Analysis & Manipulation of Adhesion Receptors & ECM Ligands

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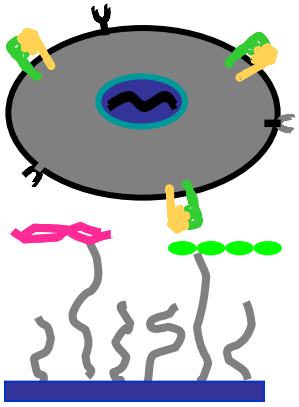
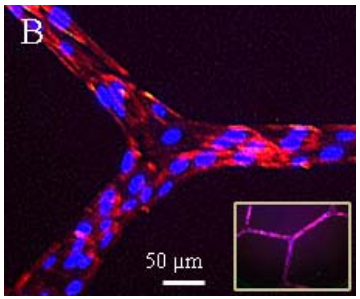
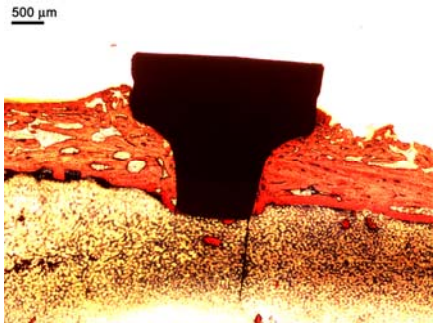
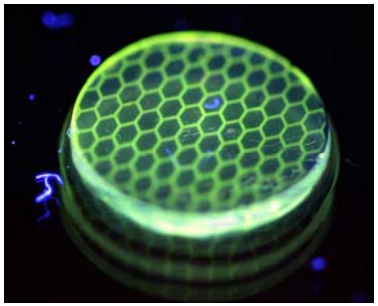
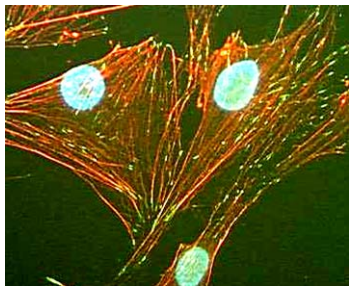










Cellular & Biomaterials Engineering Laboratory

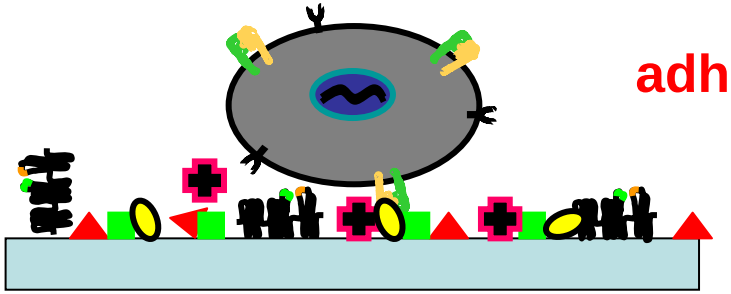
Outline – Cell Adhesion

- Motivation – Why is cell adhesion important?
- Mechanisms of cell-material interactions
- Cell adhesion - Analysis
  - adhesion as a mechanical process
  - downstream adhesive signaling
- Cell adhesion - Strategies to exploit adhesion
- Considerations

Cell Adhesion - Motivation

Tightly regulated, dynamic biological process

- central to physiological & pathological processes
- critical to biomedical & biotechnological appl.
  - host responses to implanted devices
  - tissue engineering constructs
  - cell arrays, culture supports for biotech applications
  - cell chromatography / cell targeting
- adhesive interactions
  - anchorage (migration, tissue organization)
  - signaling (activation, survival, proliferation, differentiation)



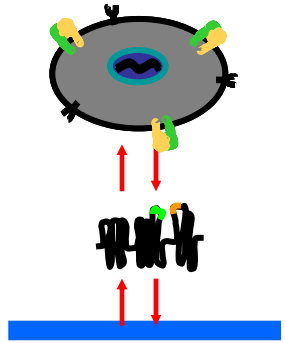
adhesion as signal transduction element

De Arcangelis & Georges-Labouesse (2000)
Garcia (2005)

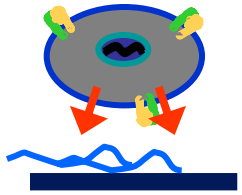
Cell-Material Interactions

Cell adhesion to synthetic & biological surfaces

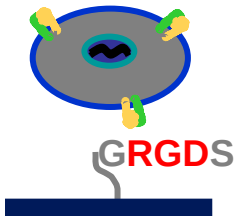
- **specific receptor interactions with adhesion proteins/motifs**



  - **proteins adsorbed from physiological fluids** (fibronectin, vitronectin, fibrinogen)



  - **ECM components present or deposited by cells** (fibronectin, collagen, laminin)

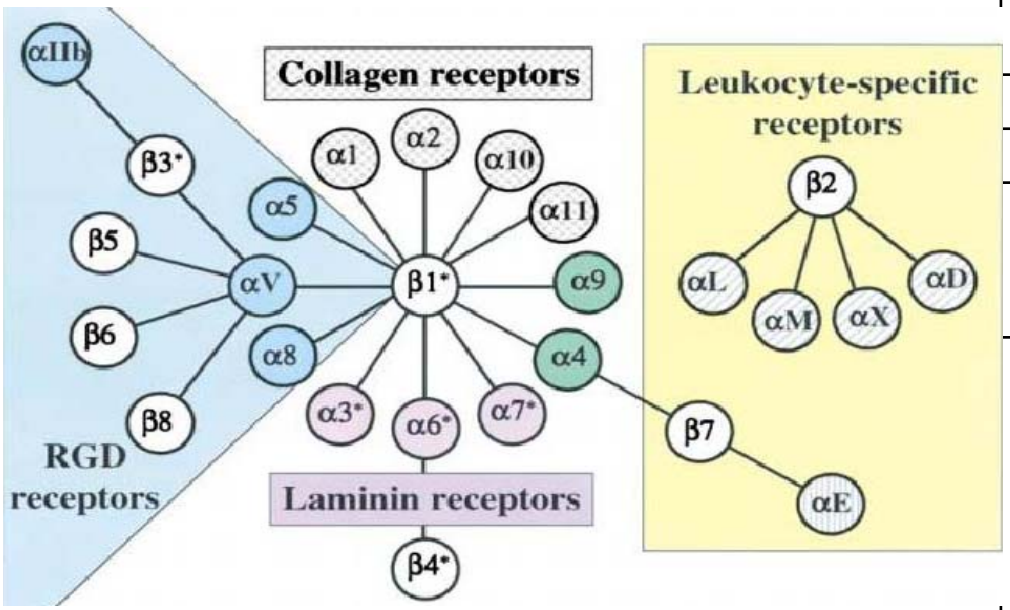


  - **biospecific sequences engineered on surfaces** (RGD, YIGSR)

