



Design Principles for Cytokine-Regulating Biomaterials

Newell R. Washburn

Department of Chemistry

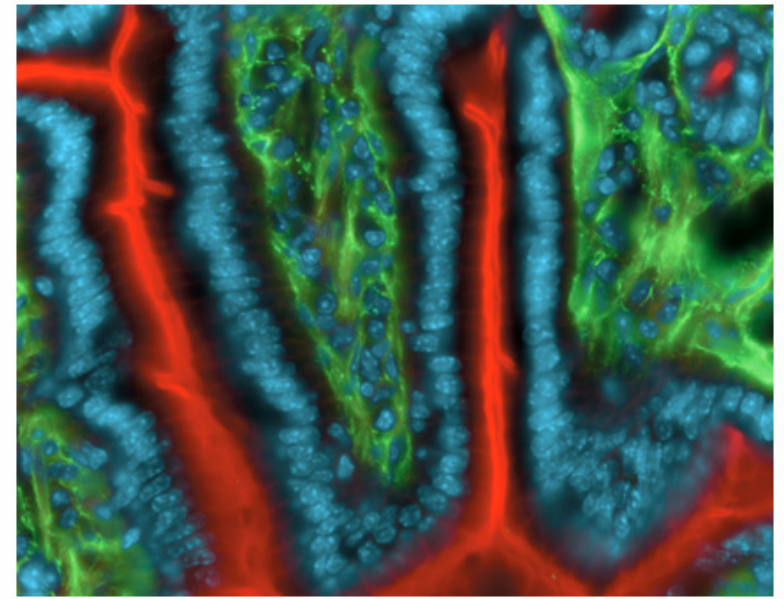
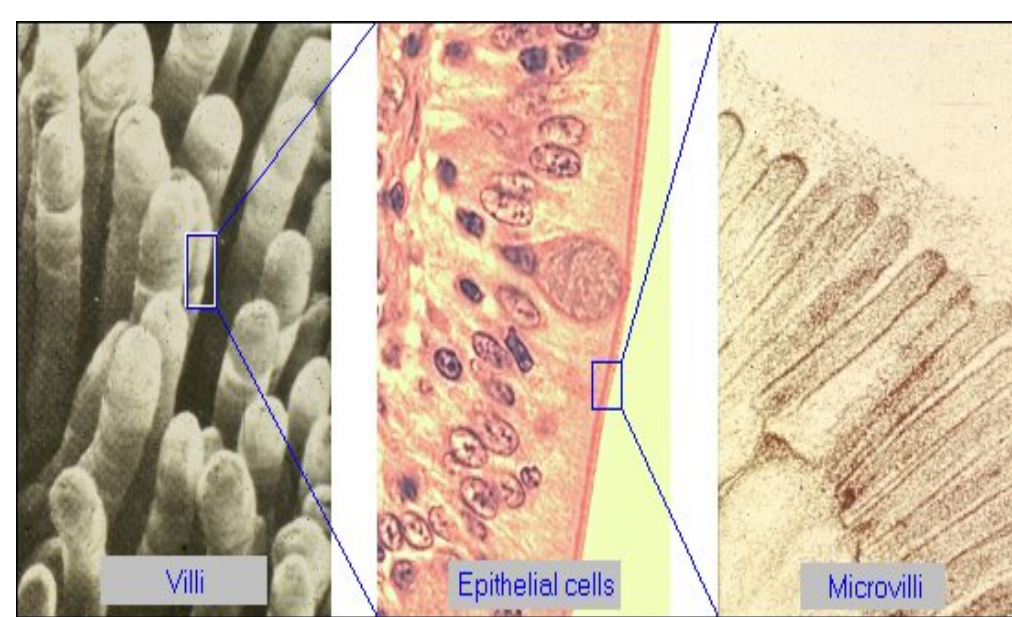
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Tissue Structure and Function



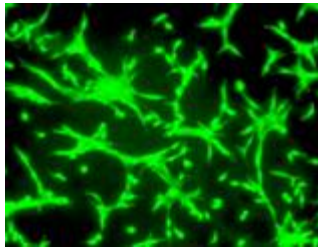
<http://biology.about.com/library/organs/bldigestsmallint.htm?iam=dpile&terms=%2Bvilli>

- Tissue-specific cells create extracellular matrix to provide mechanical support and adhesion sites.
- Matrix composed of collagen, proteins, proteoglycans, and glycosaminoglycans.
- Cells receive signals from other cells, the matrix, and soluble signaling molecules (growth factors and cytokines).
- The tissue triad of cells-soluble signaling molecules-extracellular matrix is responsible for tissue function



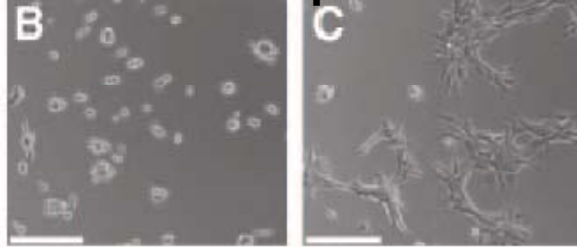
Biomaterials Mimicking ECM Functions

Cell adhesion



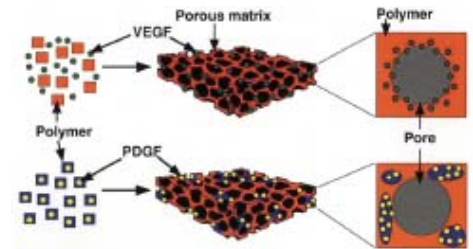
Ruoslahti and Piersbacher
Science (1987)

Growth factor presentation



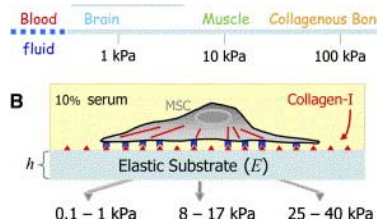
Wells, Griffith & co. Stem Cells (2007)

Growth factor delivery



Mooney & co. Nature Biotech (2001)

Modulus



Discher & co. Cell (2006)

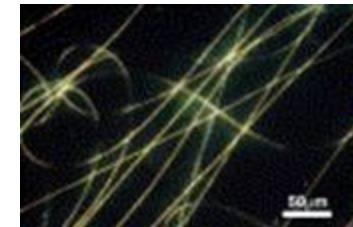
Yamada & co. Science (2001)

