-

recognition by elastase: -Ala-Ala-Ala-Ala-Ala- (polyalanine sequence)

Example: poly(ethylene glycol) networks: acrylate-**APGL**-PEG-**LGPA**-acrylate ***(ON BOARD)***



network structure formed by stitching together short strings of acrylate endgroups

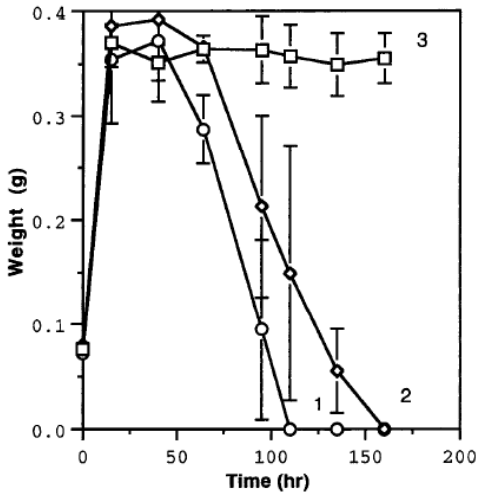


Figure 1. Enzymatic degradation of a hydrogel material formed by photopolymerization of Acr-(APGL)-PEG-(APGL)-Acr in the presence of 2 mg/mL collagenase (curve 1) or 0.2 mg/mL collagenase (curve 2), but not in the presence of 0.2 U/mL plasmin (curve 3). Each point represents the mean of four samples, and the standard error of the mean is shown.

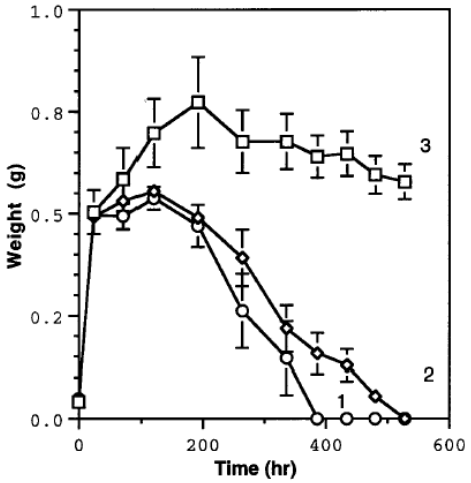


Figure 2. Enzymatic degradation of a hydrogel material formed by photopolymerization of Acr-(VRN)-PEG-(VRN)-Acr in the presence of 2 U/mL plasmin (curve 1) or 0.2 U/mL plasmin (curve 2), but not in the presence of 0.2 mg/mL collagenase (curve 3). Each point represents the mean of four samples, and the standard error of the mean is shown.

- Initially, swelling of network increases and wet weight goes up as first cross-links are broken, then as chains are freed and begin to diffuse out, weight goes down

degradation rate of network depends on collagenase concentration (from Mann et al.):

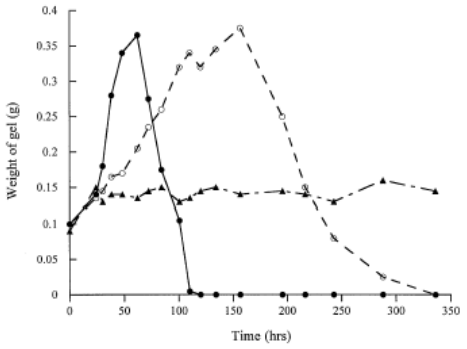


Fig. 1. Degradation of GGLGPAGGK-derivatized PEG hydrogels in solutions containing collagenase. (●) 2 mg/ml collagenase; (○) 0.2 mg/ml collagenase; (▲) no collagenase.