- **interactions are highly dynamic**

Adhesion Receptors

- **receptor families**
  - **cadherins, selectins, HSPG, integrins, Ig superfamily**
- **integrins (αβ)**



$\alpha_5\beta_1$-FN: myoblasts, osteoblasts, chondrocytes, fibroblasts
$\alpha_2\beta_1$-Col: osteoblasts, chondrocytes, smooth muscle cells
$\alpha_6\beta_1$-LN: myoblasts, epithelial cells, neurons

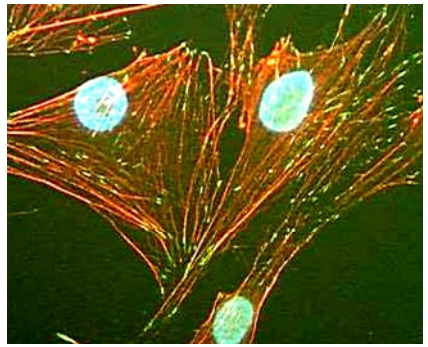
Common integrin-ligand paris

Integrin	Ligand	Binding Site
α1β1	Col-IV LN	CNBr frag. $\alpha_1(IV)_2$ E1-4, P1
α2β1	Col-I	GFOGER (triple helix)
α3β1	LN Tsp	E3, GD6 peptide TSP-768
α4β1	FN OPN	IIICS (EILDV, REDV) Hep II (IDAPS)
α5β1	FN	RGD + PHRSN
α6β1	LN	E8
αIIbβ3	Fg FN VN vWF	RGD (α); KQAGD (γ) RGD RGD RGD
αVβ3	FN VN Fg vWF Tsp OPN BSP TN Col (non fib.)	RGD RGD RGD RGD RGD RGD RGD RGD RGD
αMβ2	Fg C3bi F-X	P2

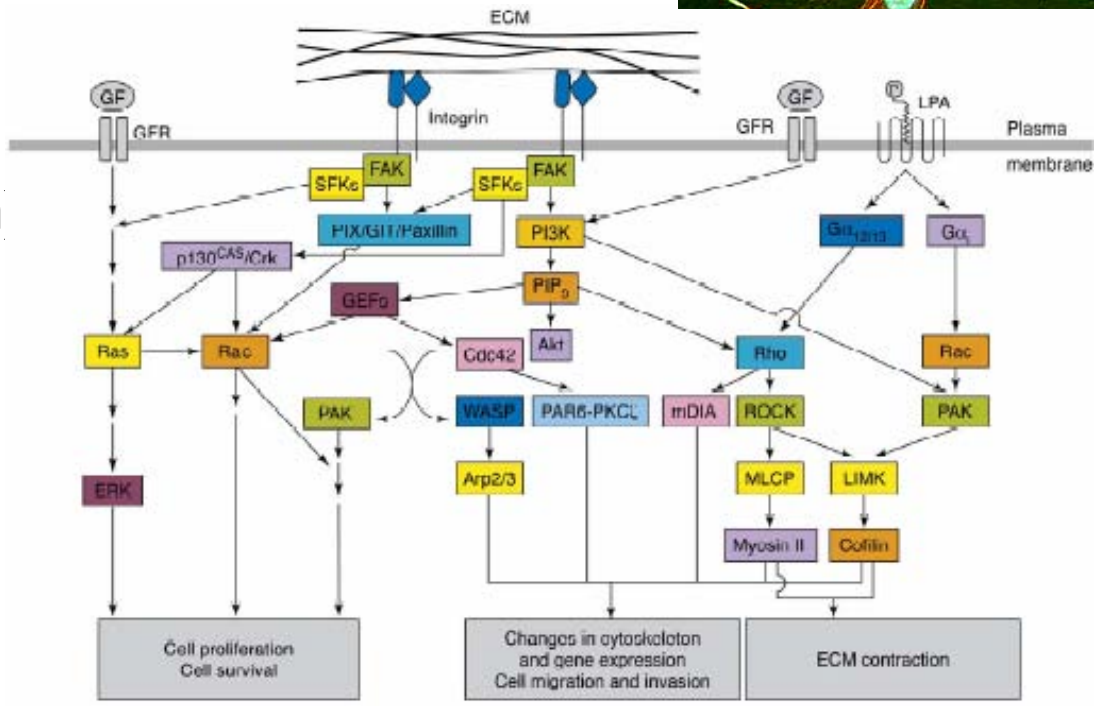
Hynes *Cell* (2002)

Cell Adhesion Process

- **complex biochemical/mechanical process**
  - **integrin binding**
  - **association with actin cytoskeleton**
  - **integrin clustering**
  - **focal adhesion assembly**



- **focal adhesions**
  - **structural proteins**
    (vinculin, talin, α-actinin
  - **signaling complexes**
    (FAK, src, fyn)

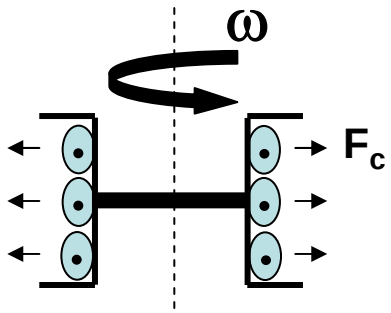


integrated adhesive & growth factor receptor signaling directing signaling responses

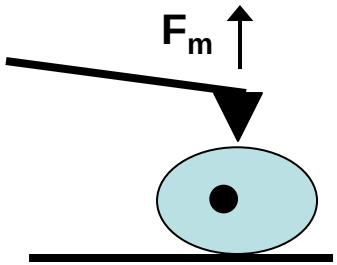
Larsen + Curr Opin Cell Biol (2006)

Cell Adhesion: Measurement

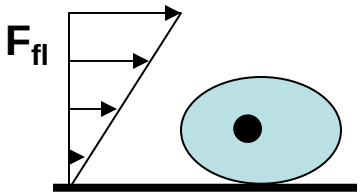
- **Implicit assays – spreading & migration assays**
- **Qualitative "wash" assays**
- **Quantitative assays**
  - **centrifugation**



  - **micromanipulation – AFM, micropipet, laser tweezers**



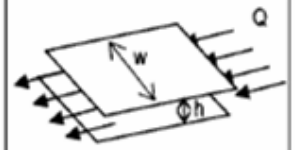
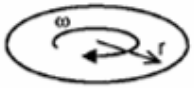
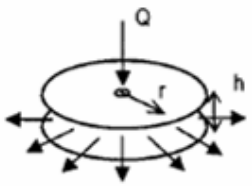
  - **hydrodynamic flow**



Garcia & Gallant (2003)