Osmotic pressure



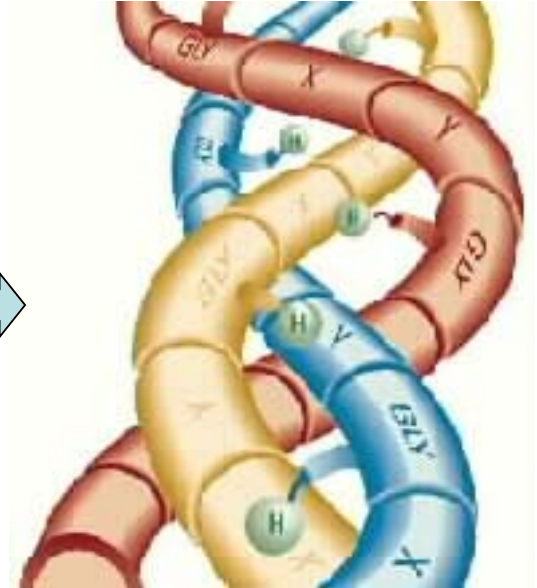
3M Tegaderm™

Structure



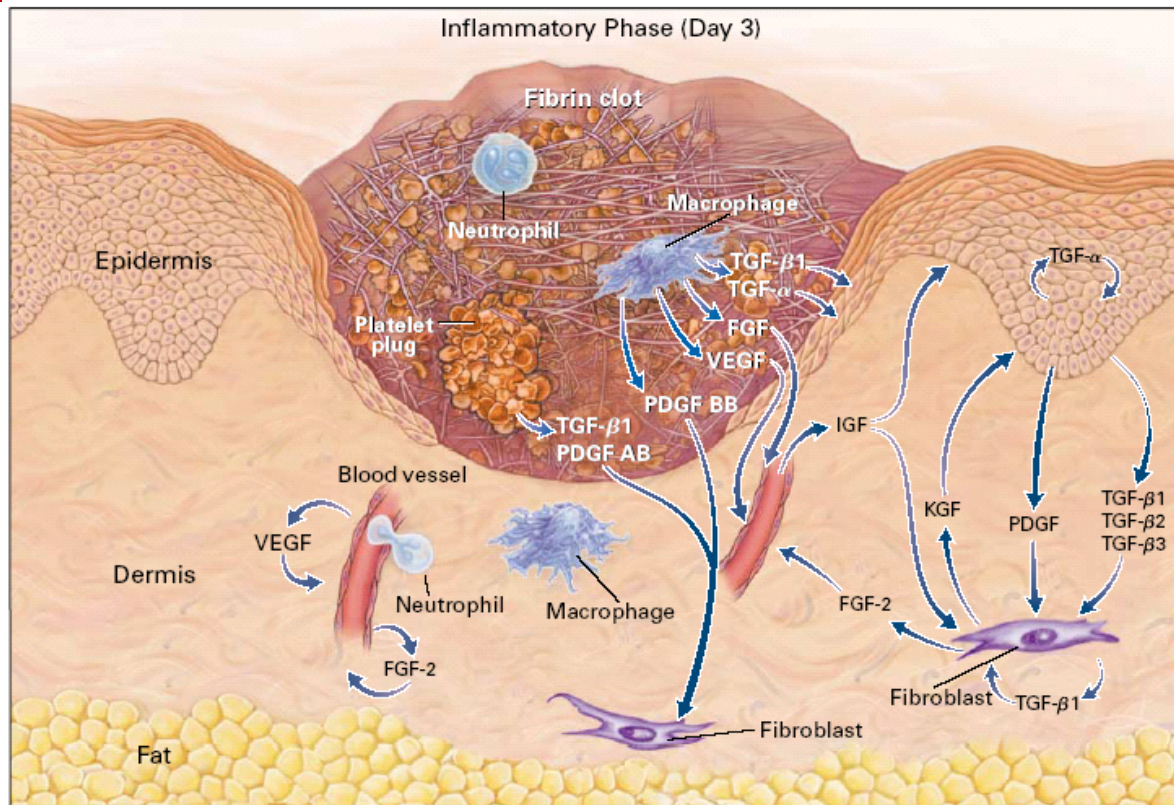
Kaplan & co. Biomaterials (2006)

- What about regulating activities of soluble signaling proteins?
 - ECM is central mediator of growth factors and cytokines during inflammation and tissue repair



- Tissues regulate the activities of soluble signaling proteins through binding interactions with extracellular matrix.
 - Burying receptor ligands results in decreases in activity
 - Exposing receptor ligands can increase activity
- Can we engineer materials to perform this same regulatory function?
 - Design principles?

Soluble Signaling Proteins in Inflammation and Wound Healing



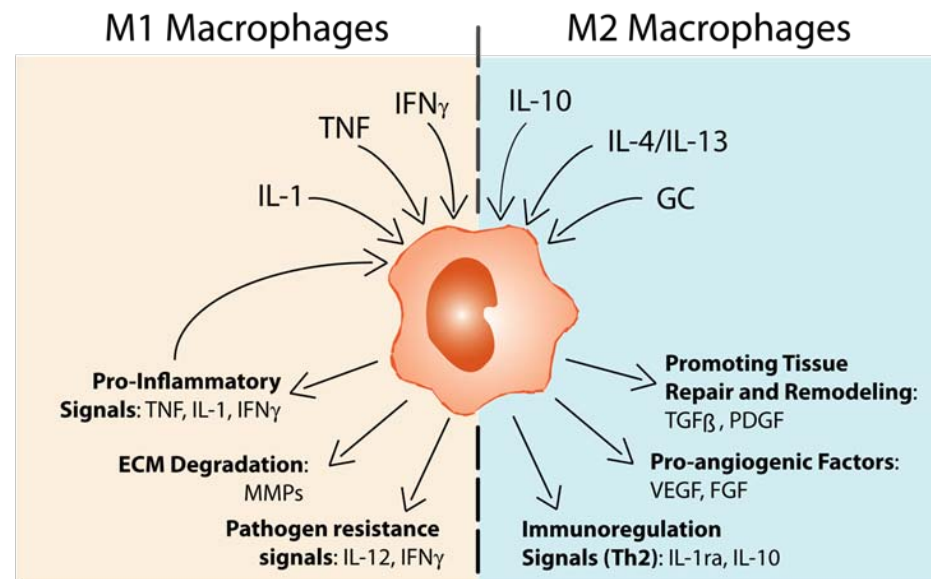
Singer & Clark NEJM (1999)

- Inflammation is a critical early step in wound healing
 - Best chance for interactive biomaterials to make a difference
- Can the wound healing trajectory be altered by changing the character of the inflammatory response?
 - Control cell activities by controlling protein activities



Potential Effects of Locally Inhibiting Pro-Inflammatory Cytokines

- Macrophages can adopt two general phenotypes
 - M1 promoted by pro-inflammatory cytokines
 - Associated with phagocytic phenotype, production of matrix-degrading compounds and pro-inflammatory cytokines
 - M2 promoted by absence of pro-inflammatory signals and stimulation by IL-10 and other anti-inflammatory cytokines
 - Phenotype associated with tissue repair including production of pro-angiogenic factors
- While M1 may be desirable in cancer biology, it may contribute to excessive tissue damage during inflammation and scar formation in wound healing



Can materials inhibit M1 phenotype and promote M2?



