Interactions of cells with their environment; Engineering materials with biological recognition

Last time: Polyelectrolyte hydrogel swelling thermodynamics
Applications of polyelectrolyte hydrogels: BioMEMS and drug delivery

Today: Biological recognition *in vivo*
Engineering biological recognition of biomaterials: controlling cell adhesion, migration, and cytokine signaling

Reading: Y. Hirano and D.J. Mooney, 'Peptide and protein presenting materials for tissue engineering,' *Adv. Mater*. **16**(1) 17-25 (2004)
Discher, Janmey, Wang, 'Tissue Cells Feel and Respond to the Stiffness of Their Substrate,' *Science* **310** 1139-1143 (2005))

Supplementary Reading: 'The Extracellular Matrix,' pp. 1124-1150, *Molecular Biology of the Cell*, Lodish et al.

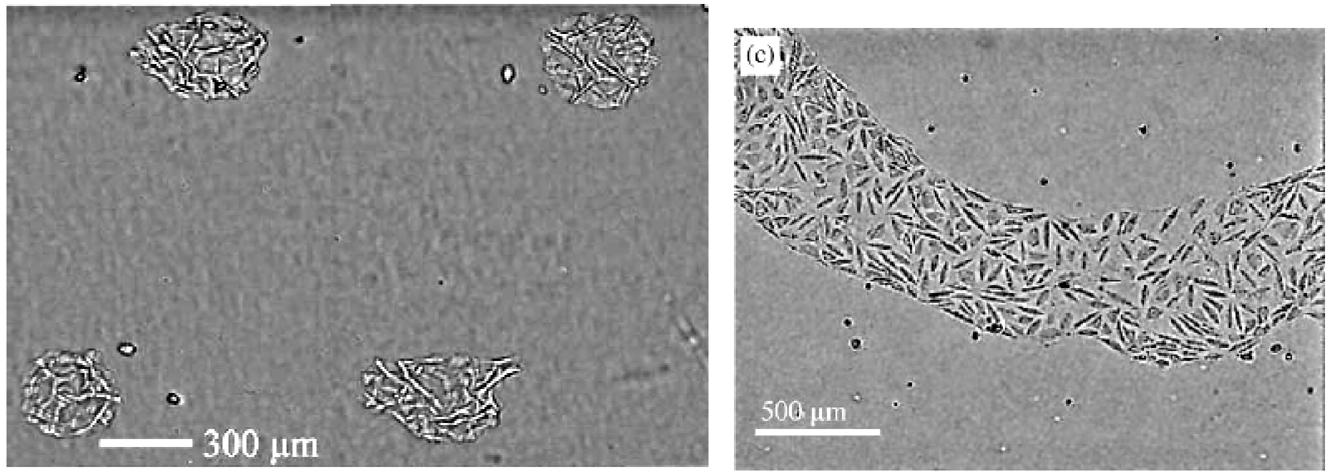
ANNOUNCEMENTS:

In situ formability: example: 'printable' gels

INKJET PRINTING:

- EJECT LIQUID TO

Collagen printed on an agarose gel substrate:



MATERIAL VISCOSITY

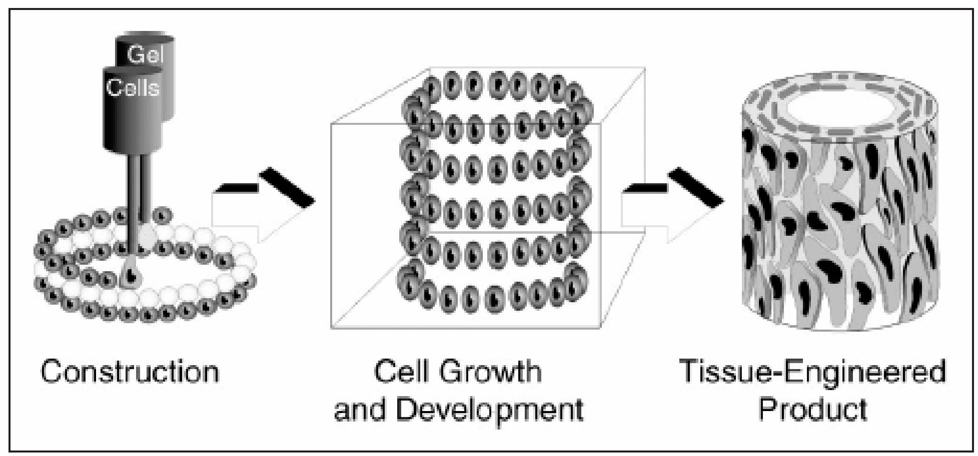
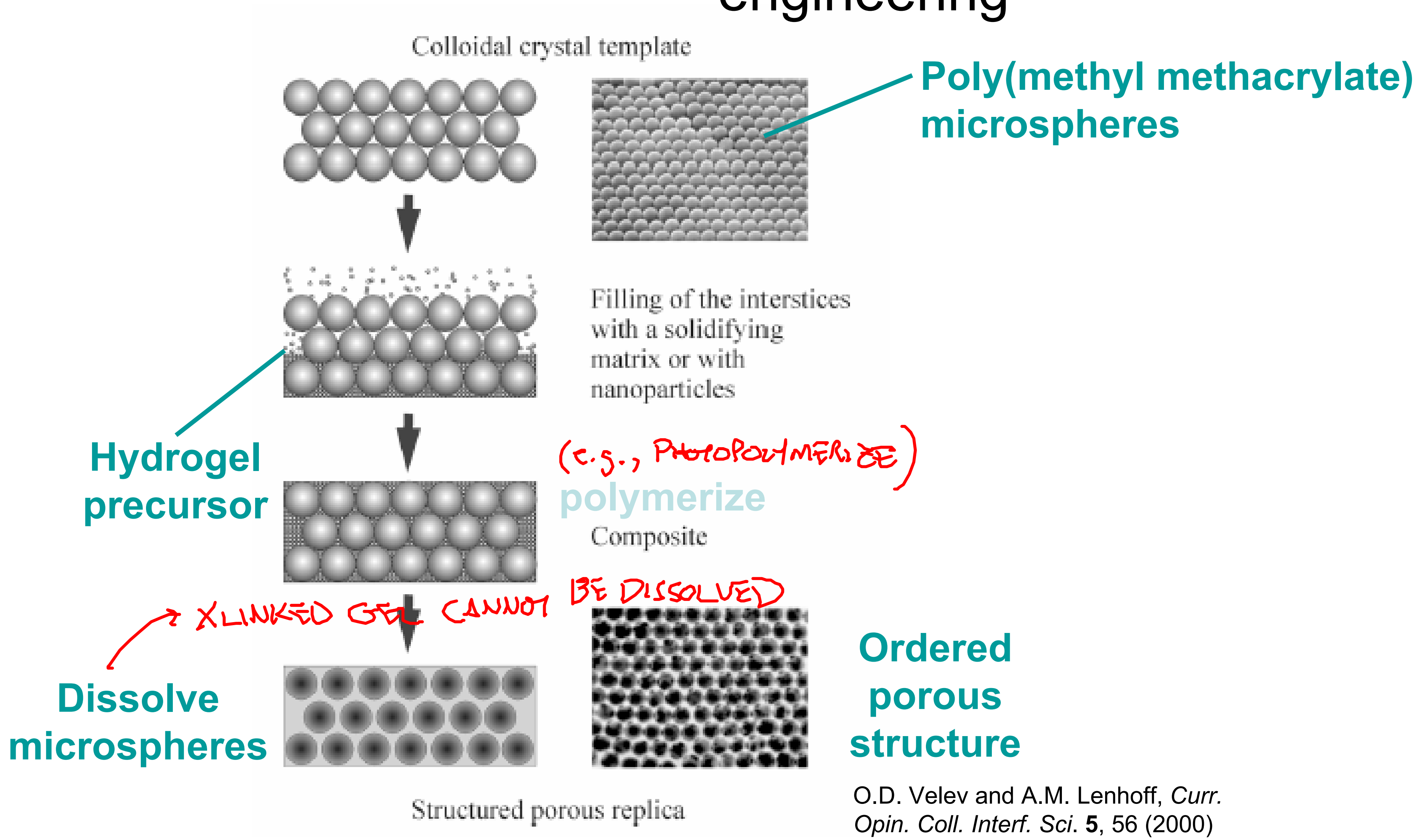


Figure 14 in Burg, K. J., and T. Boland. "Minimally Invasive Tissue Engineering Composites and Cell Printing." *IEEE Eng. Med. Biol.* 22, no. 5 (2003): 84-91.