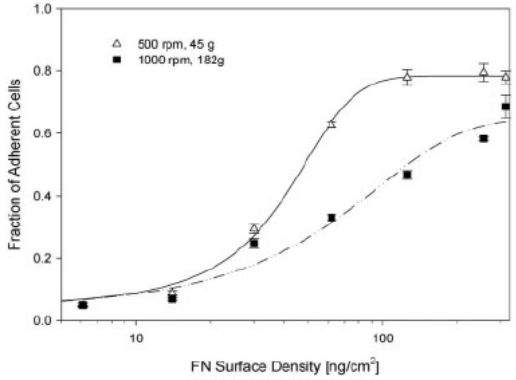
Adhesion Assays

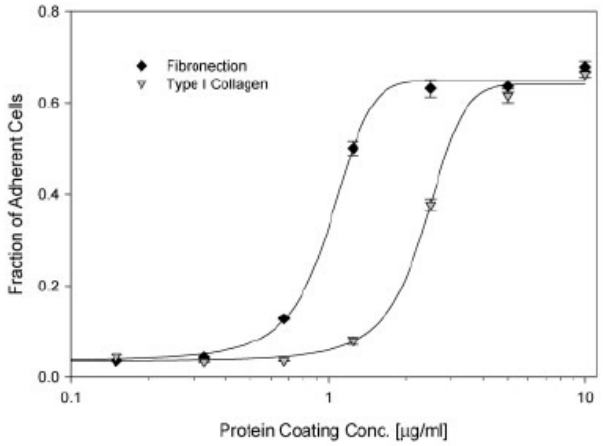
Garcia & Gallant (2003)

Assay	Configuration/Principle	Applied Force	Advantages	Disadvantages
Wash	wash off "non-adherent" cells	uneven/unknown	+ simple/convenient + widely used	- reproducibility - sensitivity - limited to short term adhesion
Micromanipulation	apply force with micropipette, microprobe, AFM cantilever, or laser tweezers	directly obtained from force transducer or calibrated deflections (F_{max} approx. 10 nN)	+ sensitive real-time force-displacement measurements + control over loading protocol	- limited to receptor-ligand binding - specialized equipment - single cell measurements
Centrifugation	apply centrifugal (normal) force using conventional centrifuge	$F = V\,d\,R\,\omega^2$ V = cell volume d = cell density – media density ω = centrifugation speed R = centrifugation radius ($F_{max} < 10^{-3}$ dynes/cell)	+ simple/convenient + population-averaged measurements	- single applied force per run - low applied forces/limited to short term adhesion
Hydrodynamic flow	shear forces generated by fluid flow over adherent cells	proportional to wall shear stress (τ_w)	+ reproducible/controlled forces + population-averaged measurements	- specialized flow cells - detachment forces depend on cell morphology - flow validation required for high flow rates
	parallel plate 	$\tau_W = \frac{6\mu Q}{w h^2}$ Q = flow rate w = channel width h = channel height μ = fluid viscosity ($\tau_{w\,max} < 120$ dyne/cm^2)	+ direct observation of attachment/detachment process + flow conditions can be readily validated	- single force per experiment - low applied forces/limited to short term adhesion
	spinning disk 	$\tau_W = 0.8 r \sqrt{\rho \mu \omega^3}$ ω = rotational speed r = radial position ρ = fluid density μ = fluid viscosity ($\tau_{w\,max} < 2500$ dyne/cm^2)	+ linear gradient of applied forces + wide range of applied forces + uniform chemical conditions at surface	- validation of flow patterns required - end point assay
	radial flow 	$\tau_W = \frac{3\mu Q}{\pi r h^2}$ Q = flow rate r = radial position h = gap height μ = fluid viscosity ($\tau_{w\,max} < 600$ dyne/cm^2)	+ gradient of applied forces inversely proportional to radial position + direct observation of the attachment/detachment process	- validation of flow patterns required - complex hydrodynamic conditions at central flow impingement point

Cell Adhesion: Measurement

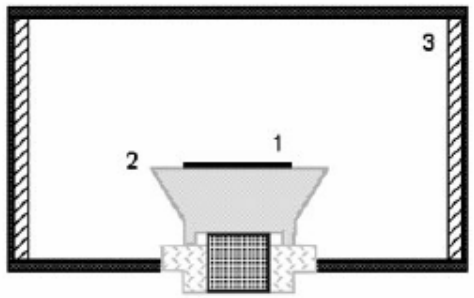
- **Centrifugation**



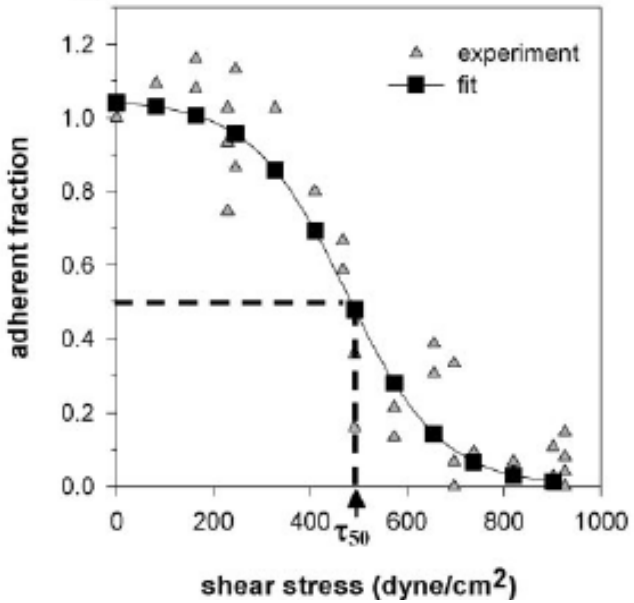


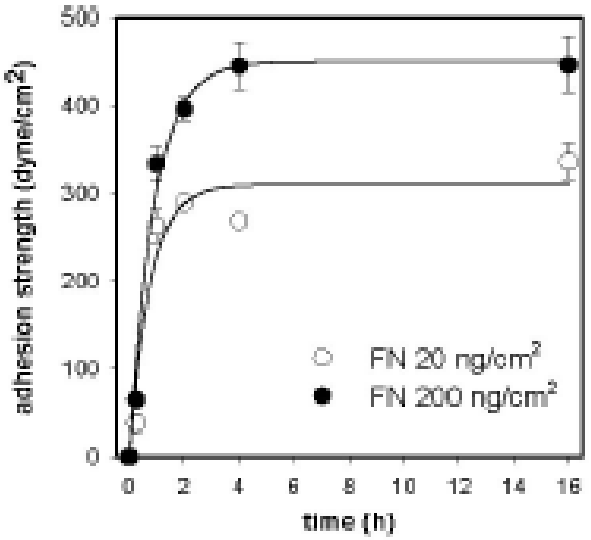
Reyes & Garcia (2003)

- **Spinning disk assay**



Garcia + (1997),
Gallant + (2005)