Inflammation in Acute Injuries

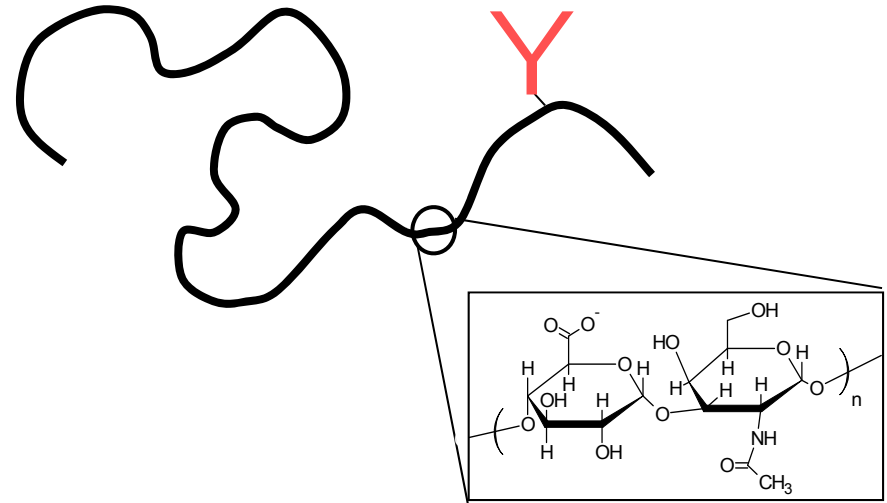
- Many acute injuries are characterized by intense inflammatory responses
- Inflammatory cascade is linked to continued apoptosis, necrosis, and tissue degradation
- Managing inflammatory responses could improve healing
 - Systemic approaches risk further immunosuppression
 - Local approaches may have significant advantages





Developing a biomaterial capable of regulating a broad range of signaling proteins

- Hyaluronic acid known to promote healing of chronic wounds
 - **Receptors for hyaluronic acid (CD44 and RHAMM) upregulate cell motility¹**
 - **Degradation products are known Damage-Associated Molecular Patterns that recruit additional repair cells²**
- Impart new dimension of biological activity to gels by covalently attaching antibodies that inhibit pro-inflammatory cytokines
 - Local control over cytokine activities
 - Synergistic function with HA-RGD

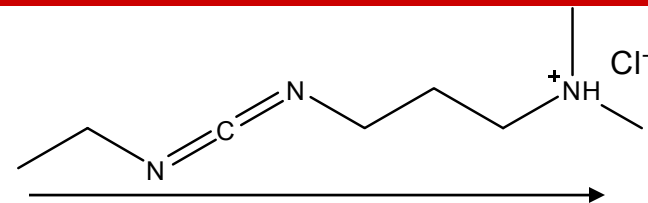
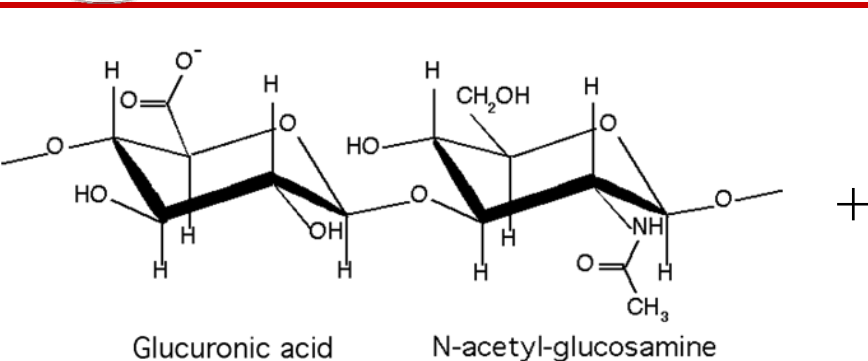


¹Savani et al. J Biol Chem (2001)

²Termeer et al. J Exp Med (2002)



Preparation of HA-mAb Conjugates



- Use carbodiimide chemistry to amide bond between carboxylate group on glucuronic acid and primary amine on monoclonal antibody
- Product purified using ammonium sulfate precipitation and dialysis
- Composition analyzed using PAGE and ELISA
 - mAb:HA = 1:7



