Materials with Biological Recognition (continued)

<table>
<thead>
<tr>
<th>Last time:</th>
<th>Biological recognition <em>in vivo</em></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Engineering biological recognition of biomaterials: adhesion/migration peptides</td>
</tr>
<tr>
<td><em>Today:</em></td>
<td>Engineering biological recognition of biomaterials: enzymatic recognition and cytokine signaling</td>
</tr>
<tr>
<td>Supplementary Reading:</td>
<td>-</td>
</tr>
</tbody>
</table>

**ANNOUNCEMENTS:**

Cell adhesion on biomaterials:
Cell responses to non-biological, synthetic biomaterials

1. Protein adsorption
2. Denaturation (unfolding)?
3. Cell responses to expected and unexpected epitopes
4. Reorganization?
   - Vroman effect: protein exchange

2 CRITICAL FACTORS CONTROLLING ADHESION ON BIOMATERIALS:
(1) PROTEIN ADSORPTION/PRESENTATION
(2) SUBSTRATE STIFFNESS
Control of cell attachment by mechanical properties of substrate

Polyelectrolyte multilayers (Rubner lab MIT):

Images removed due to copyright reasons.
Please see:
Control of cell attachment by mechanical properties of substrate

(Van Vliet and Rubner labs):

Graph removed due to copyright reasons.
Please see: Figure 3 in Thompson, M. T., et al. *Biomaterials* 26 (2005): 6836–6845.

Graph removed due to copyright reasons.
Please see: Figure 4 in Thompson, M. T., et al. *Biomaterials* 26 (2005): 6836–6845.
Controlling cell response to biomaterials by building in ECM cues on a ‘blank slate’ background

**Making a Protein-Resistant Surface:** Polymer Brush

**Grafted Hydrophilic Polymer Chains:**
- **Example:** PEO, Dextran, PVA

\[ \text{Density} = \frac{\text{# chains}}{\text{area}} \]

**2 Approaches to Protein Resistance:**

**Kinetic**
- Enthalpic gain outweighs entropic penalty
- Equilibrium state takes much longer than experimental time to reach

**Thermodynamic**
- Lowest free energy state excludes protein from surface
- Requires high & \( \frac{\text{K}}{\sigma} \) for stretching chains too large

Lecture 12 Spring 2006
Design of protein adsorption-resistant surfaces

Kinetic:
- $\Delta G < 0$
- $\Delta G_{\text{Barrier}}$
- Height controls lifetime of kinetic resistance

Thermodynamic:
- $\Delta G > 0$
- Metastable state

Depends on $\sigma$, chain length
Design of protein adsorption-resistant surfaces

Surface modification strategies:

Self-assembled monolayers (SAMs):

VERY HIGH σ Achieved by using very short chains.

Even 3 'EO' repeat units can give protein resistance!

Surface grafting:

Graft copolymers or surface polymerization:
Limiting nonspecific cell adhesion

- Methyl methacrylate
- Poly(ethylene glycol) methacrylates

Fraction of cells adhered relative to TCPS

- 9-unit side chains
  - PMMA
  - Comb w/ ~50 wt% PEG units is H2O soluble

Graph showing weight fraction PEO units vs. fraction of cells adhered.
Tailoring cell adhesion on biomaterials via immobilized ligands

Peptide  integrin-binding GRGDSP sequence
PEO      short 6-9 unit side chains for protein resistance
PMMA     backbone anchors hydrophilic side chains
### Peptides used to modulate cell adhesion on biomaterials

<table>
<thead>
<tr>
<th>Peptide sequence</th>
<th>Derived from</th>
<th>Conjugate receptor</th>
<th>Role</th>
</tr>
</thead>
<tbody>
<tr>
<td>IKVAV</td>
<td>Laminin α-chain</td>
<td>LBP110 (110 KDa laminin binding protein)</td>
<td>Cell-ECM adhesion</td>
</tr>
<tr>
<td>RGD</td>
<td>Laminin α-chain, fibronectin, collagen</td>
<td>Multiple integrins</td>
<td>Cell-ECM adhesion</td>
</tr>
<tr>
<td>YIGSR</td>
<td>Laminin β1-chain</td>
<td>α₁β₁ and α₃β₁ integrins</td>
<td>Cell-ECM adhesion</td>
</tr>
<tr>
<td>RNIAEIKDI</td>
<td>Laminin γ-chain</td>
<td>unknown</td>
<td>Cell-ECM adhesion</td>
</tr>
<tr>
<td>HAV</td>
<td>N-cadherin</td>
<td>N-cadherin</td>
<td>Cell-cell adhesion</td>
</tr>
<tr>
<td>DGEA</td>
<td>Type I collagen</td>
<td>α₂β₁ integrin</td>
<td>Cell-ECM adhesion</td>
</tr>
<tr>
<td>VAPG</td>
<td>Elastase</td>
<td>Elastase receptor</td>
<td>Cell-ECM adhesion</td>
</tr>
<tr>
<td>KQAGDV</td>
<td>Fibrinogen γ-chain</td>
<td>β₃ integrins</td>
<td>Cell-ECM adhesion</td>
</tr>
</tbody>
</table>