Cell Adhesion: Downstream Signals

- **cell spreading: morphology, FA assembly**
- **early signaling: FAK, PI 3-kinase, Src activation**
- **proliferation: DNA synthesis, cell number**
- **gene expression: RT-PCR, gene chips**
- **differentiation functional assays**

➢ **adhesive mechanisms: integrin & ECM components**

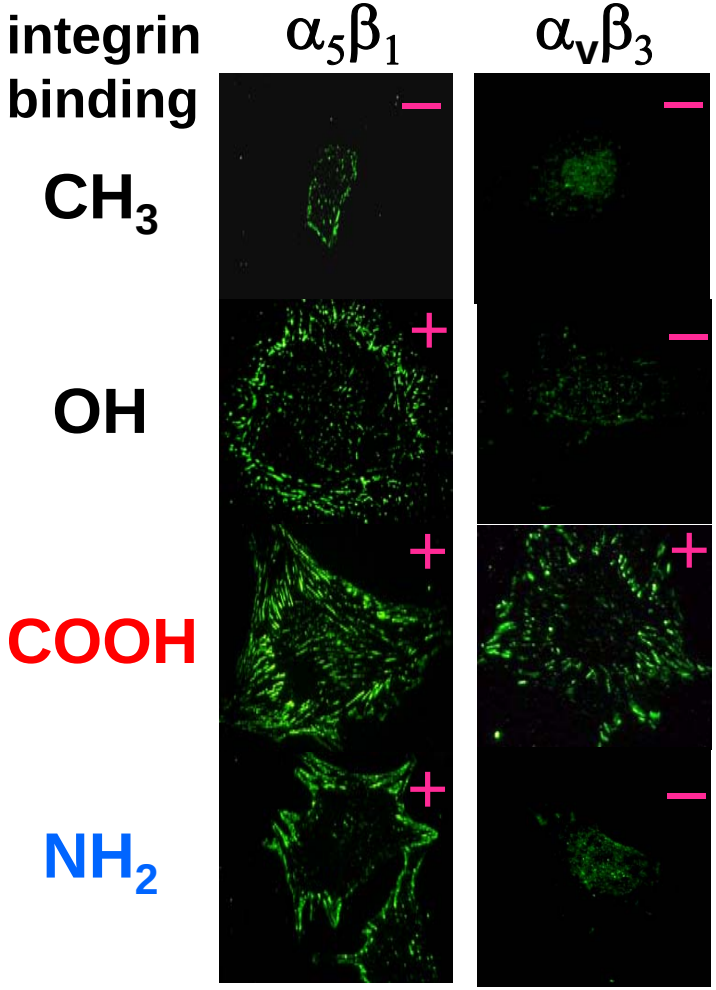
*** integrin binding**

*** function-perturbing expts: blocking AB, RNAi**

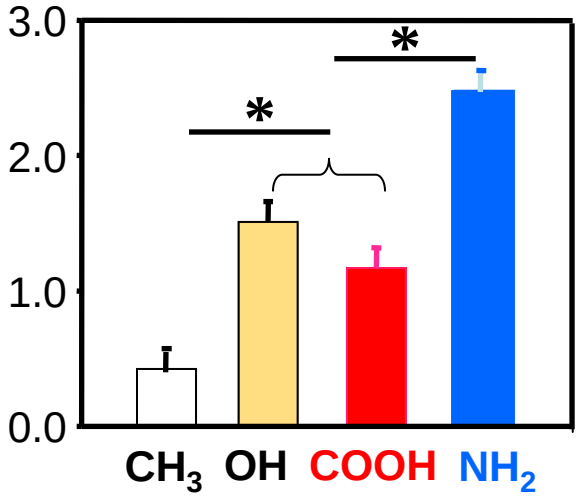
Integrin Binding Specificity Controls Cell Responses to Biomaterials

Surface chemistry modulates FN structure/activity to direct integrin binding & signaling

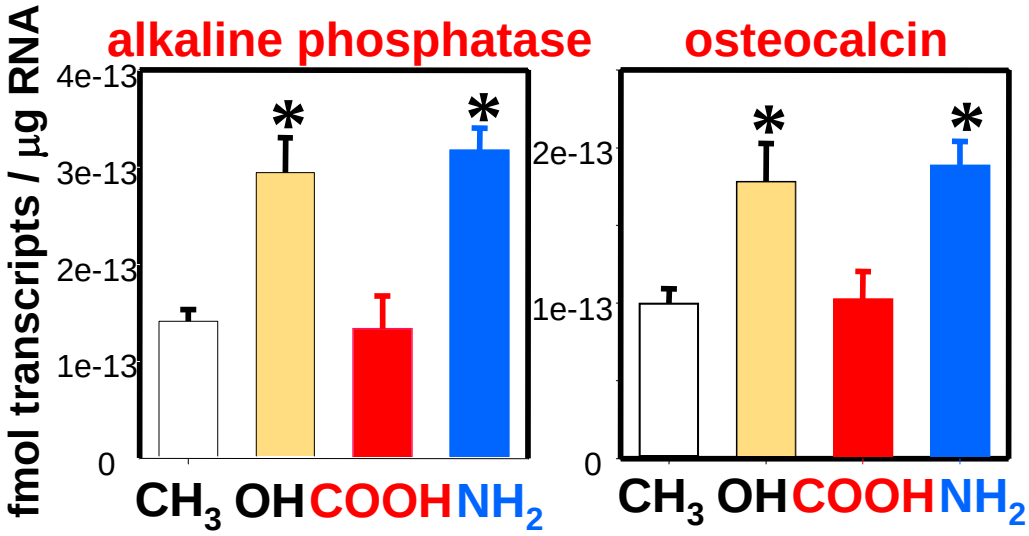
MC3T3-E1 cells, 40 ng/cm² FN-coated SAMs



pFAK [Y397] / total FAK



gene expression (7d)