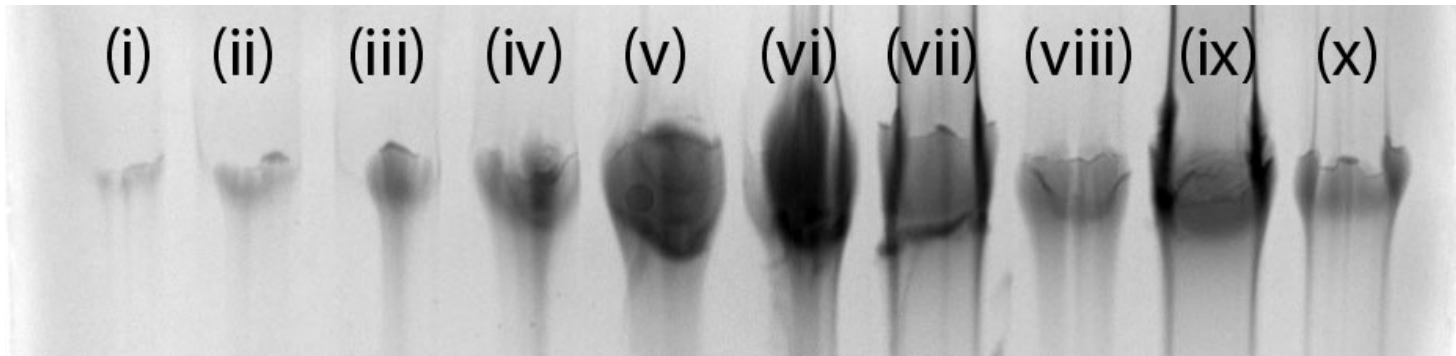
Sidi Bencherif



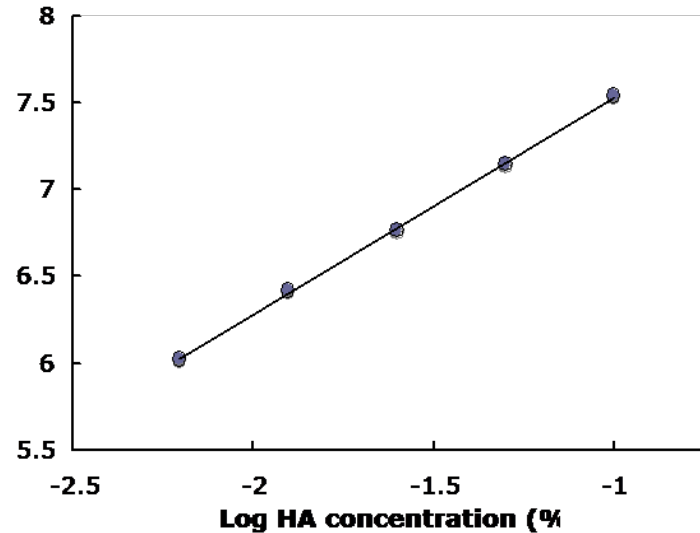
Steve Sun



PAGE Characterization of Hyaluronic Acid Concentration



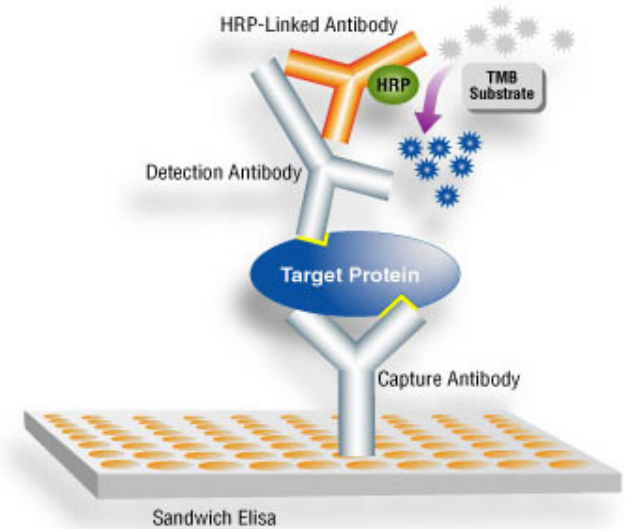
- 4% Polyacrylamide Gel
- 125V for 20 min and 200V for 1hr
- 0.5% Alcian Blue in 3% Acetic Acid for 15min
- Quantitative image analysis used to measure band intensities



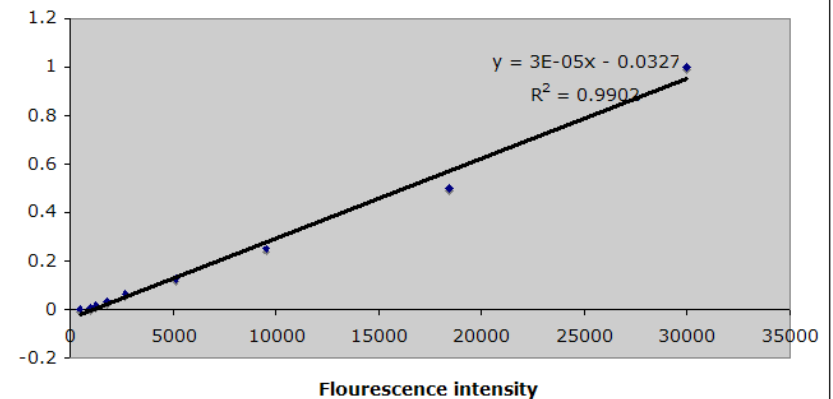


Enzyme Linked Immuno-Sorbent Assay (ELISA) of Monoclonal Antibody Functionalization

- Capture Antibody: Rabbit anti-Mouse
- Target Molecule: Mouse anti-IL1 β and Mouse whole IgG
- Detection antibody: Donkey anti-Mouse HRP or Goat anti-Mouse Alexa 488
- Measure 1:7 (Antibody:HA)