Tissue engineering

Formability of hydrogels for tissue engineering

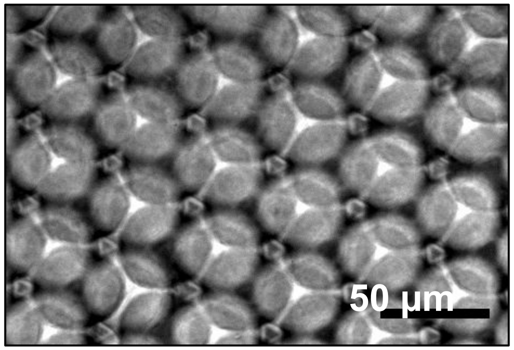


O.D. Velev and A.M. Lenhoff, *Curr. Opin. Coll. Interf. Sci*. **5**, 56 (2000)

Tissue engineering

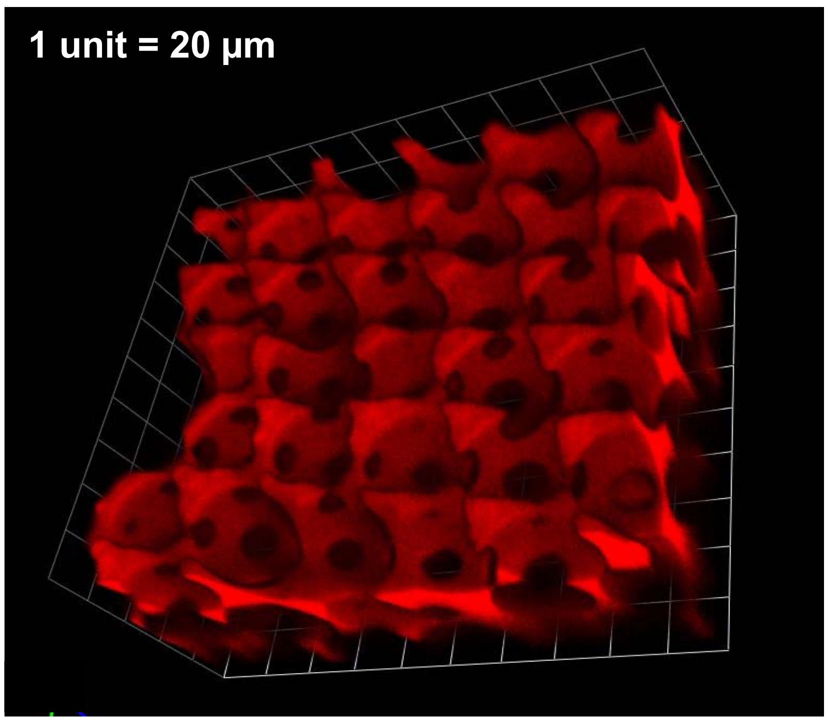
Scaffolds with ordered, highly interconnected porosity

Brightfield image:

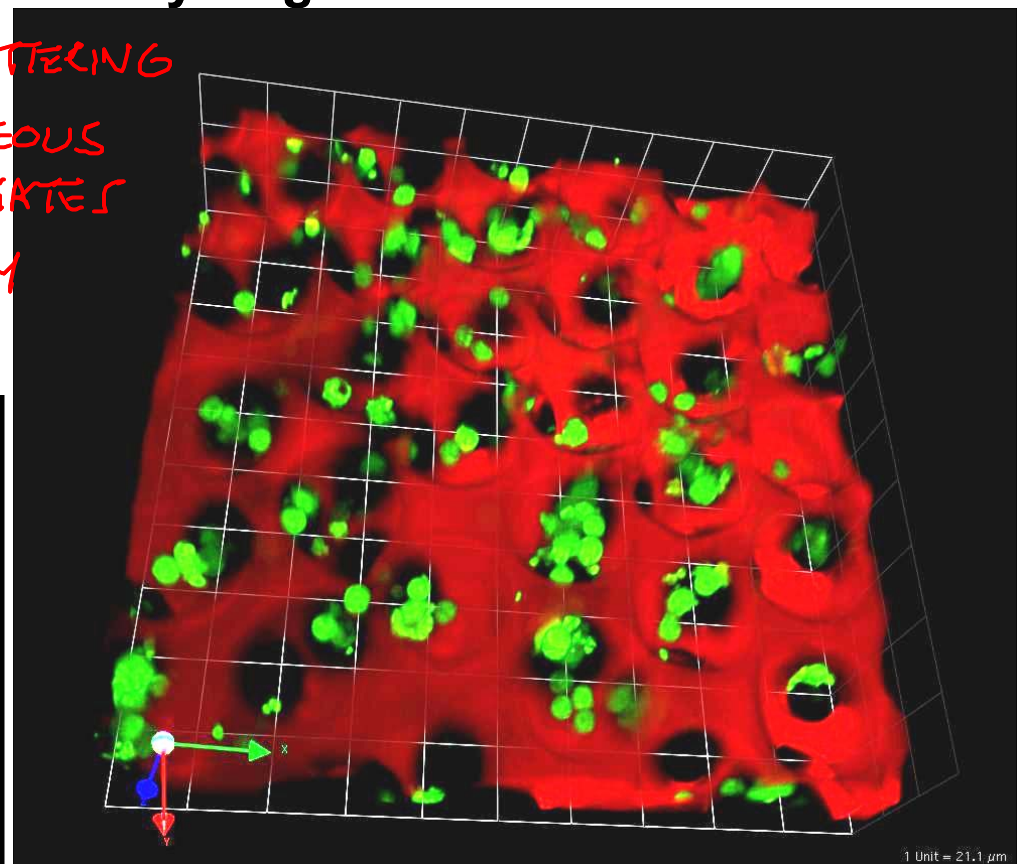


LOW LIGHT SCATTERING OF HOMOGENEOUS GELS FACILITATES MICROSCOPY

Confocal fluorescence:



PEG hydrogel scaffolds

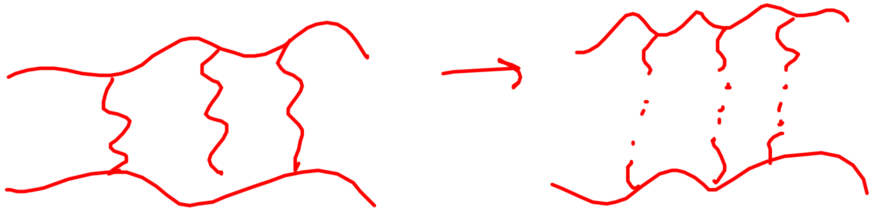


A. Stachowiak et al, *Advanced Materials* (2005)

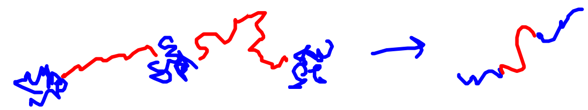
Degradable hydrogels: degradation by hydrolysis of cross-links (mechanism I)

(1) MOST COMMON ROUTE TO DEGRADABLE GELS:

XLINK DEGRADATION

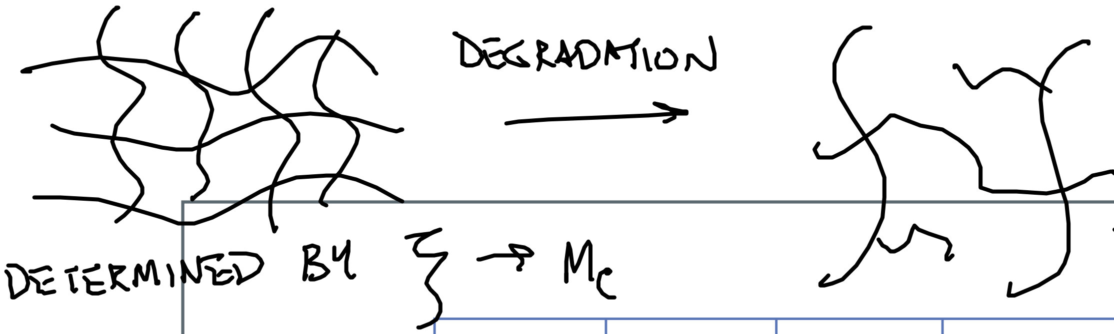


(2) PHYSICAL GELS:
THERMAL BREAKDOWN OF NONCOVALENT JUNCTIONS



(USUALLY RELATIVELY RAPID)