- **Peptides more robust than intact protein**
- **Easy to synthesize in high purity**
- **K<sub>D</sub> (binding affinity) or receptor binding to minimal peptides typically much weaker than native protein**
Peptide linking chemistry

\[
\text{OH} + \text{O} \rightarrow \text{O} \quad \text{(1)}
\]

\[
\text{OH} + \text{O} \rightarrow \text{O} \quad \text{(2)}
\]

\[
\text{N- HYDROXY SUCCINIMIDE} + \text{CARBODIMIDE} \rightarrow \text{NHS} \quad \text{(3)}
\]
Cell responses to RGD

Fraction Seeded Cells Adhere

GRGDSP

GRGESP

Tethered RGD

+ soluble RGD

Antibody Blockade

Excess Soluble Peptide
Cells respond to control of ligand density at the surface

![Graph showing the fraction of seeded cells adhered to different RGD densities](image)

- TCPS

![Microscope images](images)
Cells respond to control of ligand density at the surface

Cell migration on fibronectin-coated substrates:

Graph removed due to copyright reasons.
Please see:

Graphs removed due to copyright reasons.
Please see:
Alternative functionalization approaches: avidin-biotin chemistry

Image removed due to copyright reasons.
Please see:
Controlling gross physical distribution of cells

Images removed due to copyright reasons.
Please see:
Cellular responses to physically patterned ligand- with nonadhesive background

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Please see:
Biomaterials recognized by cell-secreted enzymes:
synthetic ECMs
Enzymatic remodeling of synthetic ECMs
Enzymatic recognition of synthetic polymer backbones

Cleavage of synthetic polymers by enzymes

<table>
<thead>
<tr>
<th>Cell source</th>
<th>Enzyme</th>
<th>Native function</th>
<th>Acts on</th>
<th>Degradation Mechanism</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Various bacteria</td>
<td>lipases</td>
<td>protease</td>
<td>Polyesters, polyesteramides</td>
<td>III</td>
<td>Monomers or dimers</td>
</tr>
<tr>
<td><em>Tritirachium album</em> (mold)</td>
<td>Proteinase K</td>
<td>Protease</td>
<td>Poly(lactide)</td>
<td>III</td>
<td>Monomers or dimers</td>
</tr>
<tr>
<td>Mammalian cells</td>
<td>esterases</td>
<td>protease</td>
<td>Poly(alkyl cyanoacrylates)</td>
<td>II</td>
<td>Water-soluble polymers</td>
</tr>
<tr>
<td>Mammalian cells</td>
<td>Papain, pepsin</td>
<td>proteases</td>
<td>polyesteramides$^2$</td>
<td>III</td>
<td>Untested</td>
</tr>
<tr>
<td>Mammalian cells</td>
<td>$\alpha$-chymotrypsin</td>
<td>Serine protease</td>
<td>Aromatic peptides in polyesteramides$^3$ (e.g. Ala, Val, Leu)</td>
<td>III</td>
<td>Untested</td>
</tr>
<tr>
<td>Mammalian cells</td>
<td>elastase</td>
<td>protease</td>
<td>Polyesteramides</td>
<td>III</td>
<td>Untested</td>
</tr>
</tbody>
</table>
Enzymatic degradation of polyesteramides


Enzymatic breakdown by papain:

Compare with hydrolysis: (poly(ortho ester))
Esterase attack on poly(alkyl cyanoacrylates)

Degradation of 250 nm-diam. porous particles:

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## Engineering enzymatic recognition of hydrogel biomaterials: recognition of peptide motifs