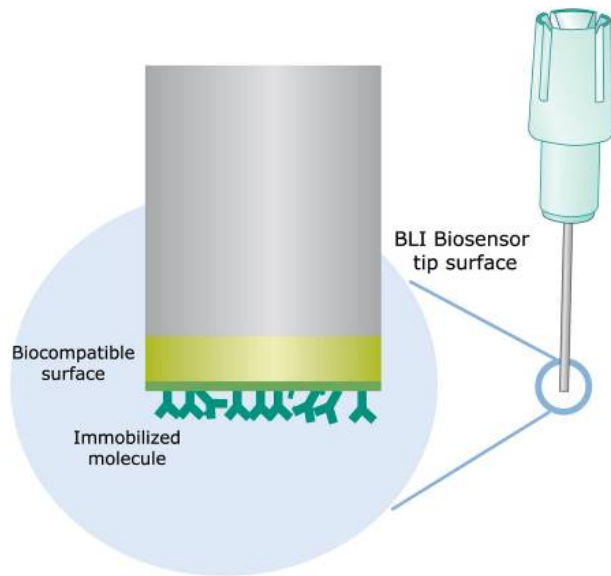


ELISA Standard Curve using Alexa 488

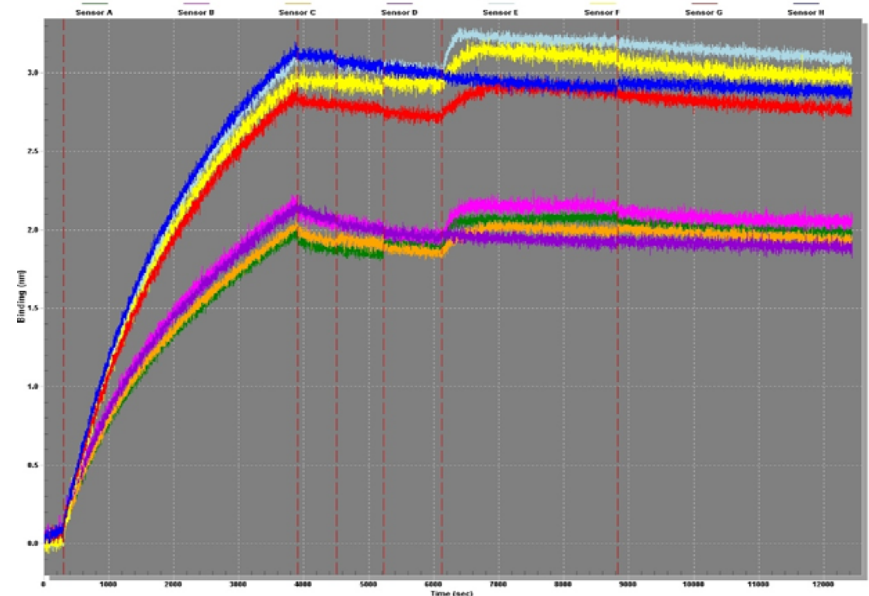




Measurement of HA-mAb Binding Affinity

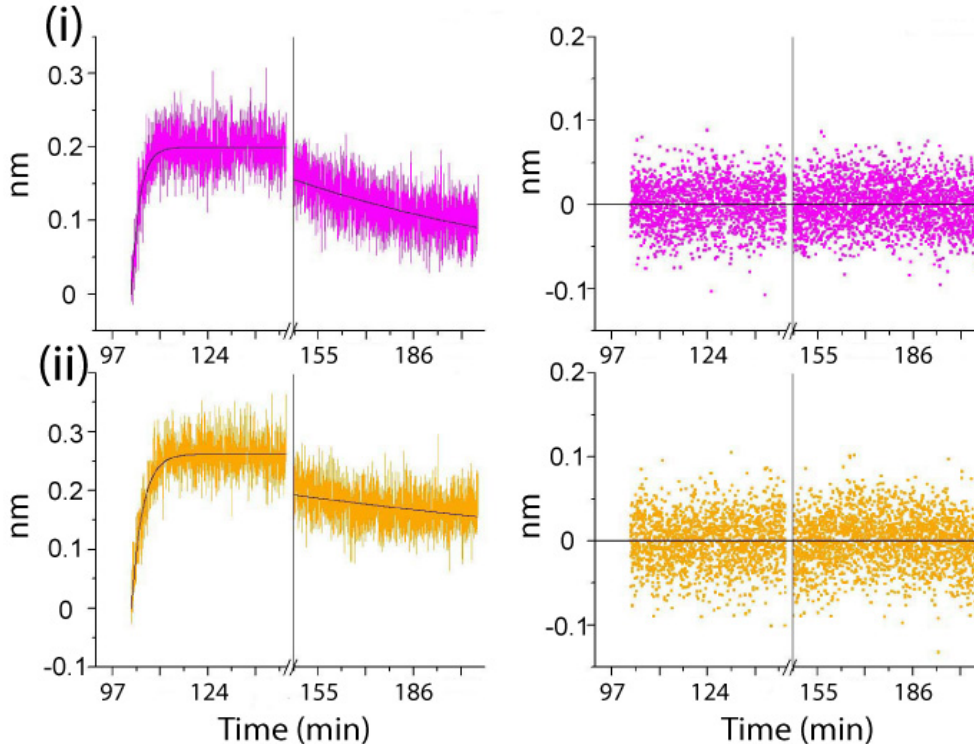


- Equilibrium binding constant measured using an optical biosensor
 - ForteBio Octet
- mAb and HA-mAb loaded onto protein A-conjugated sensor
- Correlates changes in refractive index at sensor surface with adsorption and desorption events





Synergistic enhancement of binding affinity?



- Separately measure adsorption and desorption kinetics

$$R(t) = R_0 + \Delta R \left\{ 1 - \exp[-k_{on}(t - t_{on})] \right\}$$

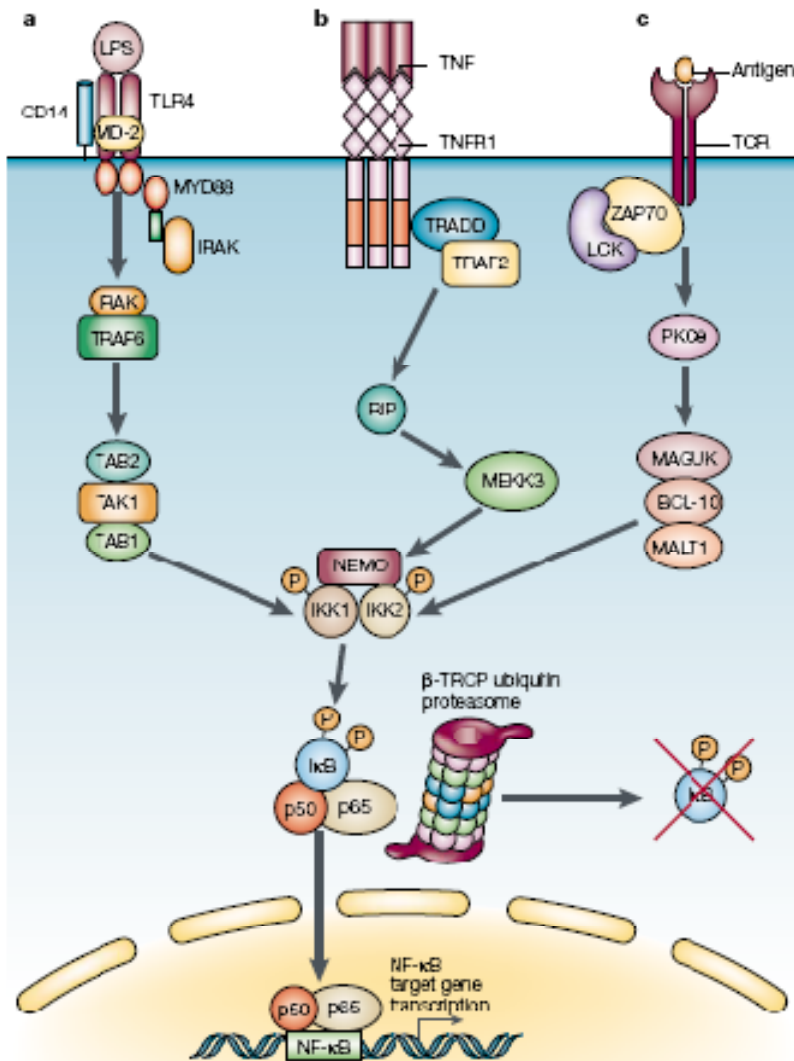
$$R(t) = R_0 + \Delta R \exp[-k_{off}(t - t_{off})]$$

$$- K_D = k_{on}/k_{off}$$

- k_{on} is nearly identical for mAb and HA-mAb
- k_{off} is 3x slower for HA-mAb than mAb
 - Suggests cooperative interaction between HA and mAb in HA-mAb when binding antigen



Quantitative Measures of IL-1 β Signal Transduction

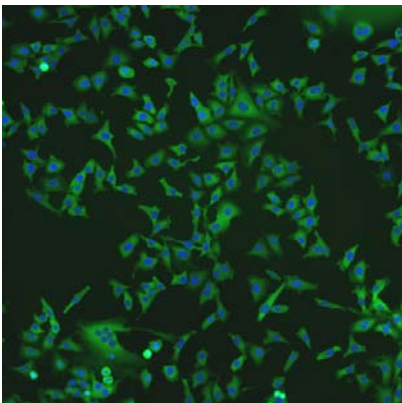


- Bacterial products, antigens, and most pro-inflammatory cytokines upregulate gene transcription through nuclear factor- κ B.
 - Protein complex maintained in the cytoplasm in an inactive state by the inhibitor protein I- κ B.
 - Signal transduction releases NF- κ B, allowing it to move through the nuclear membrane and to initiate transcription