Dextran-based degradable hydrogels: degradation by hydrolysis of cross-links

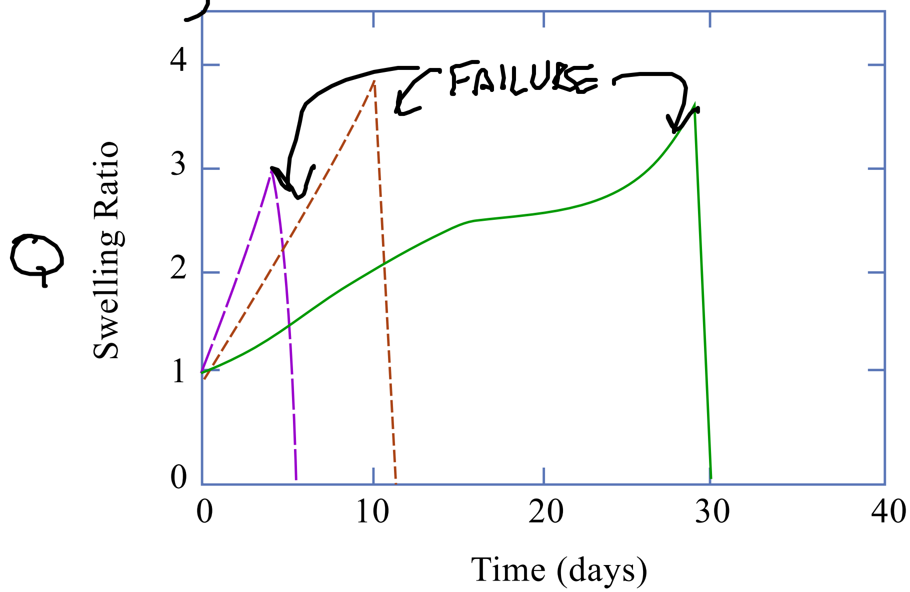


REDUCED ENTROPIC CONSTRAINTS AS XLINKS DEGRADE

↓

OSMOTIC PRESSURE DRIVES INCREASED SWELLING

Q DETERMINED BY ξ → M_c



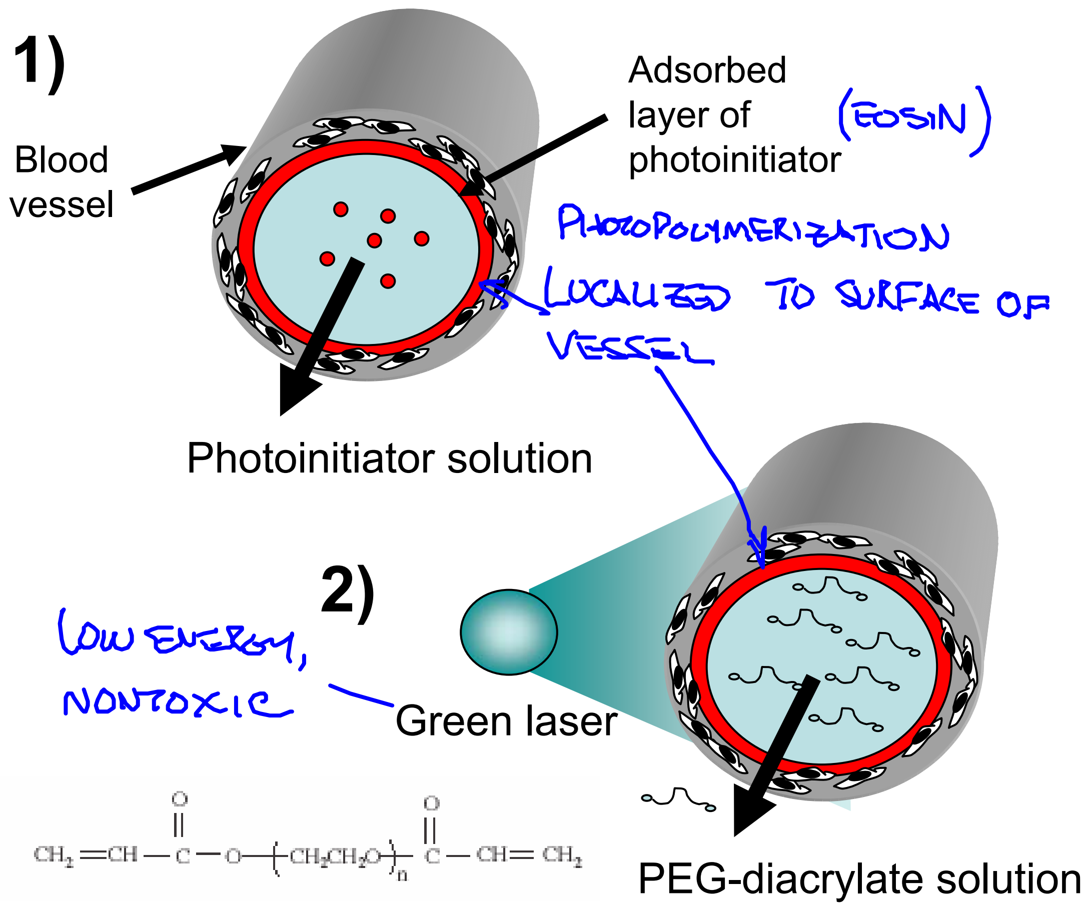
Swelling behavior of dex-HEMA (—), dex-lactate-HEMA (- - -) and dex-lactate$_2$-HEMA (— —) hydrogels in aqueous solution (pH 7.2, 37^{o}C). The initial water content of the hydrogels was 80%, the degree of methacryloyl substitution was approximately 6.

Figure by MIT OCW.