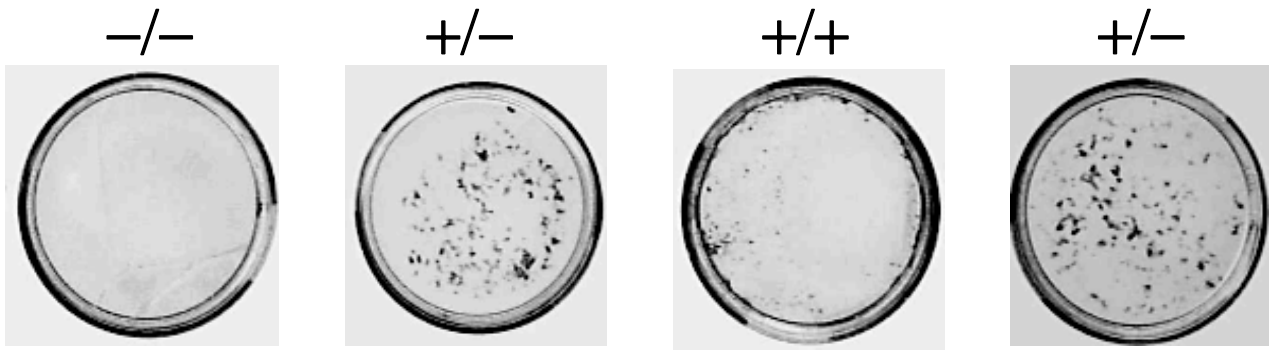


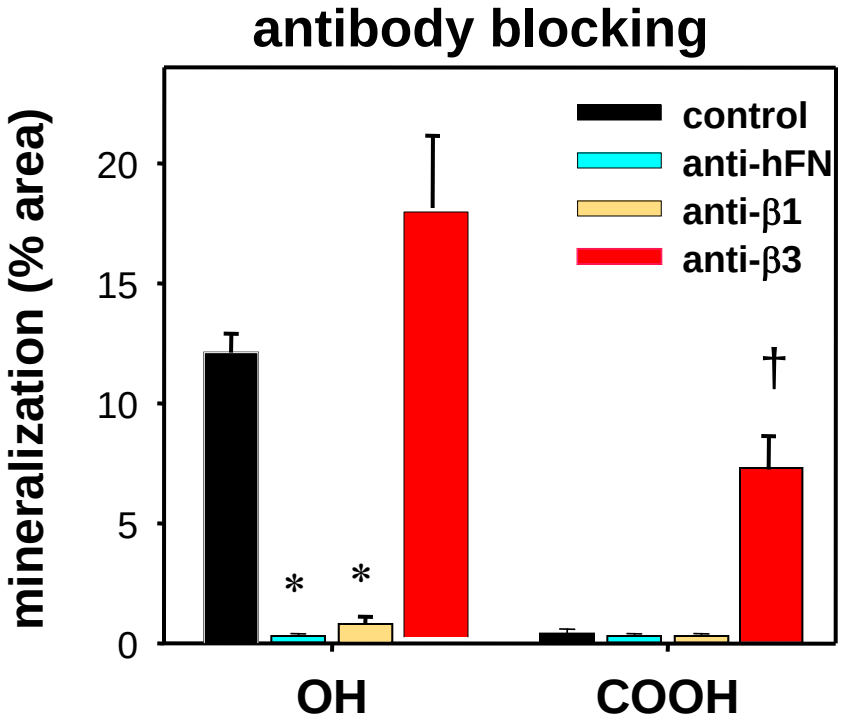
Keselowsky +, JBMR (2003); Biomaterials (2004)

Integrin Binding Specificity Controls Cell Responses to Biomaterials

$\alpha_5\beta_1/\alpha_v\beta_3$

MC3T3-E1 cells – 14 d
40 ng/cm² FN-SAMs





β_1 vs. β_3 binding to FN directs mineralization

→ other cell systems
skeletal myoblasts Lan + (2005)
neural stem cells Tate + (2004)

→ biomaterials to control integrin binding

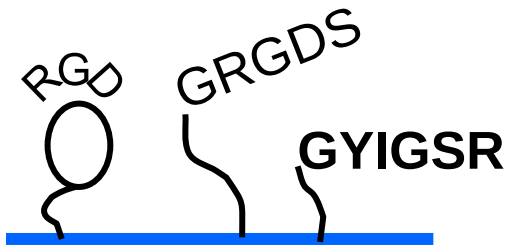
Keselowsky +, PNAS (2005)

Cell Adhesion: Manipulation

Bioadhesive materials

- **Goal: Direct cell adhesion (integrin binding)**
- **Strategy: present bioadhesive motifs**
  - **proteins, peptides**

→ functionalization of non-adhesive substrates with short adhesive motifs



integrin binding
cell adhesion & migration
proliferation & differentiation

+ biospecific
+ avoid other interactions
+ synthesis

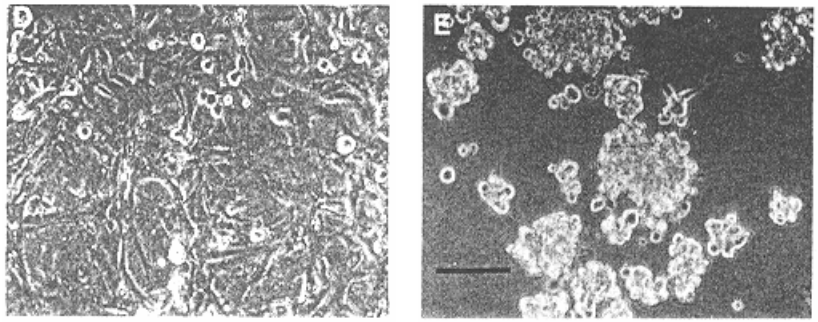
- loss of activity vs. ECM
- in vivo results disappointing
- known motifs

Lutolf & Hubbell (2005); Garcia (2005)

Bioadhesive Surfaces - RGD

RGD functionalization

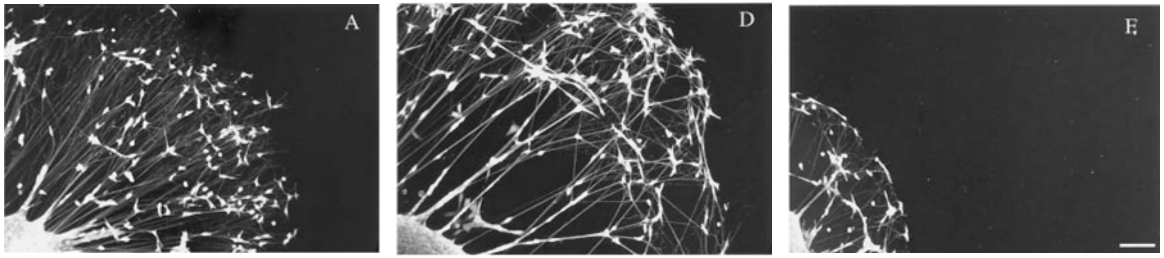
RGD-PTFE polymer (Massia & Hubbell, 1991)



RGD **control**

→ **cell adhesion**

RGD-fibrin scaffold (Schense & Hubbell, 2002)