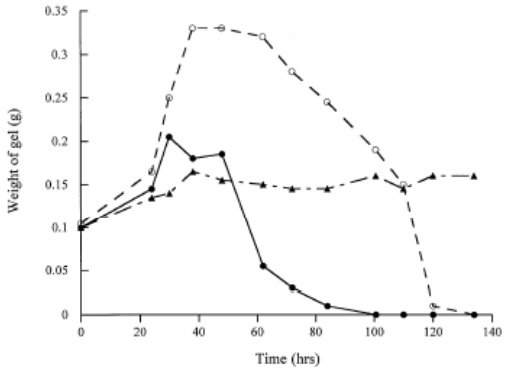


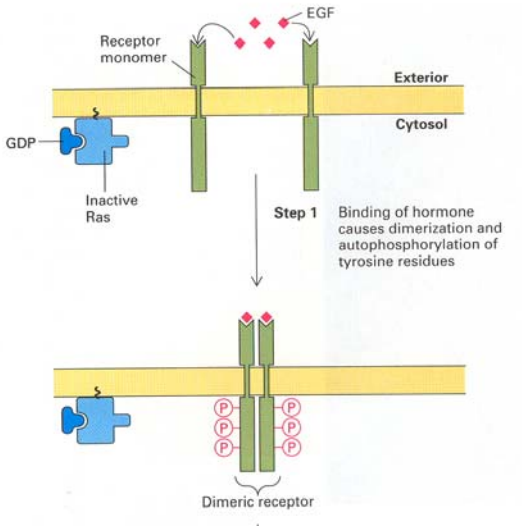
Fig. 2. Degradation of AAAAAAAAK-derivatized PEG hydrogels in solutions containing elastase. (●) 2 mg/ml elastase; (○) 0.2 mg/ml elastase; (▲) no elastase.

- note that these are hydrogels which has a major impact on the degradation rate…
- degradation rate is controlled by enzyme concentration and is selective for the enzyme targeted

Any examples tested in vivo?

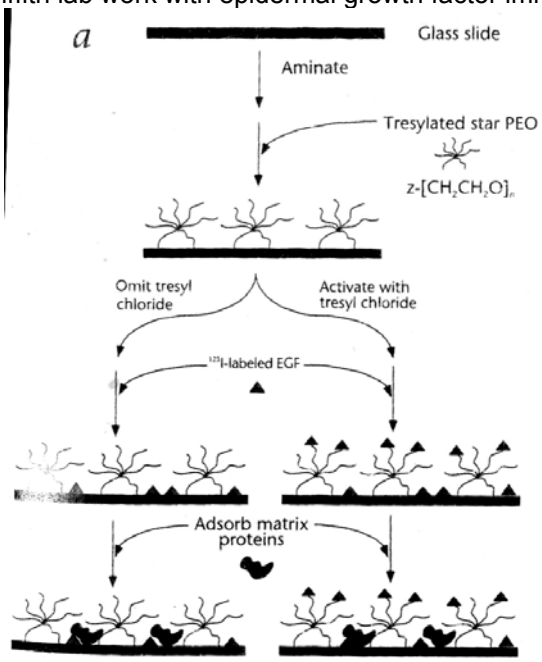
Recognition of Biomaterials by Cytokine Resceptors: Engineering growth and differentiation of cells on biomaterials via cytokine peptides[10-12]

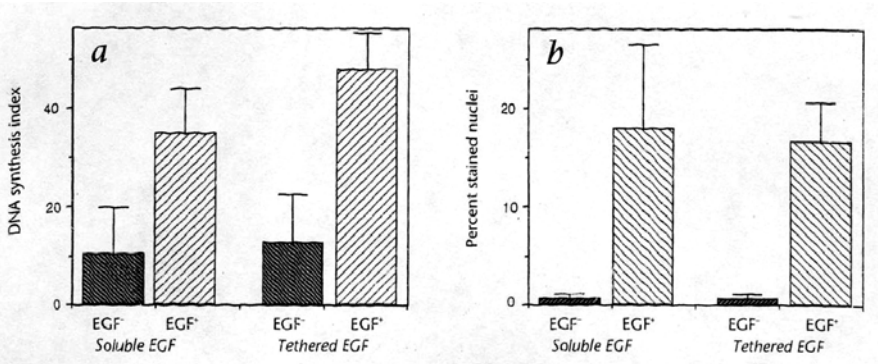
- Growth factors small proteins (ca. 50 aa)
- Can be immobilized to polymer chains present at surfaces of biomaterials much like adhesion peptides and presented to receptors of cells
- Many growth factors signal by DIMERIZATION and autophosphorylation
  - Again, spatial distribution may be key in controlling signaling
  - Cytokines immobilized in close proximity may favor signaling