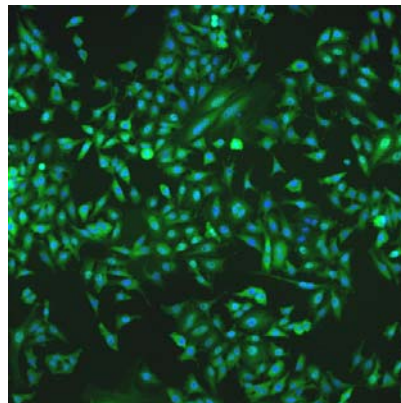


Screening Biological Activity of Cytokine Activities

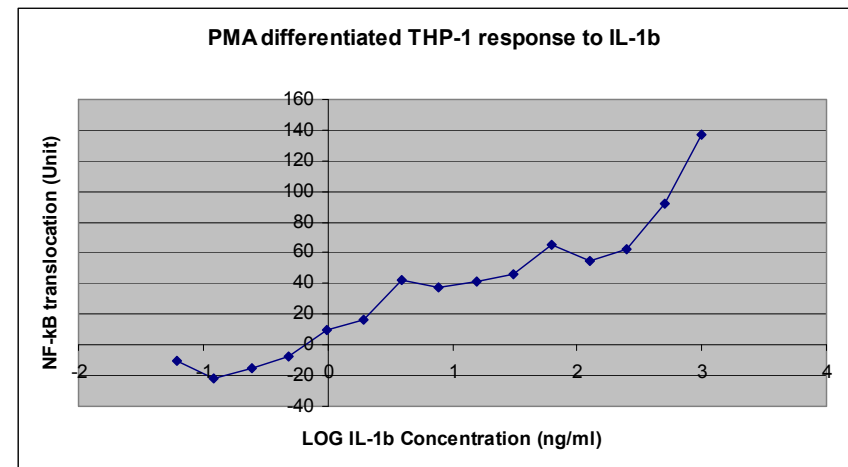
- Expose cells that are sensitive to cytokines to cytokine inhibitors
 - THP-1 macrophages
 - HeLa cells
- Monitor nuclear translocation of NF- κ B using fluorescent staining and automated image analysis
- Assay works for both soluble factors and engineered substrates



Untreated

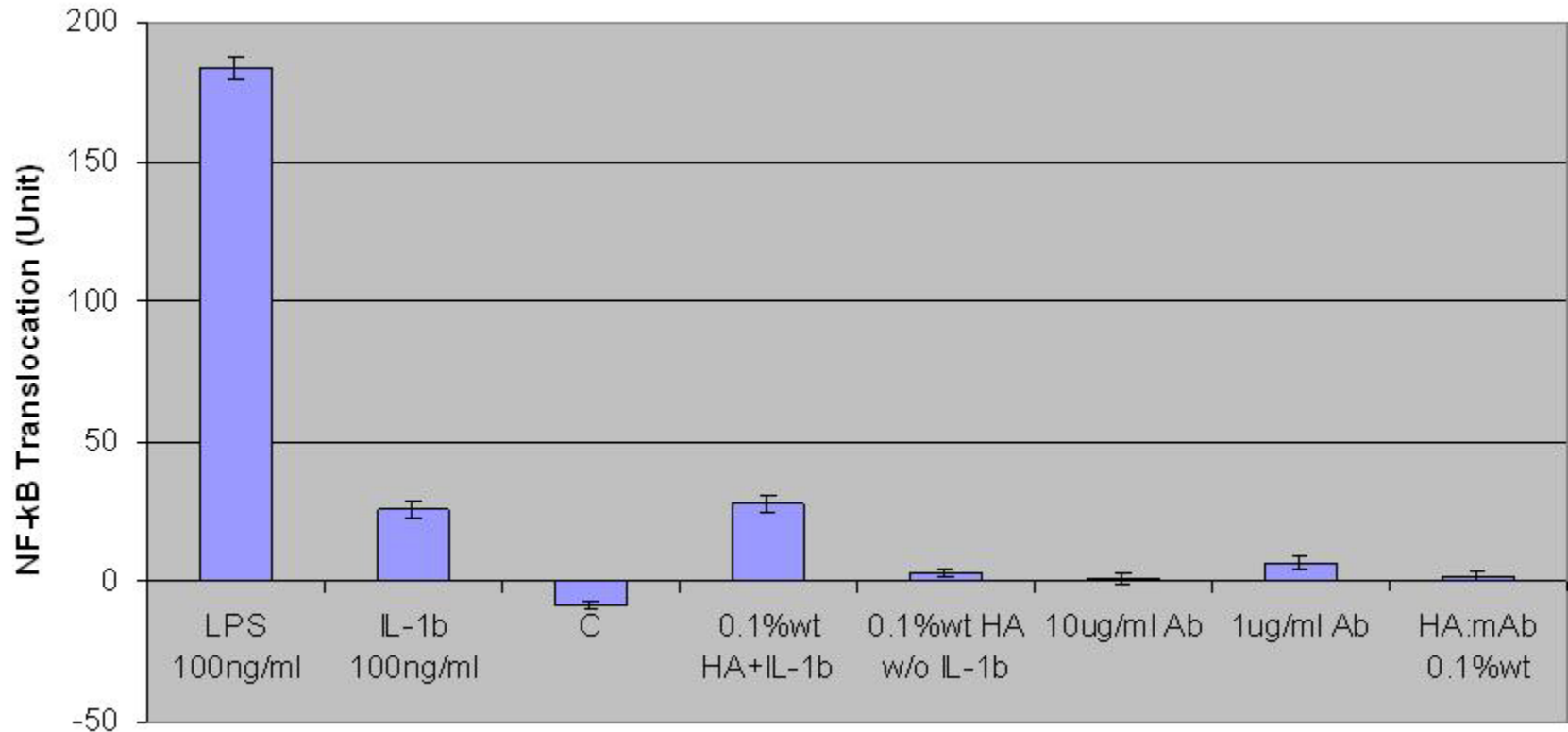


IL-1b (1000IU/ml)





Preliminary Results on Regulating IL-1 β Activities



- HA-mAb conjugates provide similar inhibition of IL-1 β as unmodified mAb



Preliminary Animal Studies

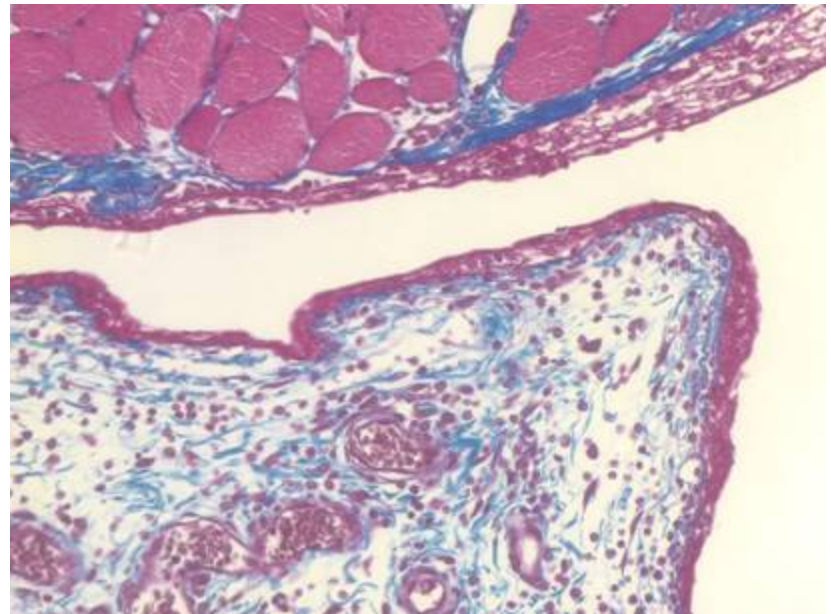
- Incisional wound model in Sprague-Dawley rats
 - Injure fascia, inject gel, suture
- HA-mAb-RGD and HA-RGD as bilateral control
- Average healing time was 4 days