Tissue barriers/conformal coatings

Applications: tissue barriers

Tissue barriers and conformal coatings



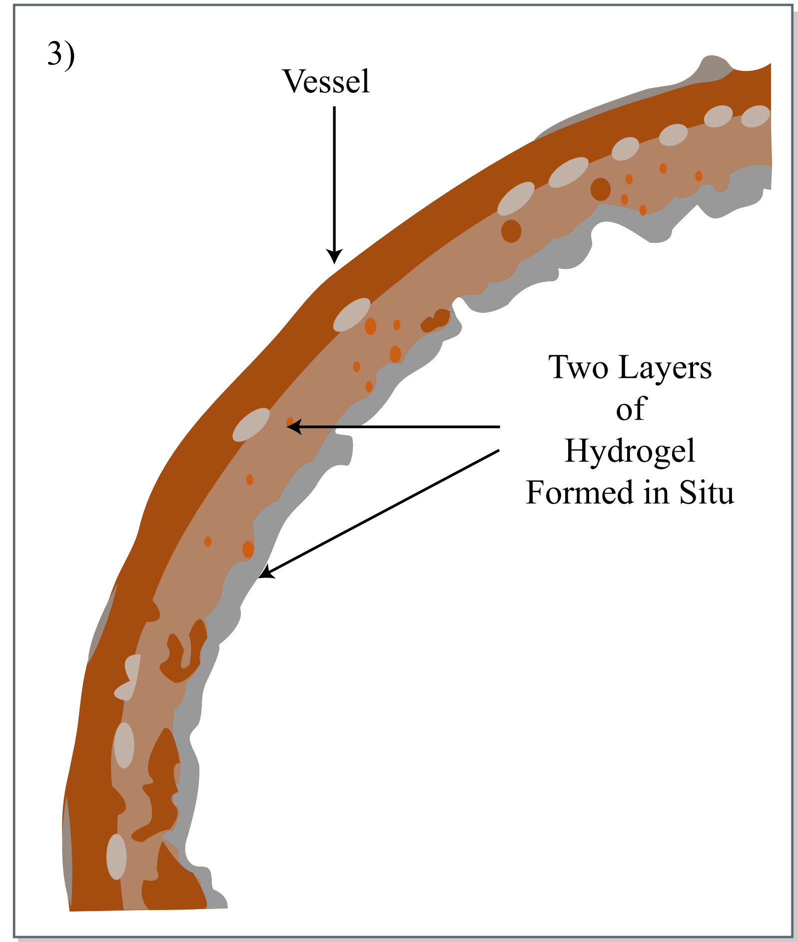


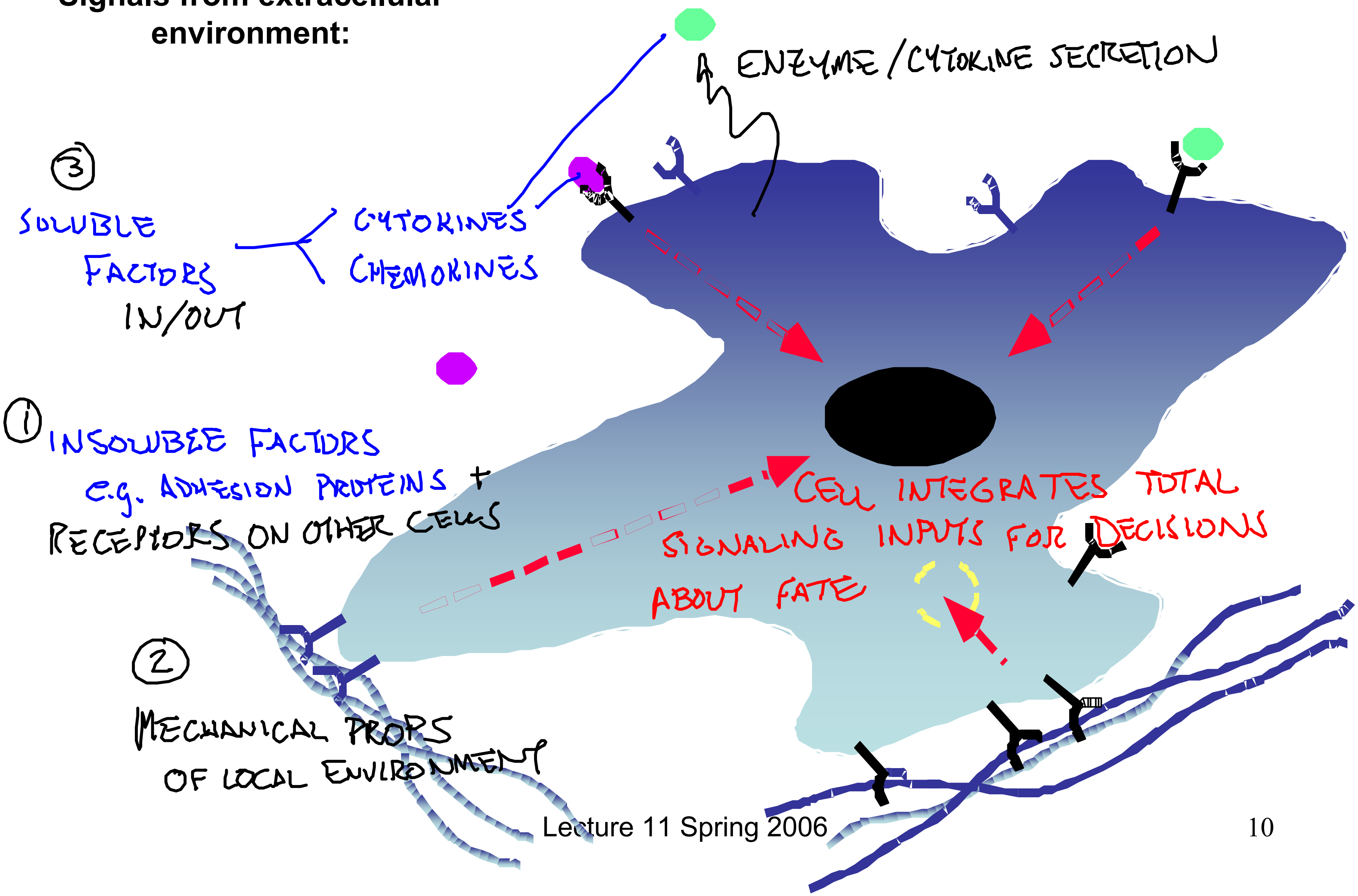
Figure by MIT OCW.

(After An and Hubbell 2000)

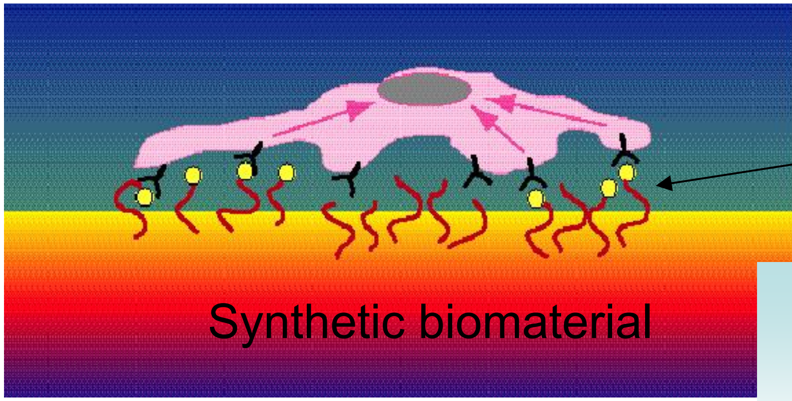
Engineering Biological Recognition in Synthetic Materials

Interactions of cells with their environment

Signals from extracellular environment:



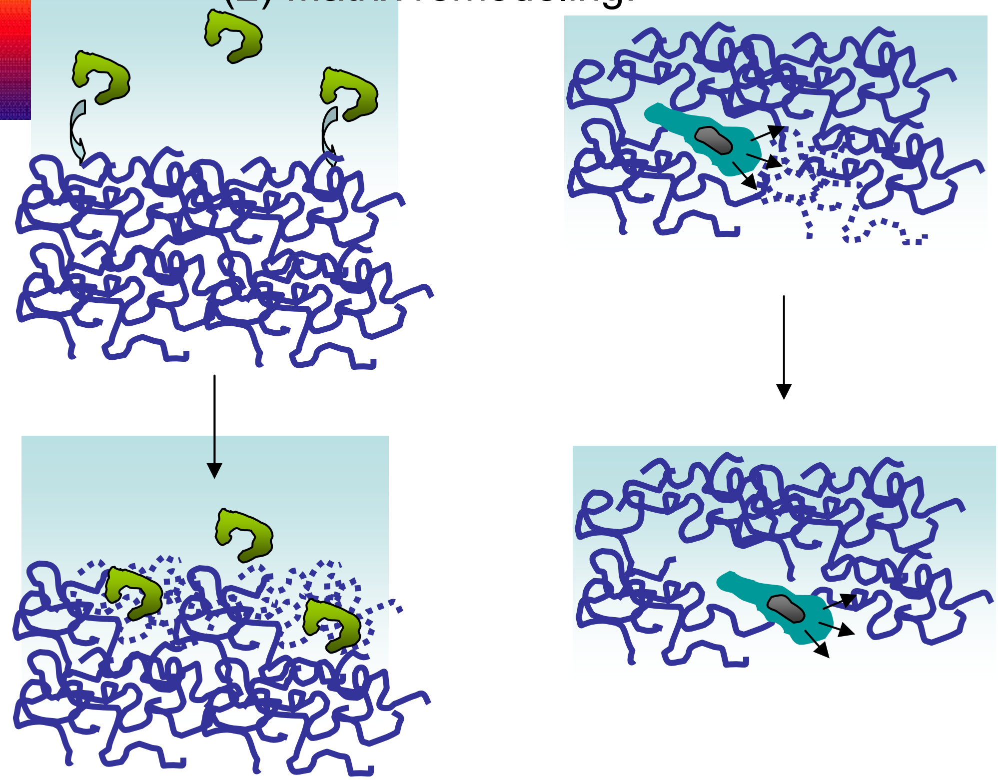
Incorporation of ECM signals in biomaterials