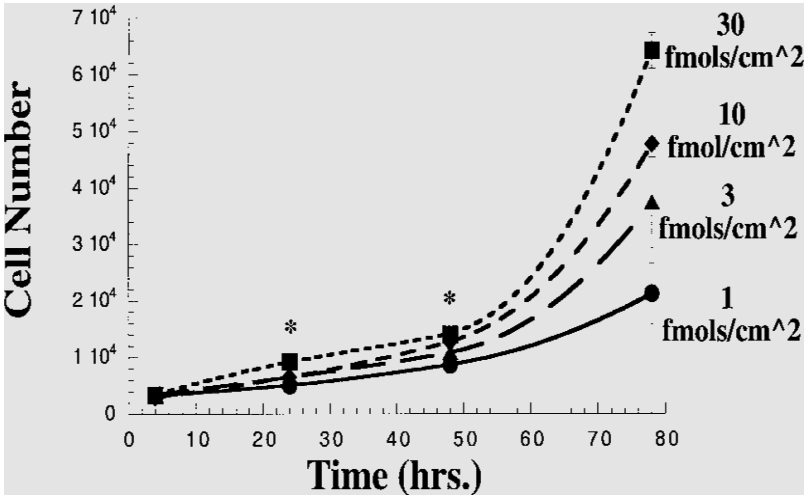


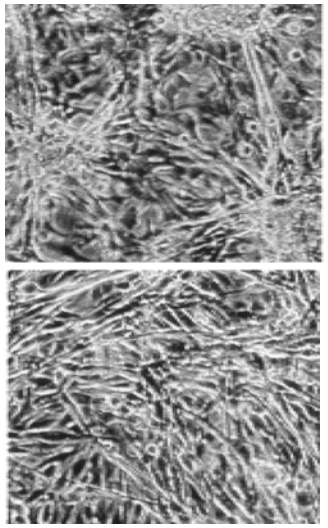
fibrin **fibrin low RGD** **fibrin high RGD**

→ **neurite outgrowth**

Bioadhesive Surfaces - RGD

– RGD-alginate surface (Rowley & Mooney, 2002)



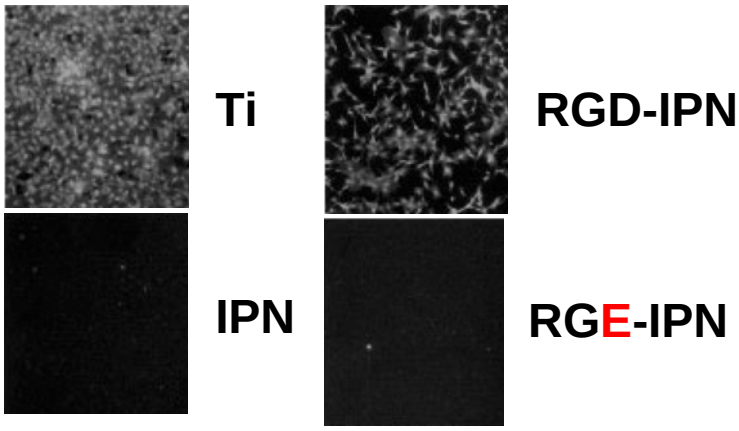


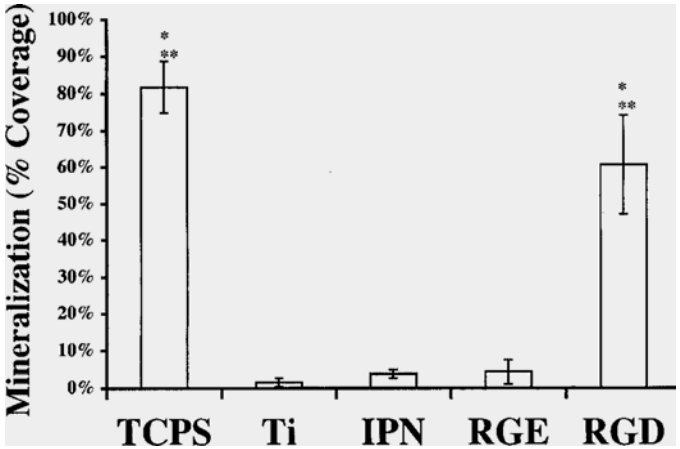
0.1 fmol/cm²

10 fmol/cm²

cell proliferation & differentation

– RGD-p(AAm-co-EG/AAc) IPN on Ti (Barber +, 2003)





Bioadhesive Surfaces - RGD

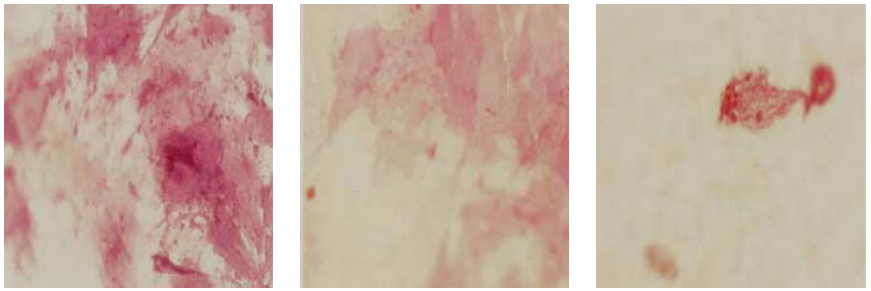
- **In vivo results with RGD-functionalized materials have been mixed**

+ cell targeting (tumor, vasculature)

+ wound healing (Mertz +, 1996)

– osseointegration (Schliephake +, 2002; Barber +, 2002)

- **RGD displays significant loss of activity when compared to FN**



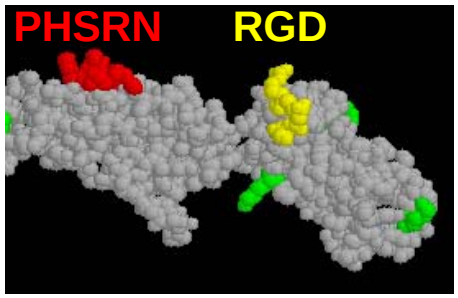
FN-PLA RGD-PLA PLA

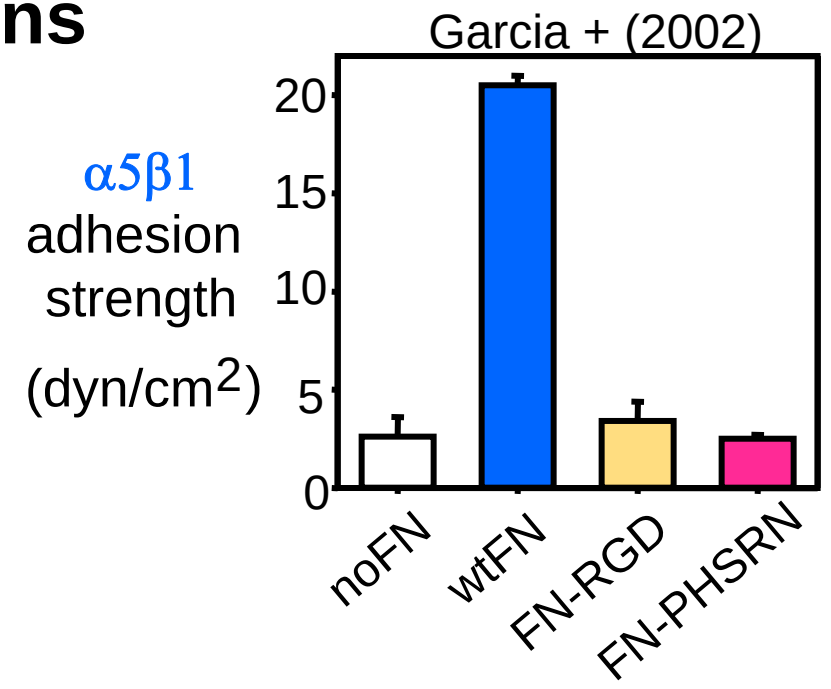
Yang et al. (2001)

