Lecture 3: Degradable Materials with Biological Recognition

Last time:	Theory of hydrolytic polymer erosion Enzymatic degradation of polymers Designing Biodegradable Macromolecules
Today:	Biological recognition *in vivo* Engineering biological recognition of biomaterials: cell adhesion/migration
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.

Biological Recognition *in vivo*

Interactions of cells with their environment at the molecular level

ECM = extracellular matrix

Motivation:

- Cell interactions with simple synthetic materials are governed by nonspecific interactions:
  - e.g. surface energies; hydrophobic interactions, charge-charge interactions
  - ***DRAW OXIDE SURFACE, POLYMER SURFACE***
- …but this is not how cells interact with ECM
- Cells use receptor-receptor/receptor-ligand interactions to guide their functions, including:
  - Adhesion, migration
  - Growth
  - Differentiation
    - Secretion of molecules
    - Binding of molecules
    - Specialized functions

Functions of ECM:

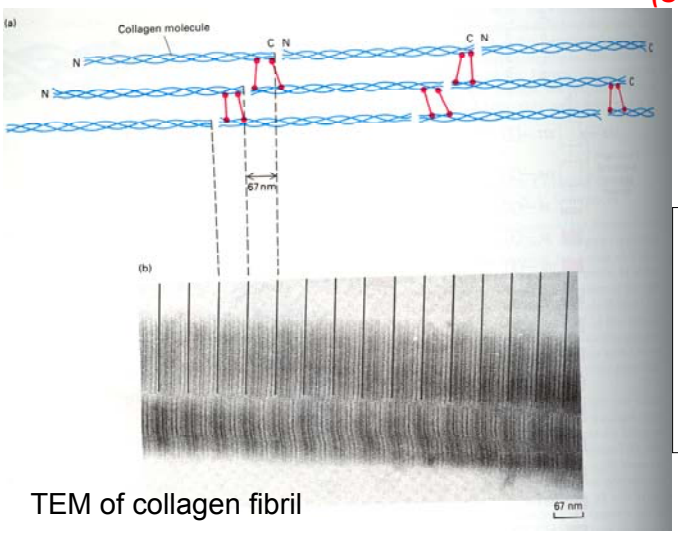
- Mechanical Support
- cues for cell survival/function
  - anchorage-dependent cell growth
  - differentiation cues
- organization of tissue
  - control of tissue morphology, localization of cell types

Structure of native ECM scaffold

Prototypical soft tissue ECM (varies from tissue to tissue):

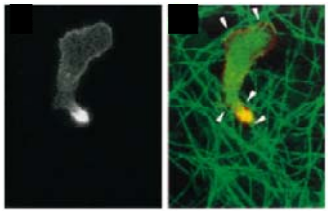
- structural fibers
  - collagen
  - other fibers?

(SLIDE)

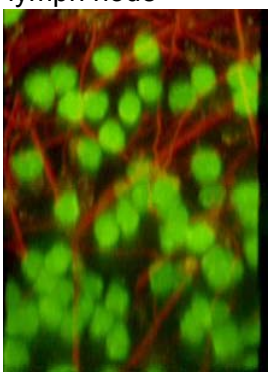


TEM of collagen fibril

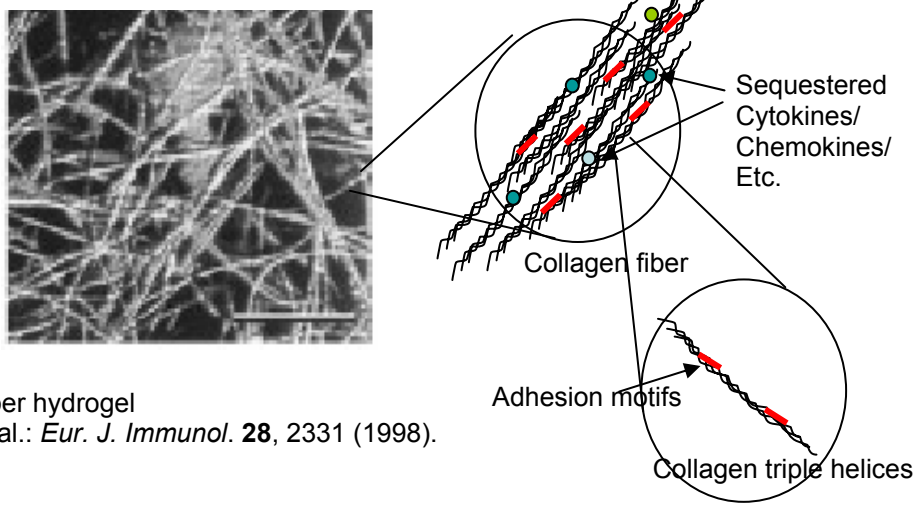
collagen gel



reticular network of lymph node



- generalized matrix assembly:
  - fibrils assemble into fibers
    - fibers may be organized or isotropic, and form tight (~10 nm separation between fibers) or open (20-30 μm between fibers) meshes
  - adhesion proteins 'decorate' fibers
  - other signals (cytokines, etc.) may also be sequestered on fiber surfaces