Peptides or proteins tethered to biomaterial surface, examples of (1) and (3)

1. Cell adhesion/migration
2. Matrix remodeling
3. Cytokine signaling

(2) Matrix remodeling:



The insoluble surroundings of the cell: Functions of the native extracellular matrix (ECM):

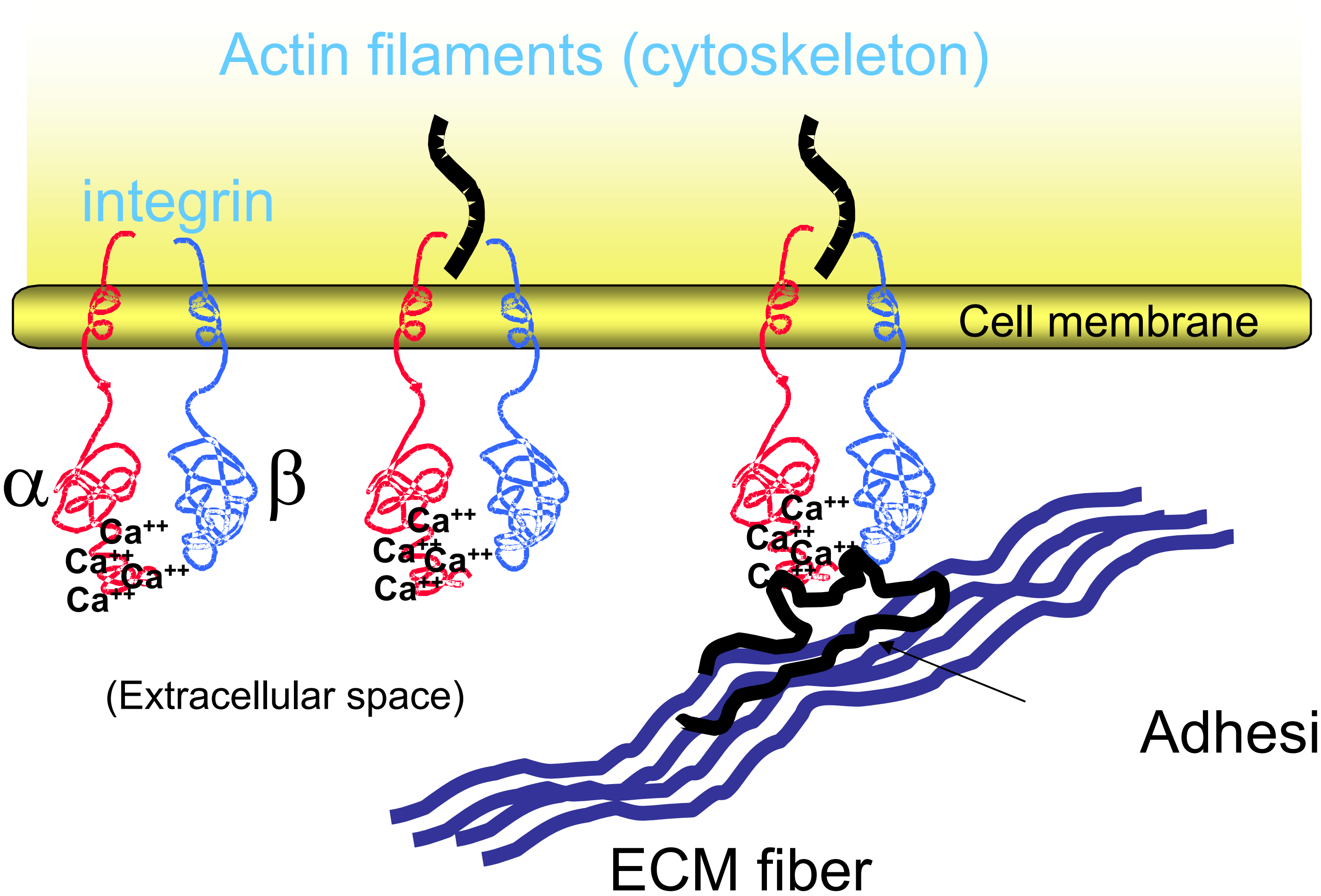
- Mechanical support
- Cues for cell survival/function
  - Anchorage-dependent cell growth
  - Differentiation cues
- Organization of tissue

Collagen and Adhesions Proteins: Structure and Function

- Sixt et al. *Immunity* 22 (2005):19-25.
- Friedl et al. *Eur. J. Immunol.* 28 (1998): 2331.
- Lodish et al. *Molecular Cell Biology*

Cell adhesion

Controlling cell attachment and migration



Structure of integrins:

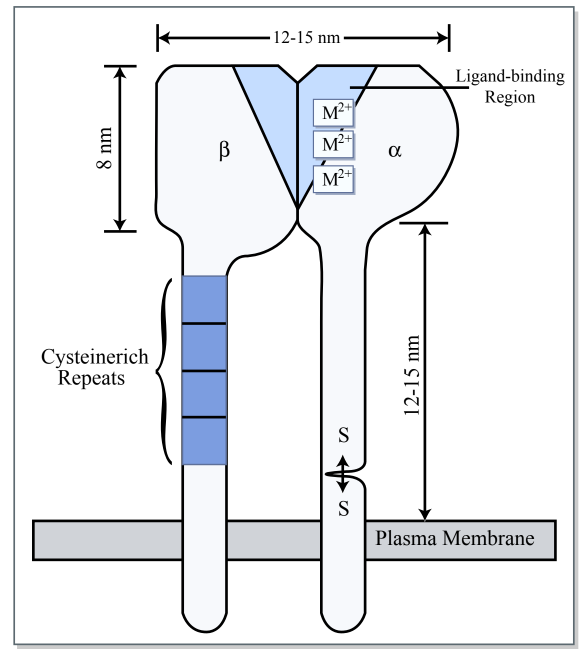


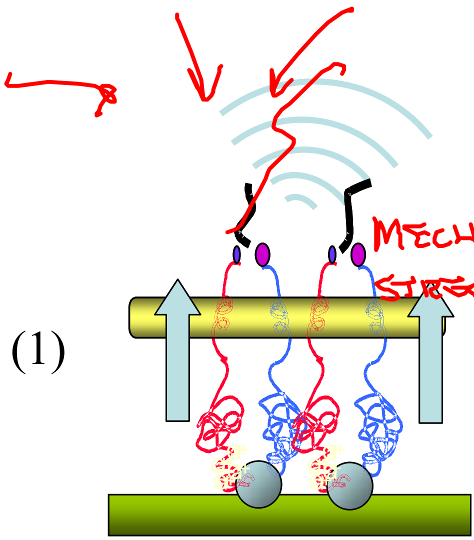
Figure by MIT OCW.

(Lodish, Molecular Cell Biology)

Cell adhesion

Adhesive interactions can play multiple roles simultaneously: supporting adhesion, delivery of biochemical signals, or delivering biomechanical signals

(1)

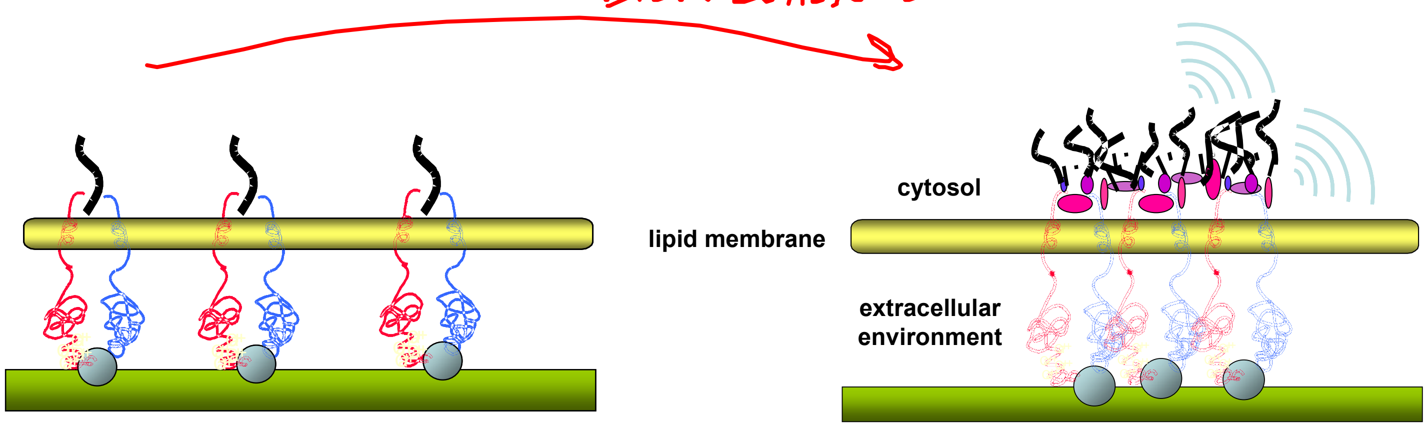


MECHANICAL STRESSES CAN

(2)