Stephen Badylak



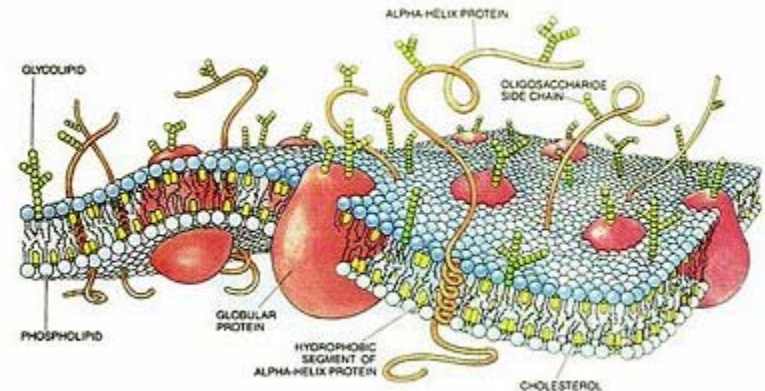
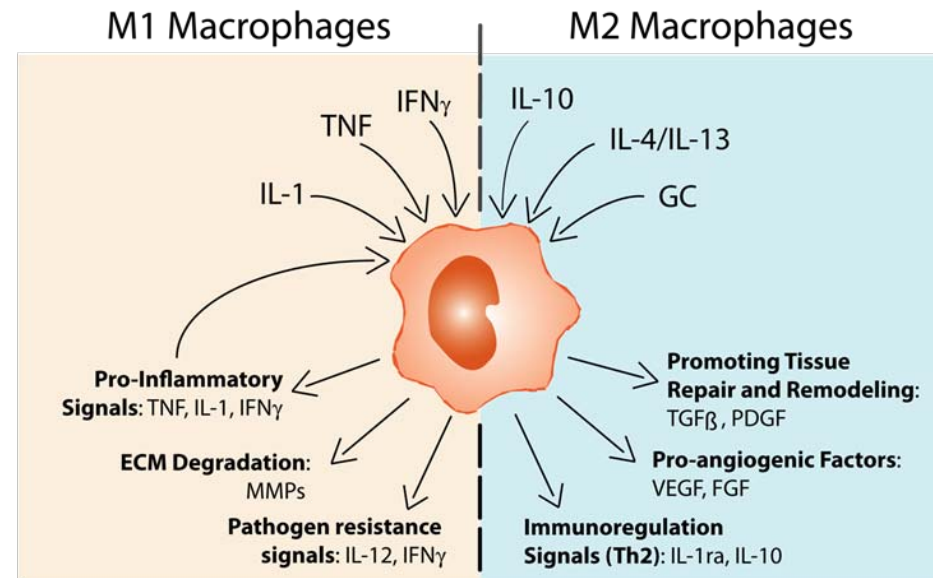
Thomas Gilbert
(& family)





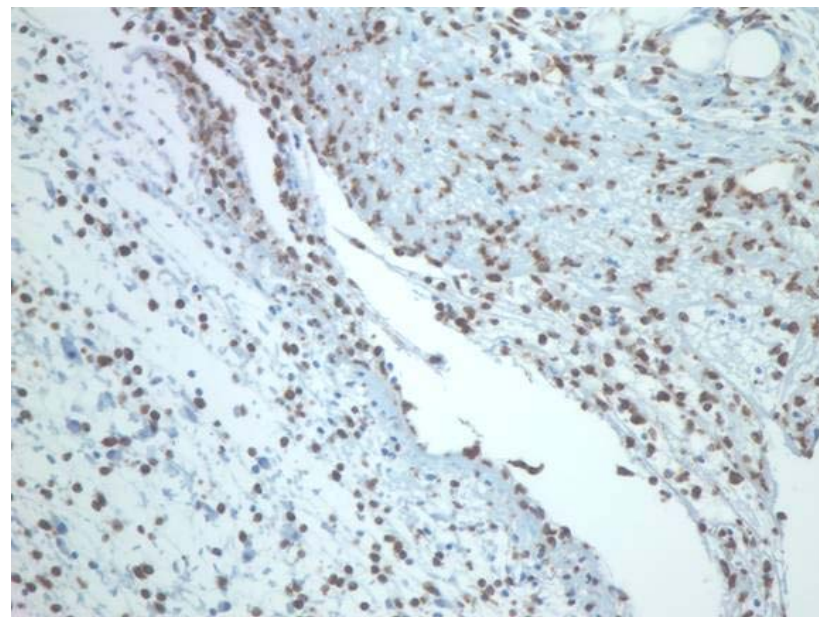
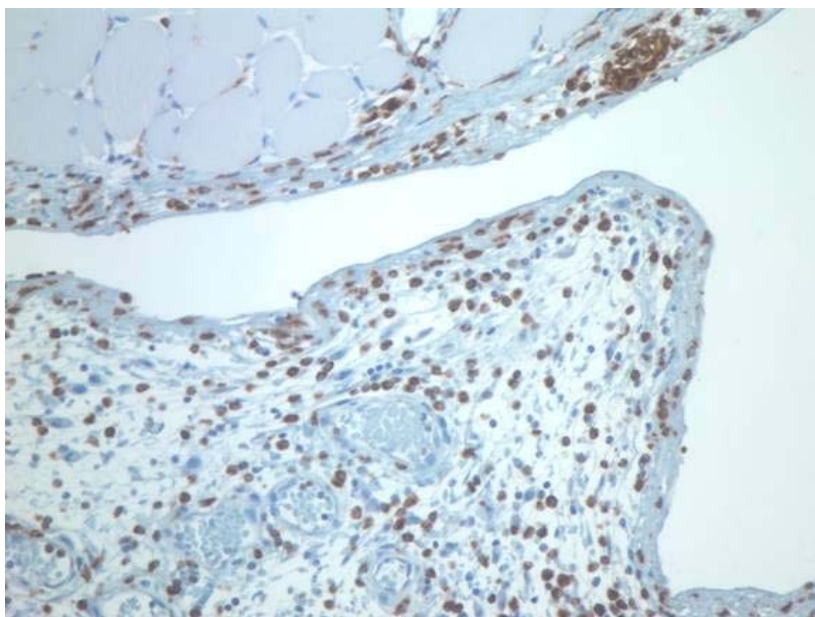
Immunohistochemical Markers of Macrophage Phenotype