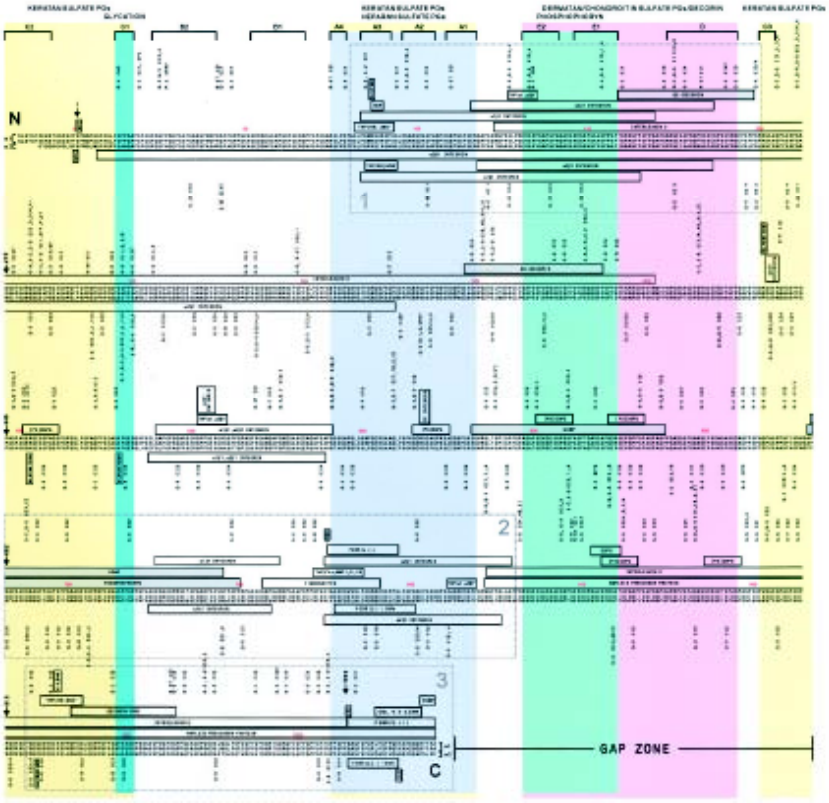


Collagen fiber hydrogel
P. Friedl et al.: *Eur. J. Immunol*. **28**, 2331 (1998).

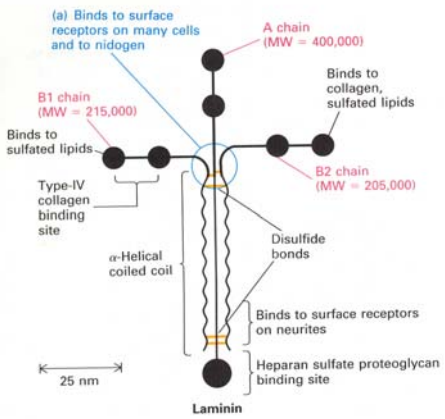
- Other major matrix-structure proteins:
  - Elastin

- Key length scales:
  - diameter of collagen fibrils: 50-200 nm
  - diameter of collagen fibers: 0.5-5 μm
  - diameter of collagen triple helices:
  - diameter of of collagen chain:
  - length of collagen triple helix: 300 nm
- Adhesion proteins
  - Complexity of adhesion proteins ***(SLIDE)***

Collagen I fibril ligand binding site map[1]



laminin structure

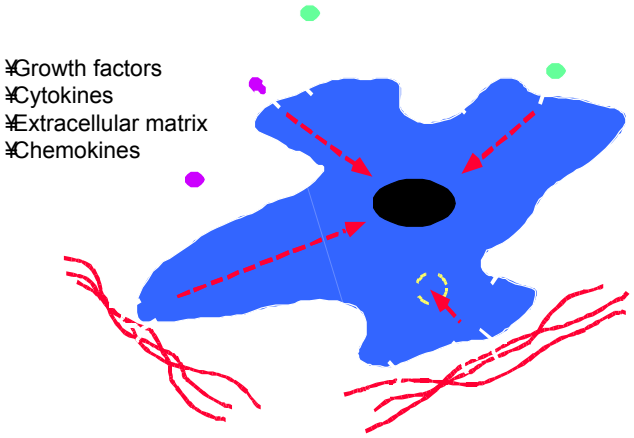


(from Lodish)

  - Adhesion proteins designed to bind to structural ECM components, and present binding sites to receptors
  - Adhesion proteins can present multiple binding sites for different receptors that work in synergy