PROTEINS

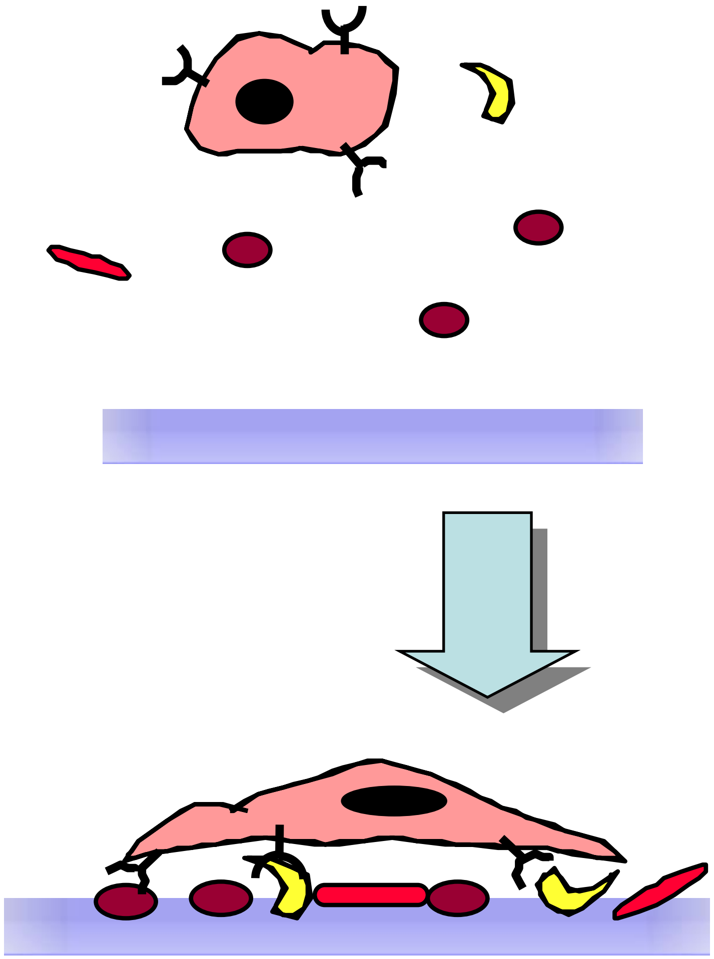
SIGNALING MAY BE REGULATED BY PHYSICAL DISTRIBUTION OF ADHESION RECEPTORS



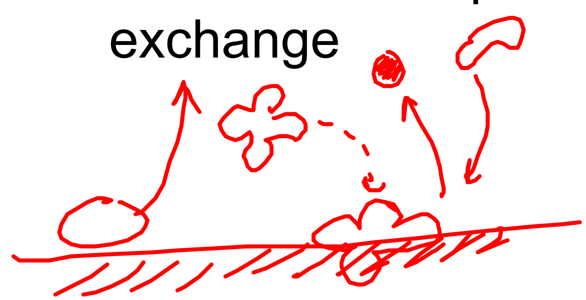
Cells sense and respond to the stiffness of their substrate

(Discher, Janmey, Wang *Science* **310** 1139-1143 (2005))

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):

CELL MUST BE CAPABLE OF GENERATING TRACTION

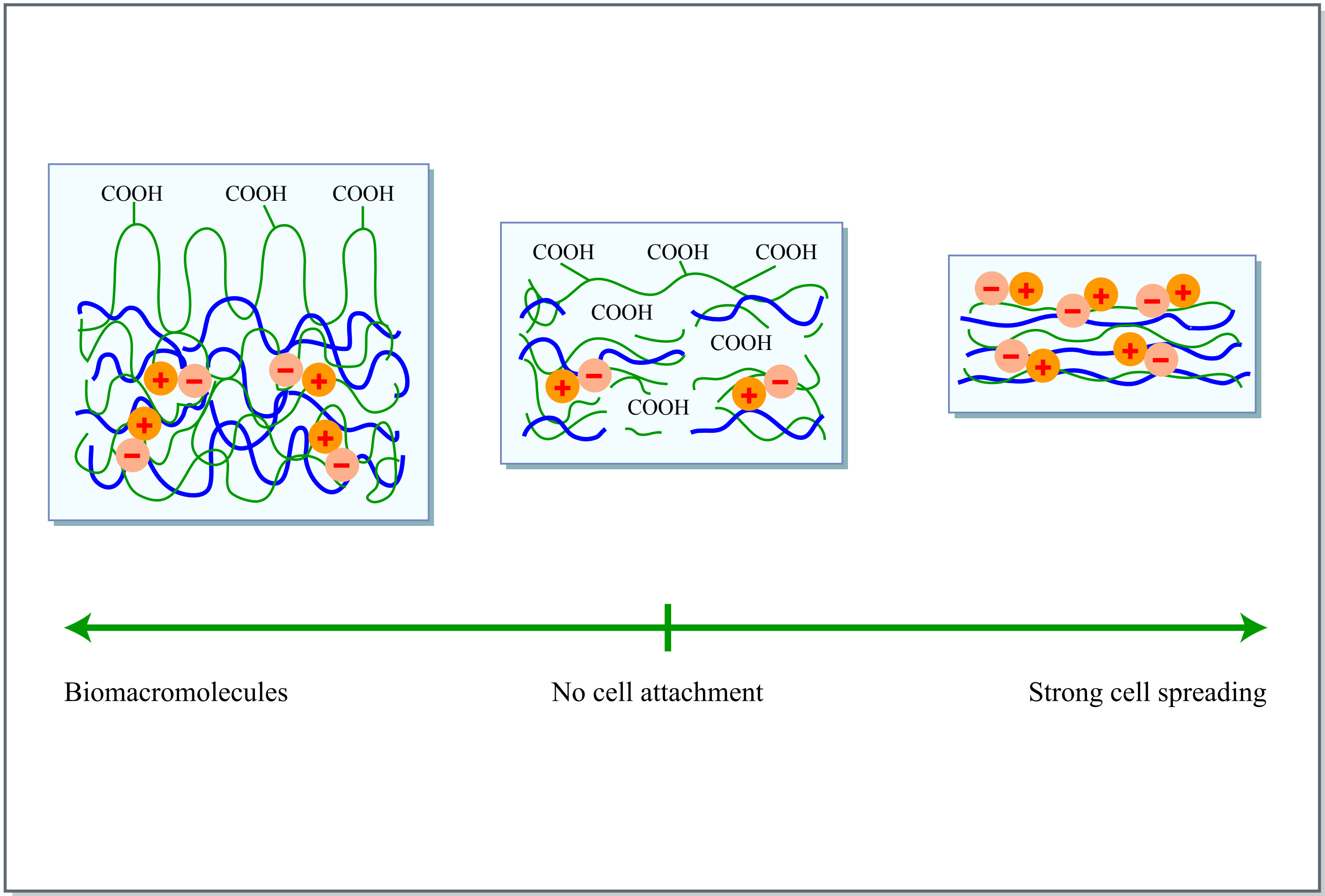


Figure by MIT OCW.