### Enzymatic activity in vivo on peptide sequences:

<table>
<thead>
<tr>
<th>Cleavage Enzyme</th>
<th>Functions in vivo</th>
<th>Target amino acid sequences</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasminogen activator (urokinase or tissue-type plasminogen activator) / plasminogen → plasmin</td>
<td>Degradation of fibrin matrices, angiogenesis, tumor progression; urokinase can bind to cell surface receptor</td>
<td>on fibrinogen: Arg$<em>{104}$-Asp$</em>{105}$, Arg$<em>{110}$-Val$</em>{111}$, Lys$<em>{206}$-Met$</em>{207}$, Arg$<em>{42}$-Ala$</em>{43}$, Lys$<em>{130}$, Glu$</em>{131}$, Lys$<em>{84}$-Ser$</em>{85}$, Lys$<em>{87}$-Met$</em>{88}$</td>
</tr>
<tr>
<td>Matrix metalloproteinases (soluble and cell-surface): e.g. Fibroblast Collagenase (MMP I)</td>
<td>Facilitate cell migration</td>
<td>Type I collagen: Gly$<em>{775}$-Ile$</em>{776}$ In smaller peptides: Gly-Leu or Gly Ile bonds</td>
</tr>
<tr>
<td>Elastase</td>
<td>Elastin remodeling</td>
<td>Poly(Ala) sequences</td>
</tr>
</tbody>
</table>

![Diagram of enzymatic recognition of peptide motifs](image-url)
Enzyme-sensitive crosslinks in hydrogel biomaterials

Acrylate endgroups

collagenase sequence

PEG

peptides

photopolymerization

(collagenase)-APGL-(CH₂CH₂O-)

(collagenase)-APGL-(West and Hubbell, 1999)
Effect of enzyme concentration

Gel containing collagenase sequence

Gel containing elastase sequence

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Please see:
Figure 1 in West, J.L. and J. A. Hubbell. “Polymeric Biomaterials with Degradation Sites for Proteases Involved in Cell Migration.” Macromolecules 32 (1999): 241-244.

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Please see:
Figure 2 in West, J.L. and J. A. Hubbell. “Polymeric Biomaterials with Degradation Sites for Proteases Involved in Cell Migration.” Macromolecules 32 (1999): 241-244.
Cellular migration through enzymatically-recognized hydrogels

Biphasic migration response in 3D matrix:

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Please see:

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Please see:
Enzymatic recognition of biomaterials II: Enzymatic cross-linking/modification of biomaterials

**In situ-forming hydrogels:**

Example enzymes and their substrates:

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Substrate <em>in vivo</em></th>
<th>Synthetic substrates</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Transglutaminase</td>
<td>Glutamines</td>
<td>Glu-containing peptides</td>
<td>Amide bond formation</td>
</tr>
<tr>
<td>Factor XIII</td>
<td>Fibrin γ-chain</td>
<td>Peptides derived from γ-chain FXIII binding site</td>
<td>Amide bond formation</td>
</tr>
</tbody>
</table>

(Zhang et al. 2002)
Biomaterials that mimic signals from soluble factors or other cells
Cytokine receptor-based recognition of biomaterials

Diverse functions of cytokines:

- Induce cell migration/stop cell migration
- Induce cell growth
- Induce differentiation
  - Upregulate tissue-specific functions

Characteristics:

- Typically potent, act at pmol concentrations
- Synergize with other receptor signals
  - e.g. integrins

Figure by MIT OCW.
Changes in signaling achieved by cytokine immobilization on surfaces

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Please see:
Immobilized insulin:

Image removed due to copyright reasons.

Please see:
Local control of gene expression by non-diffusable cytokines:

Patterned immobilization of EGF:

Image removed due to copyright reasons.

Please see:

Figure 4 in Ito, Y. "Regulation of Cell Functions by Micropattern Immobilized Biosignal Molecules." Nanotechnology 9 (1998): 200-204.
Surface immobilization can induce new function in cytokines: case of tethered EGF-triggered neuronal cell differentiation

**PC12 cell line:**
- induced to differentiate and extend axons under stimulation of **NGF** (nerve growth factor)
- induced to proliferate by **EGF**

Signal doesn’t trigger internalization of receptor; thus signal lasts longer and triggers differentiation

Signal triggers internalization of receptor; short signal triggers proliferation

Lecture 12 Spring 2006
NGF vs. EGF signaling in PC12 neuronal cells

(Traverse et al. 1994)
Further Reading

1. Voet & Voet. in *Biochemistry*.