Interactions of cells with native ECM

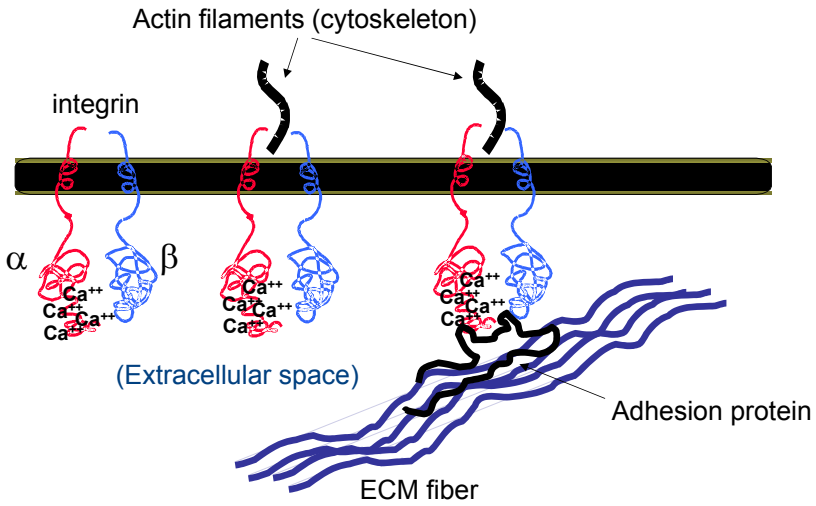
- Signals from the extracellular environment:



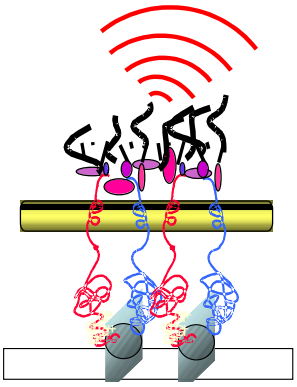
DRAW ON BOARD:

Cell adhesion: integrin-mediated cell-ECM interactions

- Cells interact with specific adhesion motifs in adhesion proteins via cell surface receptors; the microsctructure of ECM protein arrangement and its composition can tune cell adhesion
  - Adhesion in turn regulates growth, differentiation, and migration
  - Major family of cell-ECM receptors: integrins
    - Composed of noncovalently-associated α and β chains



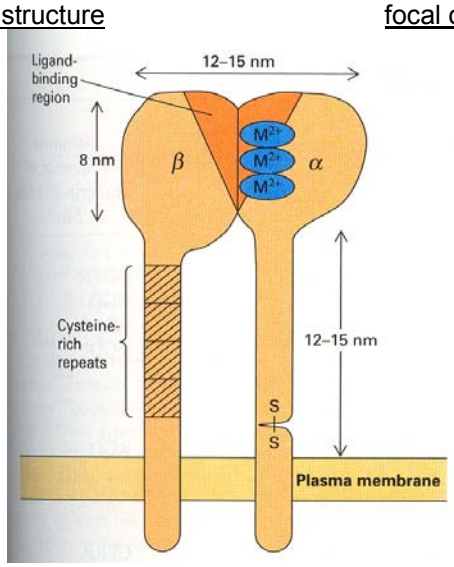
- Integrins and signaling
  - 'inside-out' signaling: biochemical signal triggers affinity change in integrins
  - focal contacts and signaling
    - integrin clustering drives actin filament assembly and can signal through multiple biochemical pathways, some of which synergize with growth factors to tell the cell 'where it is'



ON BOARD:

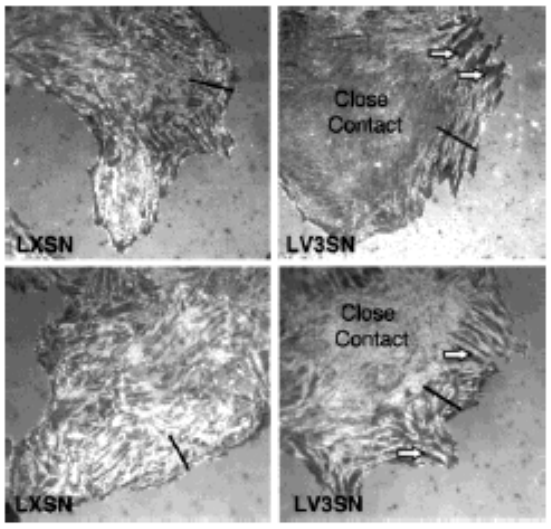
- Length scales in cell adhesion:
  - Size of integrins, focal contacts, relationship to cell size, fiber spacing ***DRAW ON BOARD***

Integrin structure

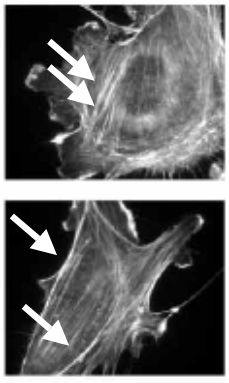


(Lodish)

focal contacts[2] (***SLIDE***)



Actin stress fibers



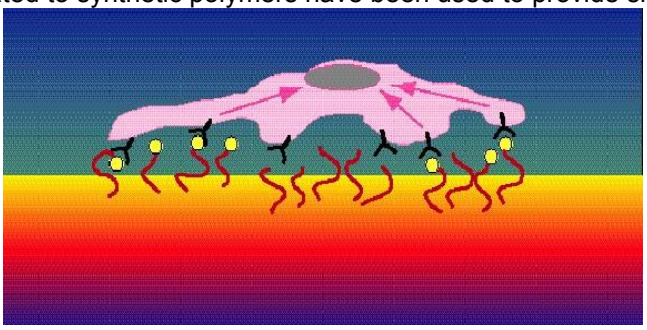
Interference reflection microscopy: dark spots indicate cell-substrate separations < 50 nm

(stress fibers pics from Maheshwari et al.)