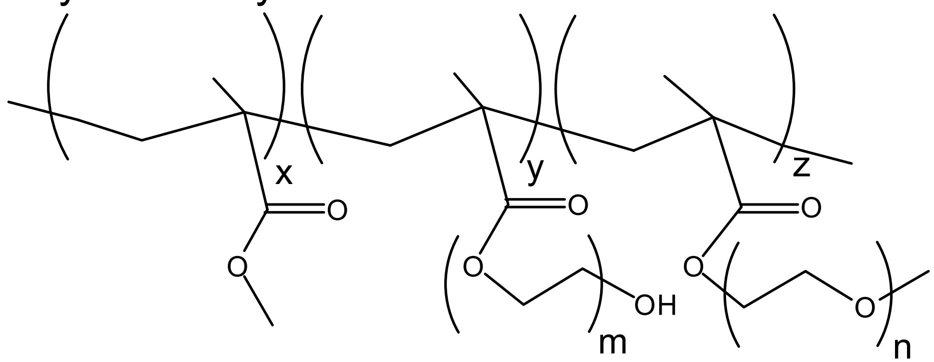
Controlling cell response to biomaterials by building in ECM cues on a 'blank slate' background

Design of protein adsorption-resistant surfaces

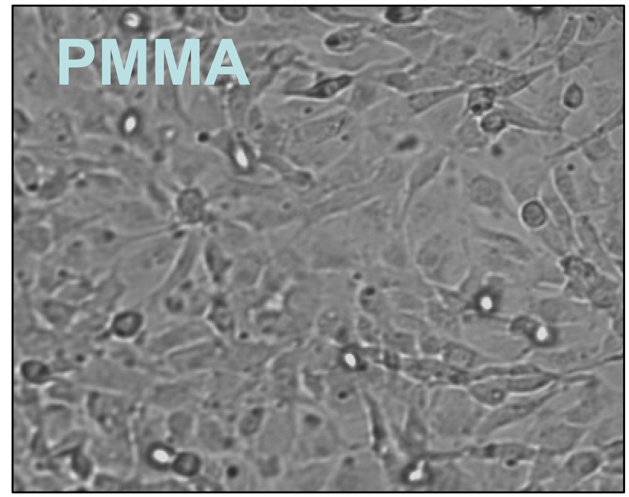
Design of protein adsorption-resistant surfaces

Limiting nonspecific cell adhesion

Methyl methacrylate



Poly(ethylene glycol)
methacrylates



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

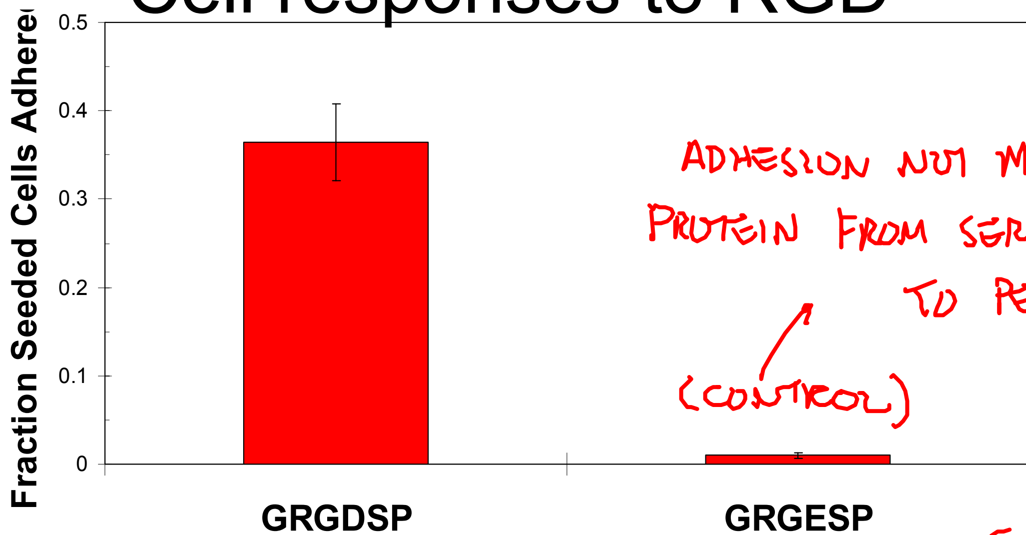
Peptide sequence	**Derived from**	**Conjugate receptor**	**Role**
IKVAV	Laminin α-chain	LBP110 (110 KDa laminin binding protein)	Cell-ECM adhesion
RGD	Laminin α-chain, fibronectin, collagen	Multiple integrins	Cell-ECM adhesion
YIGSR	Laminin β1-chain	$\alpha_1\beta_1$ and $\alpha_3\beta_1$ integrins	Cell-ECM adhesion
RNIAEIIKDI	Laminin γ-chain	unknown	Cell-ECM adhesion
HAV	N-cadherin	N-cadherin	Cell-cell adhesion
DGEA	Type I collagen	$\alpha_2\beta_1$ integrin	Cell-ECM adhesion
VAPG	Elastase	Elastase receptor	Cell-ECM adhesion
KQAGDV	Fibrinogen γ-chain	β_3 integrins	Cell-ECM adhesion

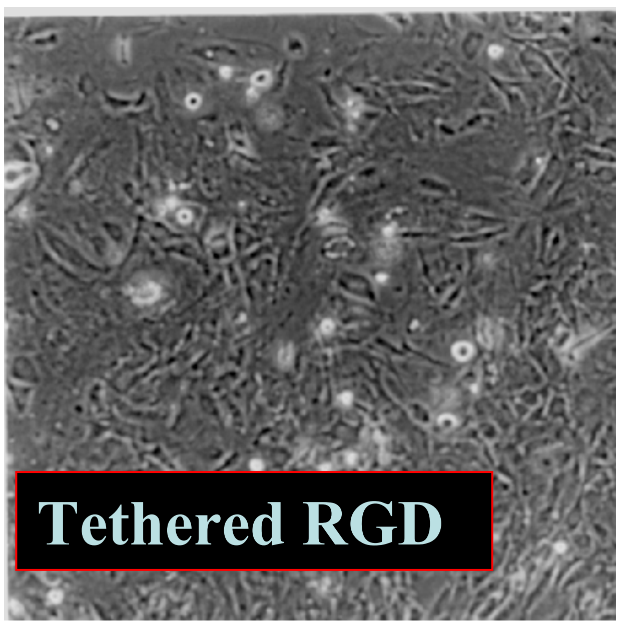
- PEPTIDES MORE ROBUST THAN INTACT PROTEINS
- K_D OF R-L BINDING USUALLY SIGNIFICANTLY REDUCED:

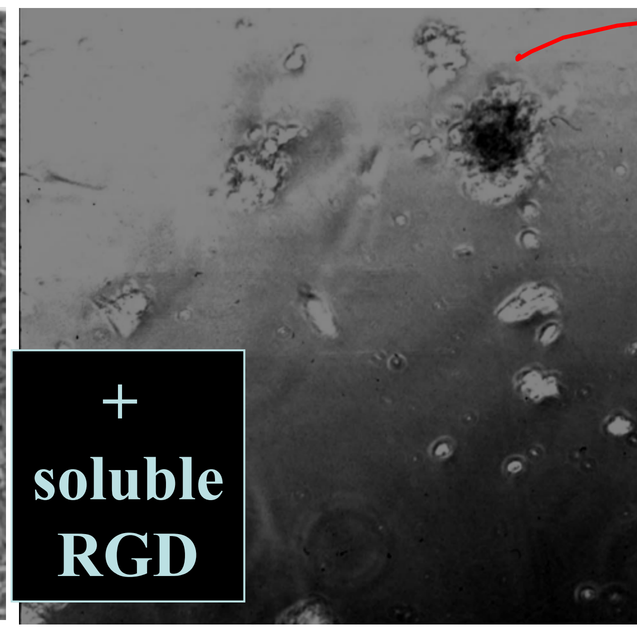
e.g. RGDS vs. FN

K_D 1000-FOLD LOWER FOR PEPTIDE

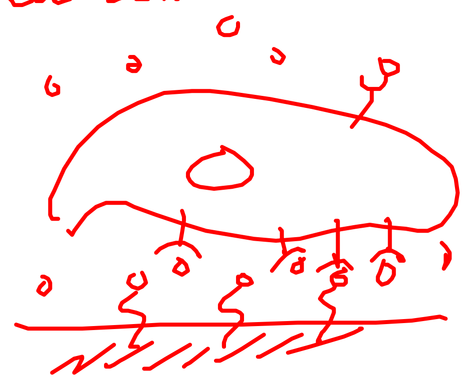
Cell responses to RGD







COMPETITION FOR RECEPTORS LEADS TO CELL DETACHMENT



Cells respond to control of ligand density at the surface

Figure 11 in Irvine, D. J., A. V. Ruzette, A. M. Mayes, and L. G. Griffith. "Nanoscale Clustering of RGD Peptides at Surfaces Using Comb Polymers. 2. Surface segregation of comb polymers in polylactide." *Biomacromolecules* 2 (2001): 545-56.