(Lodish Fig. 20-32)

- INTERNALIZATION: used to down-regulate signals
  - Immobilized cytokines may show more potent signaling due to lack of this down-regulation pathway
- One of the first examples- Griffith lab work with epidermal growth factor immobilized on star polymers:





ıg. 2 Tethered EGF produces the same increase in DNA synthesis as soluble EGF for primary rat hepatocytes. *a*, Soluble EGF case contained 10 ng/ml EGF (EGF^+) or 0 ng/ml EGF (EGF^-). Tethered EGF was prepared with star PEO (f = 180 and M_s = 10,000) as shown in Fig. 1*a*; surfaces present either 3.2 ng/cm² covalently tethered EGF and 1.9 ng/cm² nonspecifically adsorbed EGF (EGF^+) or 0 ng/cm² covalently tethered EGF and 1.9 ng/cm² nonspecifically adsorbed EGF (EGF^-). Staining index represents the number of nuclei stained per area covered by cells, and represent the mean ± s.d. for at least 100 nuclei. *b*, Soluble case is the same as (*a*). Tethered EGF was prepared by the solution-first method with star PEO (f = 70, M_s = 5200) as shown in Fig. 1*b*; surfaces present either 0.4 ng/cm² covalently tethered EGF and <0.006 ng/cm² nonspecifically adsorbed EGF (EGF^+) or 0 ng/cm² covalently tethered EGF and <0.006 ng/cm² nonspecifically adsorbed EGF (EGF^-). DNA synthesis was measured as a percentage of the total nuclei that had synthesized DNA and each point represents the mean ± s.d. for three separate surfaces with at least 100 nuclei counted per surface.

- A second example, immobilized insulin (Ito):

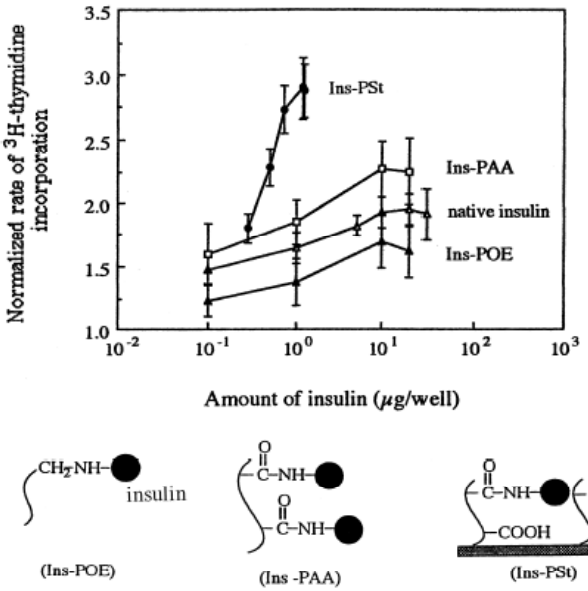


Fig. 2. Relative growth rate of mouse fibroblast STO cells in the presence of (△) native insulin, (▲) Ins–POE, (□) Ins–PAA, (●) Ins–PSt. Bars represent standard deviation. $n = 6$.

  - This data interestingly shows several biophysical effects:
    - PEG-insulin not as good as free insulin
      - Steric interference
    - PAA-insulin better than free insulin
      - Multivalent
    - Surface-immobilized PAA-insulin better than all above
      - Lack of internalization/signal downregulation?
  -

- Issues faced in incorporation of cytokines in biomaterials:
  - Protein stability (rugged, but not as good as peptides- may significant secondary structure to worry about
  - Steric interference of tether/surface with receptor binding
- Growth factors that have been studied in biomaterials:
  - EGF
  - Insulin (Y. Ito)
  - TGF-β (West)

References

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