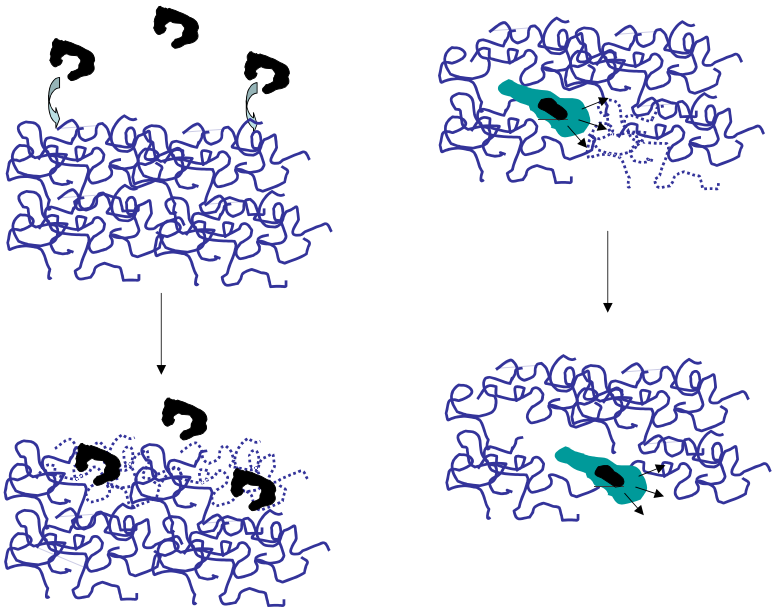
- 20 different integrins known, many different pairs possible with different ligand specificities, thus cell-specific adhesion can be modulated by ECM

Engineering Biological Recognition of Synthetic Degradable Polymers: Incorporation of peptides in synthetic polymers

- Lit on peptides in polymers[3-7]
- So far, we've focused on making degradable synthetic materials, and ignored biological recognition
  - How do we make materials that can interact in an engineered way with their biological environment?
  - ANSWER: incorporation of ECM cues
- Peptides have been introduced in synthetic polymers to provide scaffolds that appear less foreign and have some engineered response from cells
- Why use peptides instead of full proteins?
  1. Proteins fragile
  2. Proteins not soluble in organic solvents, but peptides often are
  3. Cost
  4. Immunogeneicity of peptides is less than complete protein sequences (reduce likelihood of provoking inflammatory response to devices)
- Peptide sequences conjugated to synthetic polymers have been used to provide signals for: ***(SLIDE)***



  - Adhesion
    - Fragments from ECM adhesion proteins
  - Remodeling
    - Short sequences recognized by remodeling enzymes
    - Support transformation of synthetic scaffolds into *de novo* natural matrix
    - Support cell migration through solid scaffolds



- Growth/differentiation
  - Peptide cytokines
- Other potential functions
  - Chemotaxis?: gradients of peptide attractants

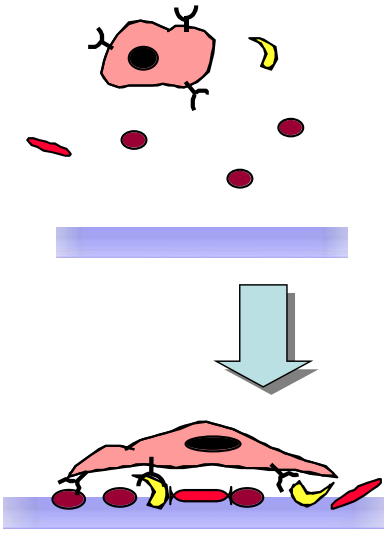
- What sizes are we talking about when we discuss peptides vs. proteins?
  - Ideal case are peptides of ~30 amino acids or less that can be prepared on a solid-phase synthesizer
  - This is usually more than adequate for adhesion peptides, enzyme-recognized peptides
  - Cytokines used on biomaterials may be slightly larger (30-60 amino acids total MW~5K g/mole), but may also be produced efficiently in mass quantities
    - These peptides sometimes have some folding or intra-chain bonding that is not reproducible with shorter peptide sequences
- We'll discuss approaches to incorporating peptides in biomaterials as we go through the representative applications:

1. **cell adhesion**
2. **matrix remodeling**
3. **cytokine signaling**

Recognition of Biomaterials by Adhesion Receptors: Controlling Cell Adhesion on Degradable Polymers

Paradigm of Cellular responses to synthetic biomaterials

- Proteins adsorb ***(SLIDE)***
- Cells respond to adsorbed protein layer
  - Why is this an issue?
    1. Adsorbed layer often unstable and reconstructs as environment changes
       - Vroman effect -> protein exchange
    2. Cells may adhere poorly to this surface
    3. Proteins denatured/presented in non-native conformations ***DRAW ON BOARD***
       - Non-native signals transmitted that are not readily controlled
       - Immunogenic epitopes may be generated