Figure 12 in Irvine, D. J., A. V. Ruzette, A. M. Mayes, and L. G. Griffith. "Nanoscale Clustering of RGD Peptides at Surfaces Using Comb Polymers. 2. Surface segregation of comb polymers in polylactide." *Biomacromolecules* 2 (2001): 545-56.

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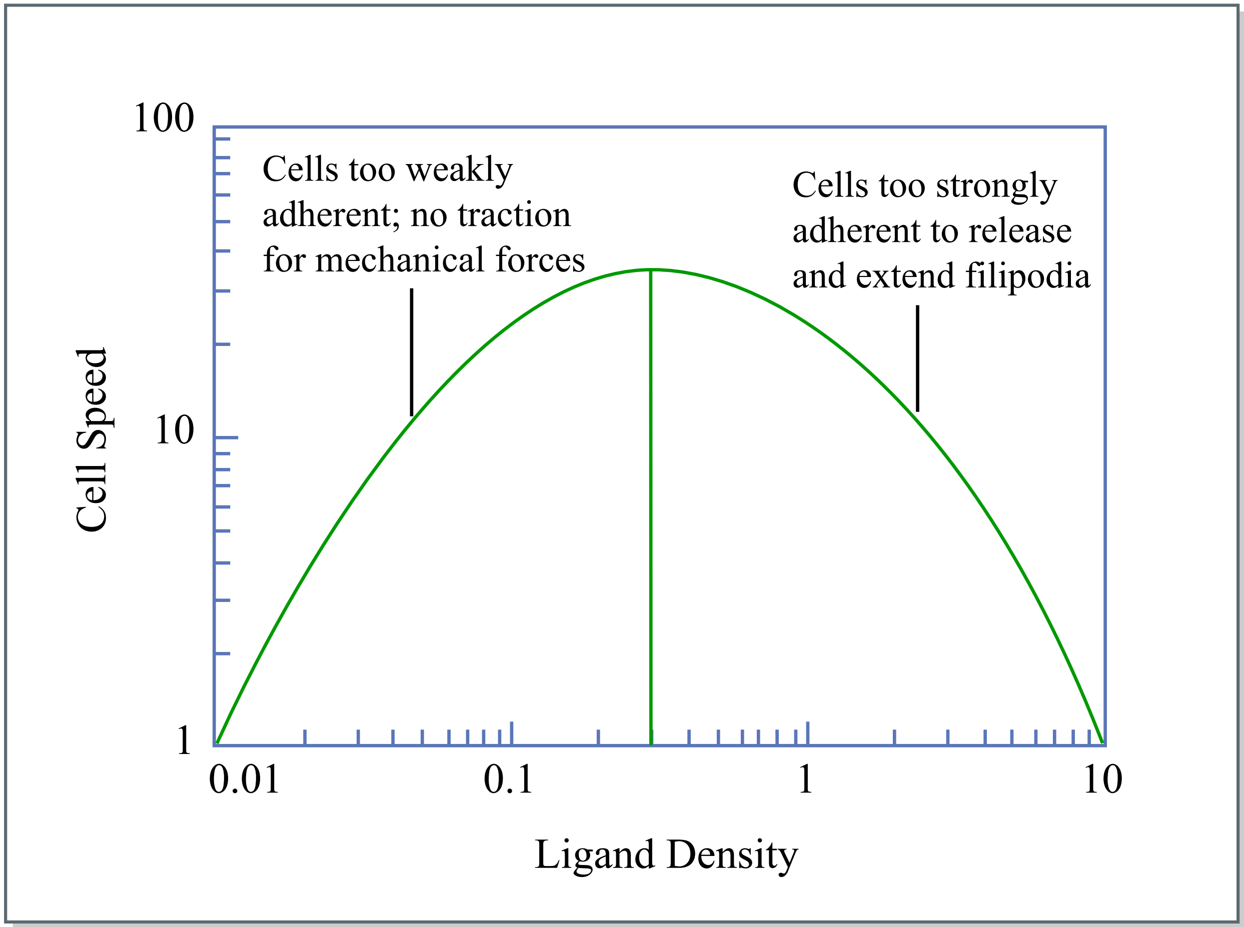

Figure by MIT OCW.

Further Reading

1. Di Lullo, G. A., Sweeney, S. M., Korkko, J., Ala-Kokko, L. & San Antonio, J. D. Mapping the ligand-binding sites and disease-associated mutations on the most abundant protein in the human, type I collagen. *J Biol Chem* **277**, 4223-31 (2002).
2. Lemire, J. M., Merrilees, M. J., Braun, K. R. & Wight, T. N. Overexpression of the V3 variant of versican alters arterial smooth muscle cell adhesion, migration, and proliferation in vitro. *J Cell Physiol* **190**, 38-45 (2002).
3. Hubbell, J. A., Massia, S. P. & Drumheller, P. D. Surface-grafted cell-binding peptides in tissue engineering of the vascular graft. *Ann N Y Acad Sci* **665**, 253-8 (1992).
4. Drumheller, P. D. & Hubbell, J. A. Polymer networks with grafted cell adhesion peptides for highly biospecific cell adhesive substrates. *Anal Biochem* **222**, 380-8 (1994).
5. Kuhl, P. R. & Griffith-Cima, L. G. Tethered epidermal growth factor as a paradigm for growth factor-induced stimulation from the solid phase. *Nat Med* **2**, 1022-7 (1996).
6. Cook, A. D. et al. Characterization and development of RGD-peptide-modified poly(lactic acid-co-lysine) as an interactive, resorbable biomaterial. *J Biomed Mater Res* **35**, 513-23 (1997).
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9. Milner, S. T. Polymer brushes. *Science* **251**, 905-914 (1991).
10. Mendelsohn, J. D., Yang, S. Y., Hiller, J., Hochbaum, A. I. & Rubner, M. F. Rational design of cytophilic and cytophobic polyelectrolyte multilayer thin films. *Biomacromolecules* **4**, 96-106 (2003).
11. Banerjee, P., Irvine, D. J., Mayes, A. M. & Griffith, L. G. Polymer latexes for cell-resistant and cell-interactive surfaces. *J Biomed Mater Res* **50**, 331-9. (2000).
12. Irvine, D. J., Mayes, A. M. & Griffith, L. G. Nanoscale Clustering of RGD Peptides at Surfaces Using Comb Polymers. 1. Synthesis and Characterization of Comb Thin Films. *Biomacromol.* **2**, 85-94 (2001).
13. Irvine, D. J. et al. Comparison of tethered star and linear poly(ethylene oxide) for control of biomaterials surface properties. *J Biomed Mater Res* **40**, 498-509. (1998).
14. Irvine, D. J., Ruzette, A. V., Mayes, A. M. & Griffith, L. G. Nanoscale clustering of RGD peptides at surfaces using comb polymers. 2. Surface segregation of comb polymers in polylactide. *Biomacromolecules* **2**, 545-56 (2001).
15. Patel, N. et al. Spatially controlled cell engineering on biodegradable polymer surfaces. *Faseb Journal* **12**, 1447-1454 (1998).
16. Palecek, S. P., Loftus, J. C., Ginsberg, M. H., Lauffenburger, D. A. & Horwitz, A. F. Integrin-ligand binding properties govern cell migration speed through cell-substratum adhesiveness. *Nature* **385**, 537-40 (1997).