Bioadhesive Surfaces - RGD

- **RGD-surfaces – limitations**
  - ➢ **loss of activity & accessory domains**

FN

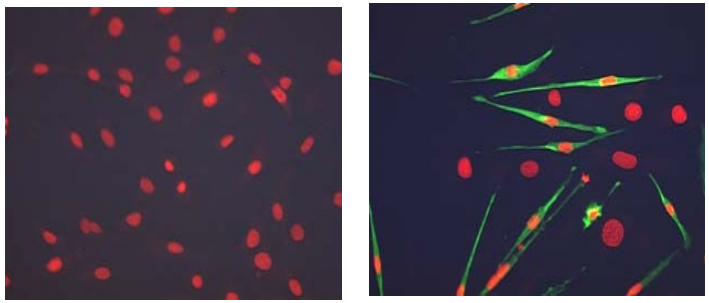




  - ➢ **integrin specificity**

α5β1 vs. αvβ3

Garcia + (1999)



αvβ3 α5β1

  - ➢ **non-RGD integrins**

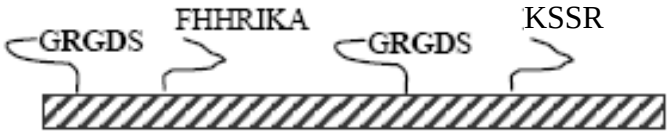
Bioadhesive Surfaces - Beyond RGD

Improve bioactivity beyond RGD

- **constrained (cyclic) RGD** Scarborough +, 1993

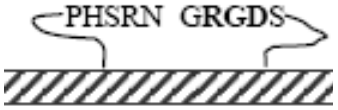
improved specificity
marginal effects in cell function

- **mixed RGD & non-integrin ligands** Dee +, 1998; Rezania & Healy, 1999



marginal effects in cell function

- **mixed RGD & PHSRN** Kao +, 2001; Dillow +, 2001



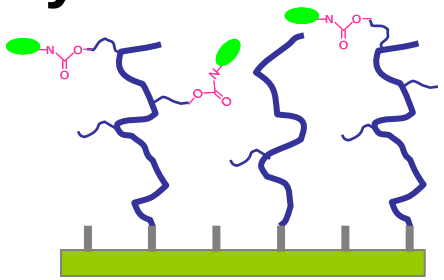
limited specificity
marginal effects in cell function

- **biomimetic ligands w. 2º/3º structure** Reyes +, 2007; Petrie +, 2006

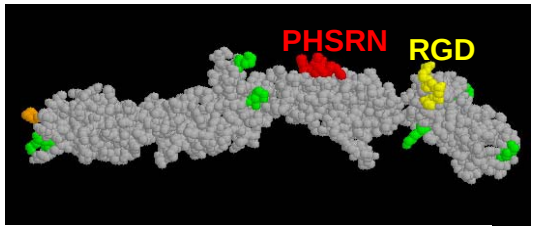
increased specificity
enhanced cell activities

Integrin Specificity Enhances Bone Marrow Stromal Cell Differentiation

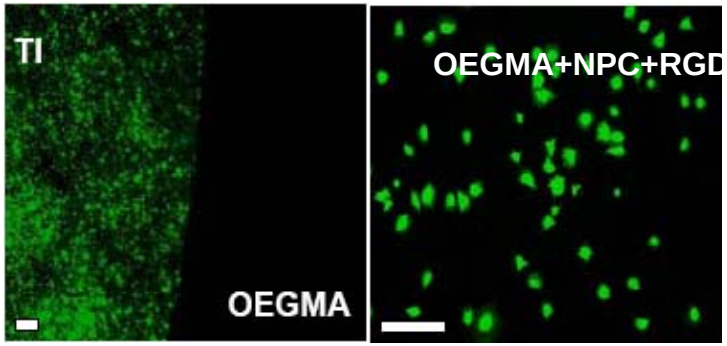
Polymer brushes on Ti with integrin-specific ligands

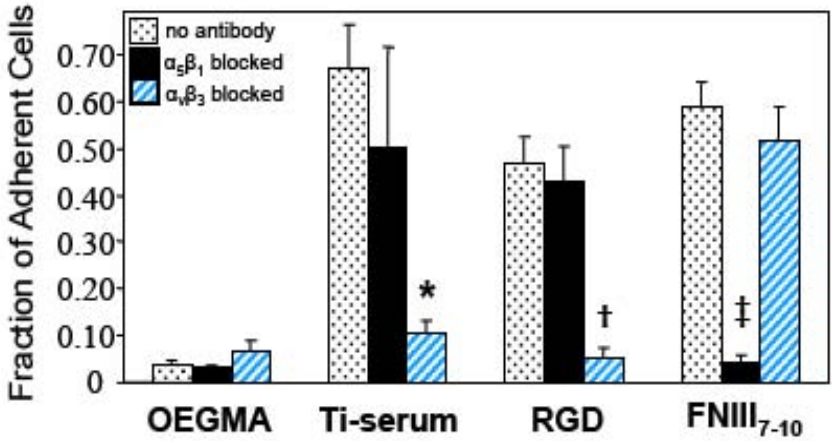


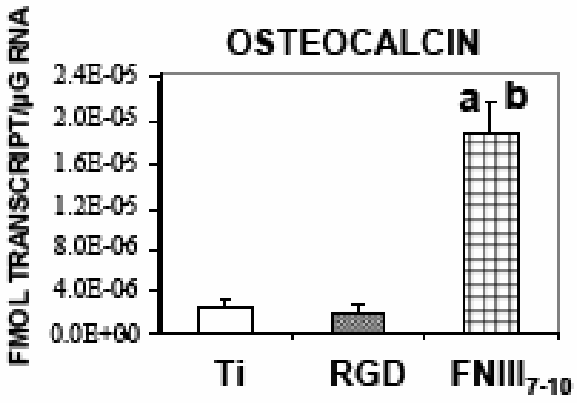
non-fouling OEGMA
RGD: αvβ3 integrin
$FNIII_{7-10}$: α5β1 integrin