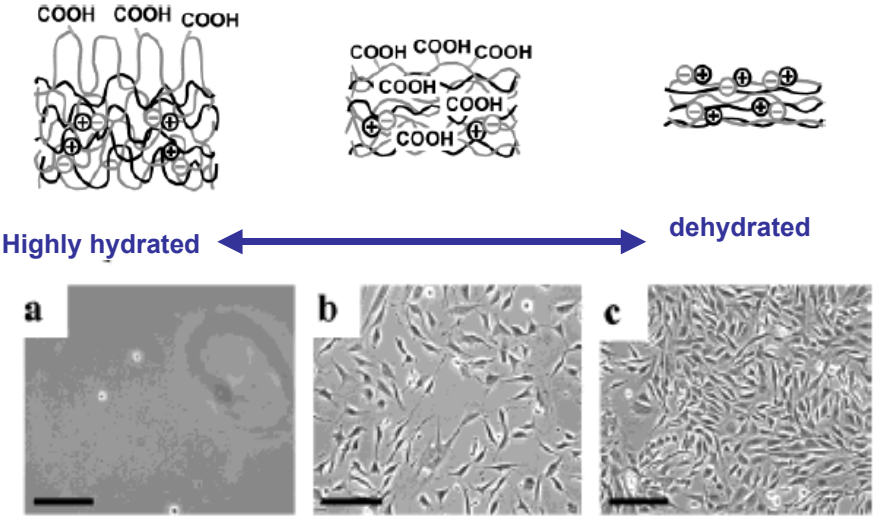


Protein-resistant surfaces[8,9]

- ONLY LANGMUIR ADSORPTION COVERED IN 3.051J
- 1st order: present peptide fragments from adhesion proteins
- more control, perhaps better: present peptide fragments from adhesion proteins on 'inert' background
    - modify material to resist protein adsorption
    - present peptides on this 'blank slate' background
- resisting protein adsorption
    - current hypotheses: achieve a surface structure with some or all of these characteristics:
        - water-rich surface layer – reduce protein-surface interactions
        - dynamics chains at surface – steric interference with adsorption
    - SCHEMATIC VIEW OF HOW PROTEIN RESISTANCE IS ACHIEVED
    - molecules known to provide protein resistance: (essentially, the most hydrophilic nonionic polymers)
        - poly(ethylene glycol)
            - most water-soluble non-ionic synthetic polymer
            - fastest chain dynamics of any polymer in water
            - can be synthesized with good control to many molecular weights and incorporate end-functional groups
            - reminder: PEG = PEO; nomenclature MW < 20K is called PEG
        - dextran
    - note that resisting protein adsorption may not *always* be necessary for eliminating nonspecific cell adhesion
- mechanically soft surfaces – work of Rubner lab[10]

(SLIDE)

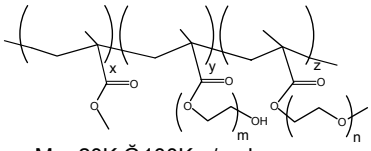
Polyelectrolyte multilayers (Rubner lab):



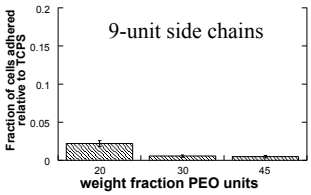
- poly(acrylic acid)/poly(allyl amine) multilayers with varying charge density- thus loopiness and swelling

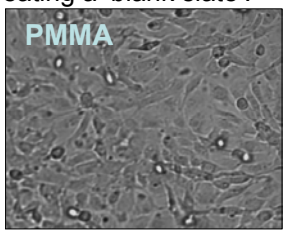
- Achieving controlled cell adhesion in practice: 1 method
  - Incorporation of poly(ethylene glycol) at surface with adhesion peptides

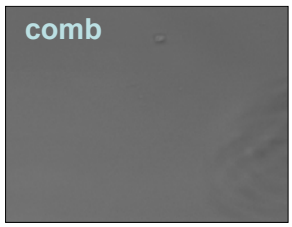
Creating a 'blank slate':[11-14] ***(SLIDE)***

