Materials with Biological Recognition (continued)

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


Supplementary Reading: -

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
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
Design of protein adsorption-resistant surfaces
Design of protein adsorption-resistant surfaces

Surface modification strategies:

Self-assembled monolayers (SAMs):

Surface grafting:

Graft copolymers or surface polymerization:
Limiting nonspecific cell adhesion

Methyl methacrylate

Poly(ethylene glycol) methacrylates

9-unit side chains

PMMA

comb

Fraction of cells adhered relative to TCPS

weight fraction PEO units

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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>RNIAEIIKDI</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>
Peptide linking chemistry

(1) + (2) → (3)

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Cell responses to RGD

Fraction Seeded Cells Adhere

0.5
0.4
0.3
0.2
0.1
0

GRGDSP

GRGESP

Tethered RGD

+ soluble RGD
Cells respond to control of ligand density at the surface
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>polyesteramides²</td>
<td>III</td>
<td>Untested</td>
</tr>
<tr>
<td>Mammalian cells</td>
<td>α-chymotrypsin</td>
<td>Serine protease</td>
<td>Aromatic peptides in polyesteramides³ (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))

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Please see:

Esterase attack on poly(alkyl cyanoacrylates)

Degradation of 250 nm-diam. porous particles:

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Please see:
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</td>
<td>Degradation of fibrin matrices, angiogenesis, tumor progression; urokinase can bind to cell surface receptor</td>
<td>on fibrinogen: Arg\textsubscript{104}-Asp\textsubscript{105}, Arg\textsubscript{110}-Val\textsubscript{111}, Lys\textsubscript{206}-Met\textsubscript{207}, Arg\textsubscript{42}-Ala\textsubscript{43}, Lys\textsubscript{130}, Glu\textsubscript{131}, Lys\textsubscript{84}-Ser\textsubscript{85}, Lys\textsubscript{87}-Met\textsubscript{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\textsubscript{775}-Ile\textsubscript{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>
Enzyme-sensitive crosslinks in hydrogel biomaterials

PEG photopolymerization

Acrylate endgroups

collagenase sequence

(APGL)–CH₂CH₂O–

(collagenase sequence)

(collagenase)

West and Hubbell, 1999
Effect of enzyme concentration

Gel containing collagenase sequence

Gel containing elastase sequence

Graph removed due to copyright reasons.
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:

Please see:

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

Example enzymes and their substrates:

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Substrate in vivo</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-diffusible cytokines:

Patterned immobilization of EGF:

Images removed for 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

Figure by MIT OCW.
NGF vs. EGF signaling in PC12 neuronal cells

Growth factor

Activated receptor-protein tyrosine kinase

GTP

Ras

GDP

Active

Inactive

GEF

Raf

MAPKK

MAPKPs

Cytoplasm

Nucleus

Activation of gene transcription

Figure by MIT OCW.

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Further Reading

1. Voet & Voet. in *Biochemistry*.