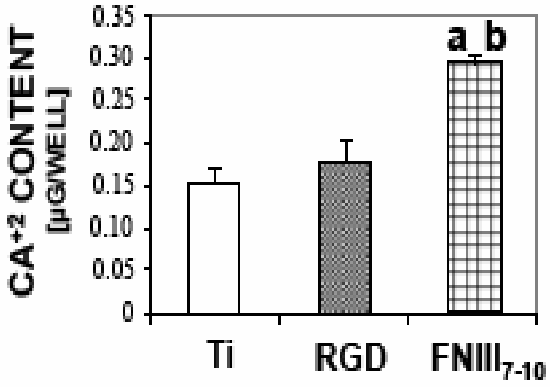


cell adhesion (serum) 56 d



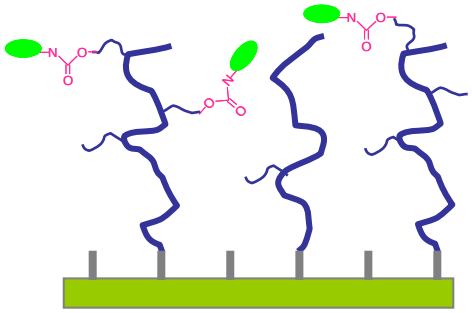




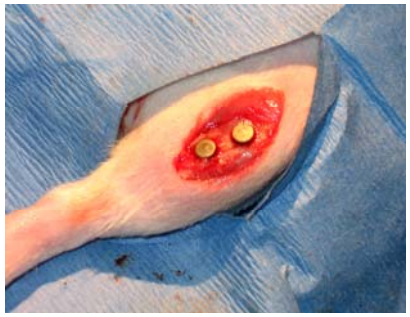


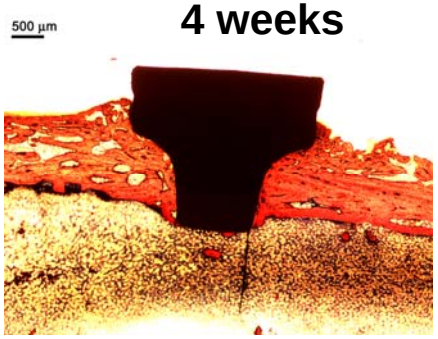
Integrin Specificity Enhances Osseointegration

Polymer brushes on Ti with integrin-specific ligands

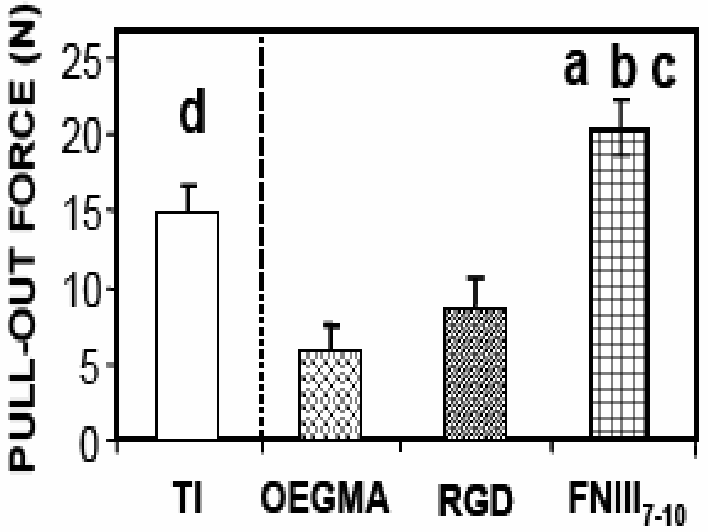


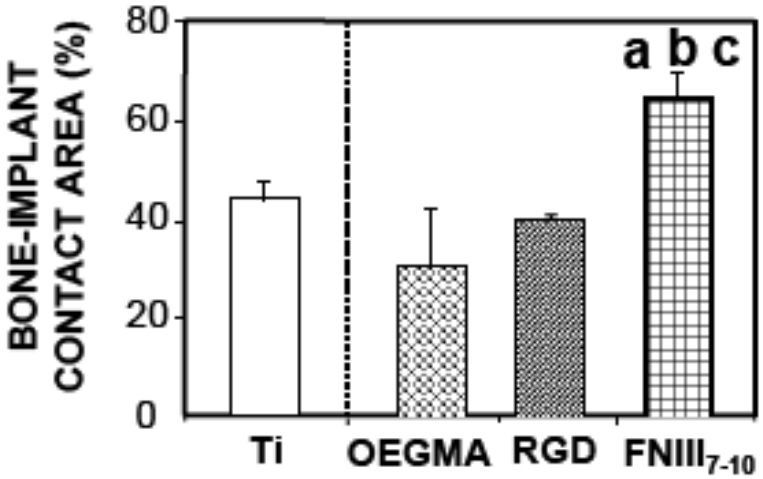
non-fouling OEGMA
RGD: αvβ3 integrin
$FNIII_{7-10}$: α5β1 integrin

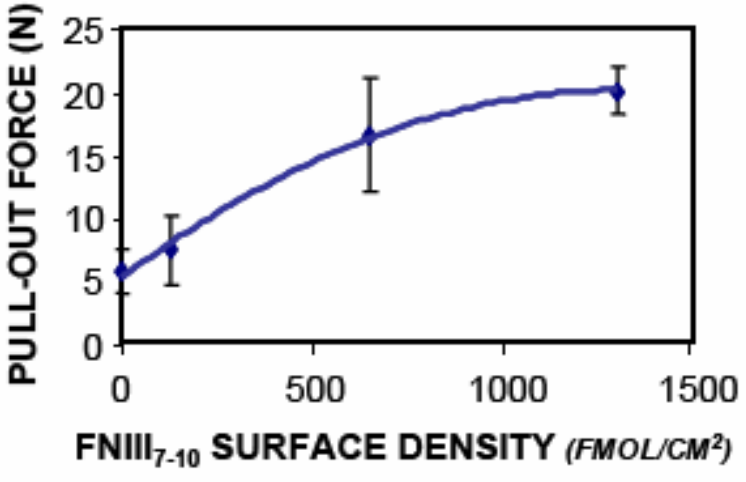




4 weeks







Cell Adhesion: Considerations 1

- Adhesive effects – multiple outcomes
  - anchorage (STD adhesion assay)
    - evolution, mechanism(s)
    - more adhesion ≠ enhanced function
    - 2D vs. 3D
  - signaling & downstream effects
    - cell morphology, spreading, migration & scaffold colonization, focal adhesion assembly
    - signaling pathways (FAK, ERK)
    - apoptosis vs. necrosis
    - proliferation (DNA synthesis, mitosis)
    - activation, differentiation, ECM remodeling
  - general considerations
    - appropriate cell types, co-cultures, tissue models
    - controls & reference conditions (human fibroblasts on Col-I?)

Cell Adhesion: Considerations 2

- Adhesive mechanism
  - ligand
    - adsorbed vs. engineered ligands
    - density, activity/conformation, specificity
  - receptor
    - expression in target cells
    - competition, dynamic changes
- Other considerations
  - cell-cell effects
  - serum/plasma, supplements
  - in vitro ↔ in vivo platforms

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