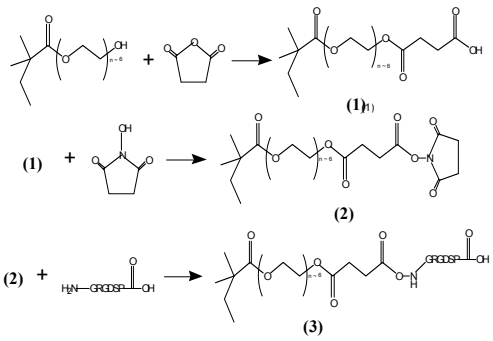


M = 20K Ğ 100K g/mol

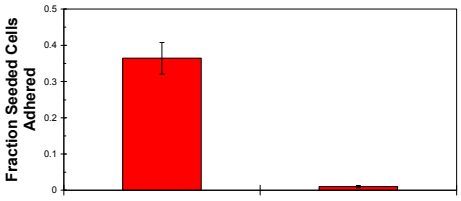


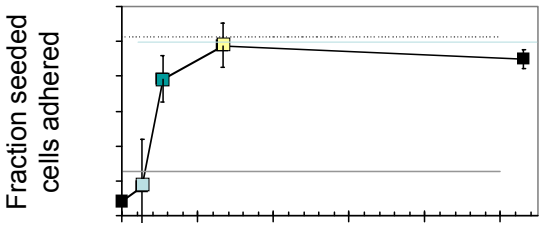


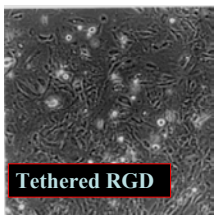


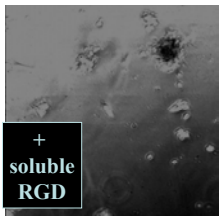


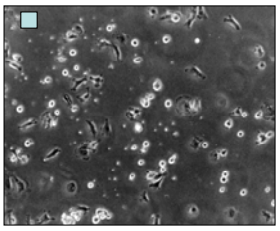
now add adhesion peptides: (Here, Arg-Gly-Asp-based peptide)

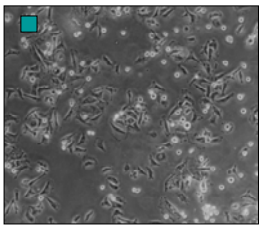


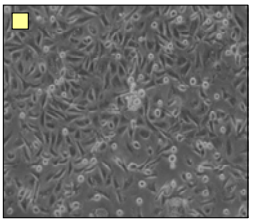












- **concept of soluble peptide control**: integrin-mediated attachment is low-affinity but high avidity: many receptors binding on and off relatively quickly

Biophysical Effects of Cell Adhesion Peptides

- controlling the physical distribution of cells (work of Shakesheff)[15]

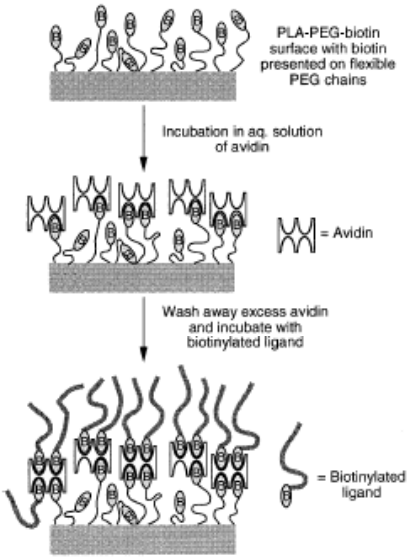


Figure 1. Schematic representation of the surface engineering of PLA-PEG-biotin. Biotin moieties presented at the polymer surface are used to immobilize tetrameric avidin molecules. Free biotin binding sites on the avidin molecules are in turn used to anchor biotinylated ligands. All steps in the surface engineering are performed in aqueous environments.

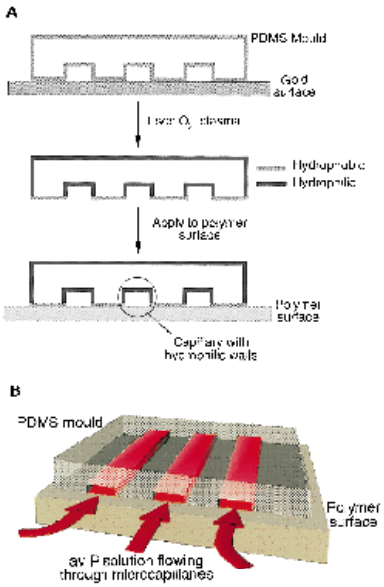


Figure 2. *A)* The microfluidic patterning technique requires treatment of the PDMS mold with an O_2 plasma. This treatment increases the hydrophilicity of any PDMS surface that is not protected by the gold surface. Transferring the treated mold to the PLA-PEG-biotin surfaces produces capillaries with hydrophilic walls. Avidin solution flow across the PLA-PEG-biotin surface is restricted to the capillary regions by the hydrophobic regions of the mold base. *B)* Schematic representation of the microfluidic patterning technique.

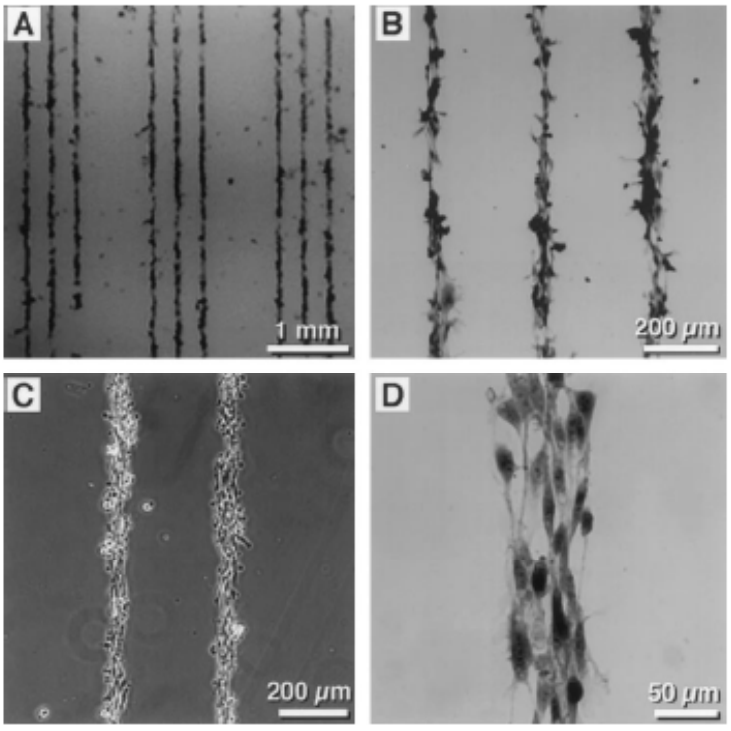
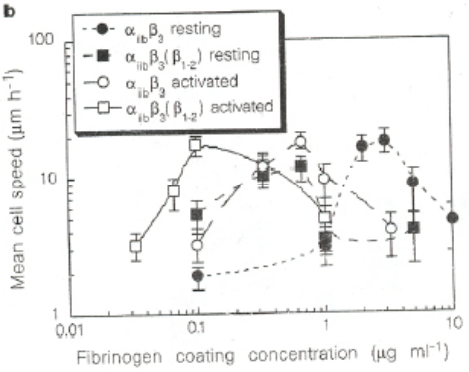


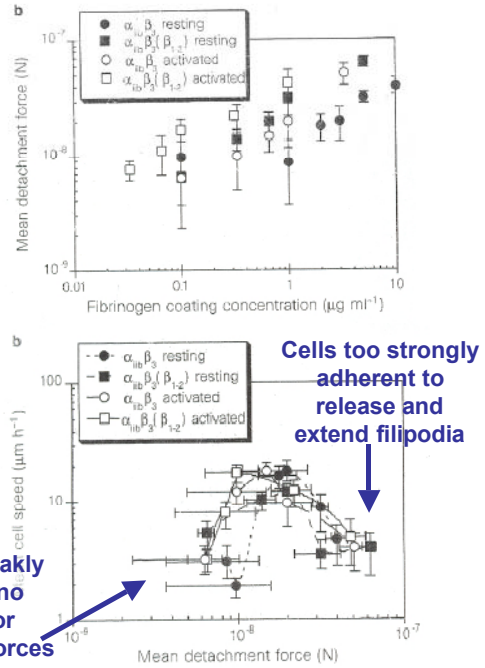
Figure 4. Spatially controlled adhesion and spreading of bovine aortic endothelial cells on 70 and 50 μm-wide lines containing RGD peptides. Panels *A, B, D* are transmission images. Panel *C* is a phase contrast image.

- Cell adhesion ligand density effects on cell migration[16]

Cell migration on fibronectin-coated substrates:



Lauffenburger lab



Cells too strongly adherent to release and extend filipodia

Cells too weakly adherent; no traction for mechanical forces

- Clustering effects

DRAW ON BOARD:

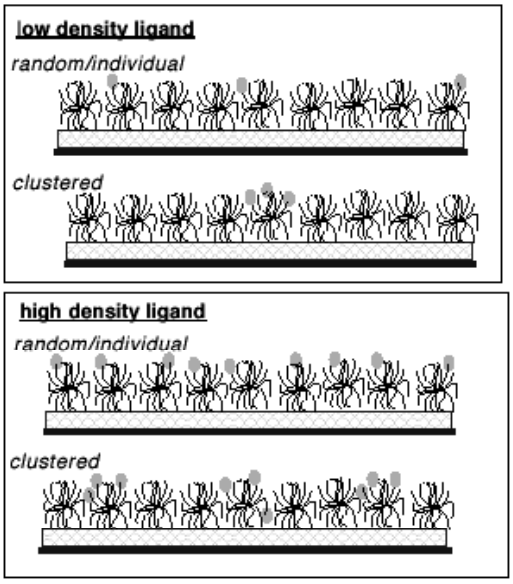


Fig. 1. Schematic illustration of star polymer as a tether to present ligand (shaded oval) in a manner in which the total average concentration (top versus bottom) and the spatial distribution, from homogeneous to highly clustered (left to right), can be independently varied.

- PEO surface modified with PEO star polymers: CONTROL OF ARCHITECTURE FOR CONTROL OF LIGAND PRESENTATION

(SLIDE)

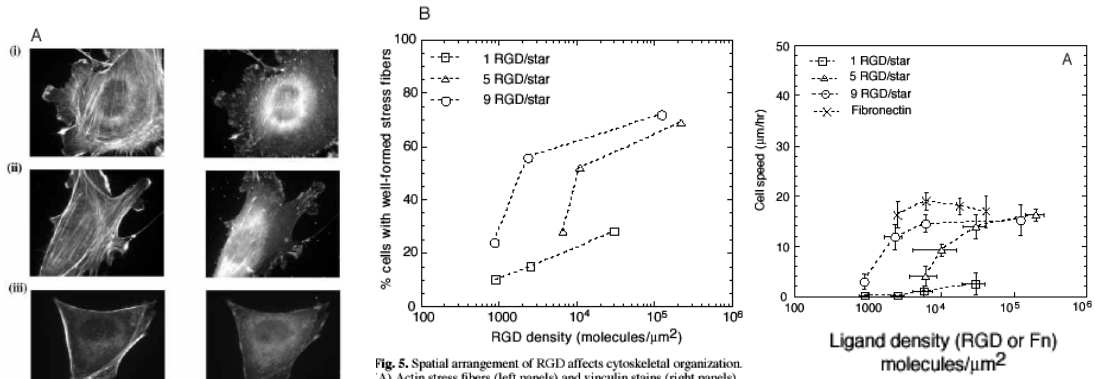


Fig. 5. Spatial arrangement of RGD affects cytoskeletal organization. (A) Actin stress fibers (left panels) and vinculin stains (right panels) were visualized at varying RGD densities and cluster sizes. Shown here are typical stains from cells plated on surfaces coated with (i) 1 μg/ml fibronectin, (ii) 100% RGD-modified star PEO molecules with n_{cl}=9 and (iii) 100% RGD-modified star PEO molecules with n_{cl}=1. (B) Cells were scored for actin stress fiber formation with varying RGD spatial arrangements. Approximately 140 cells from three independent experiments were scored for each condition.

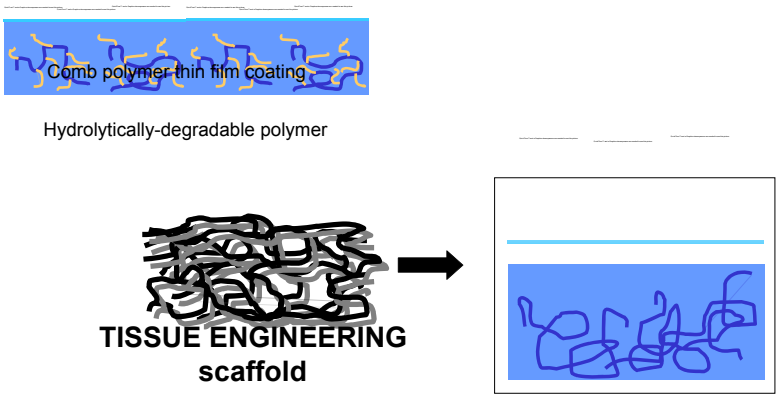
- Utility of a branched polymer architecture

- self-assembled monolayers
  - K.L Prime and G.M. Whitesides , *J. Am. Chem. Soc.*, **115**, 10714 (1993)
- end-grafted polymers
  - C.G. Golander et al., in *Poly(Ethylene Glycol) Chemistry: Biotechnical and Biomedical Applications*
- adsorbed polymers
  - D. Gingell and N. Owens, *J. Biomed. Mat. Res.*, **28**, 491 (1994)
- plasma discharge coating
  - G.P. Lopez et al., *J. Biomed. Mat. Res.*, **26**(4), 415 (1992)
- hydrogels
  - J.P. Bearinger , D.G. Castner , S.L. Golledge , A. Rezanio , S. Hubchak , and K.E. Healy, *Langmuir* , **13**, 5175 (1997)

Example peptides used to modulate cell adhesion on biomaterials

Peptide sequence	Derived from	Conjugate receptor	Role	References
IKVAV	Laminin α-chain	LBP110 (110 KDa laminin binding protein)	Cell-ECM adhesion	J. Biol. Chem. 264, 16174 (1989)
RGD	Laminin α-chain, fibronectin, collagen	Multiple integrins	Cell-ECM adhesion	J. Cell Physiol. 146, 451 (1991)
YIGSR	Laminin β1-chain	$\alpha_1\beta_1$ and $\alpha_3\beta_1$ integrins	Cell-ECM adhesion	Cell 48,989 (1987); Arch. Biochem. Biophys. 272, 39 (1989); J. Biol. Chem. 268, 8053 (1993)
RNIAEIIKDI	Laminin γ-chain	unknown	Cell-ECM adhesion	FEBS Lett. 244, 141 (1989)
HAV	N-cadherin	N-cadherin	Cell-cell adhesion	Dev. Biol. 139, 227(1990)
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	

- Applying the 'comb polymer' approach on biodegradable materials:



  - This general approach has also been applied to fully biodegradable polymers
    - use peptide or side chain-bearing ring monomers to create biodegradable backbones with PEG side chains that can be functionalized (not trivial)

References

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).
7. Mann, B. K., Schmedlen, R. H. & West, J. L. Tethered-TGF-beta increases extracellular matrix production of vascular smooth muscle cells. *Biomaterials* **22**, 439-44 (2001).
8. de Gennes, P. G. Conformations of polymers attached to an interface. *Macromolecules* **13**, 1069-1075 (1980).
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).