- Monocytes/macrophages express CD68
 - Pan-macrophage marker
- Macrophages can adopt two general phenotypes
 - M1 promoted by pro-inflammatory cytokines
 - Express CCR7
 - M2 promoted by absence of pro-inflammatory signals and stimulation by IL-10 and other anti-inflammatory cytokines
 - Express CD163





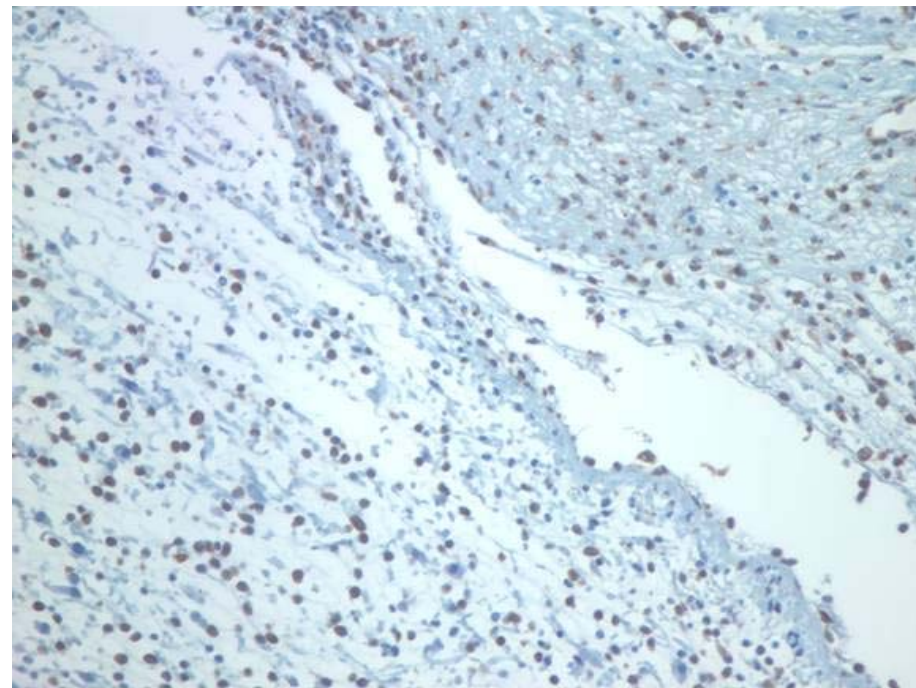
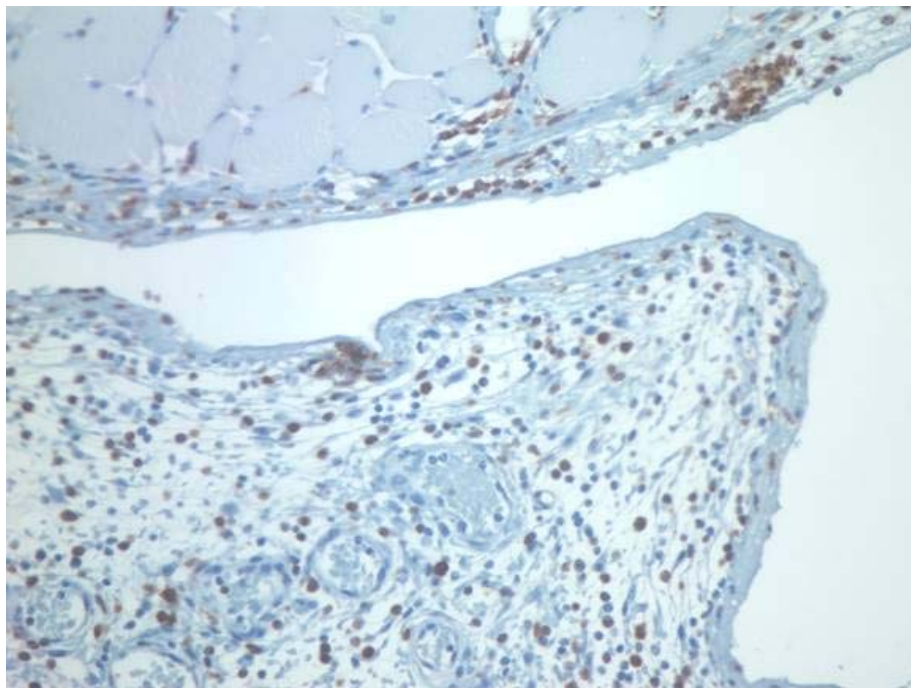
Effects of Neutralizing IL-1 β



- CD68 was used as a marker for identifying macrophages
- Two days following injury, significant invasion of macrophages is observed in anti-IL-1 β (+) and anti-IL-1 β (-) sites



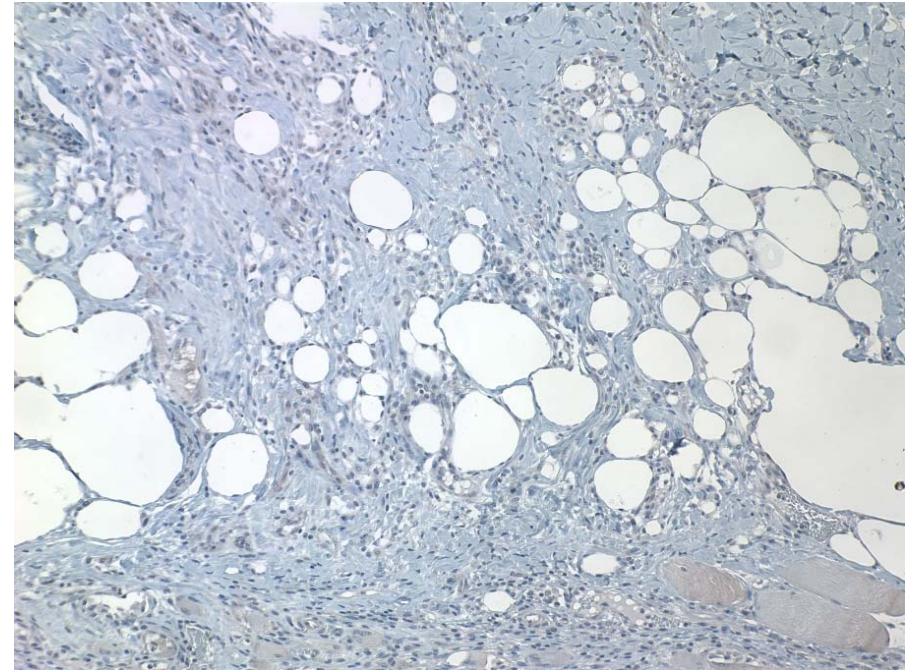
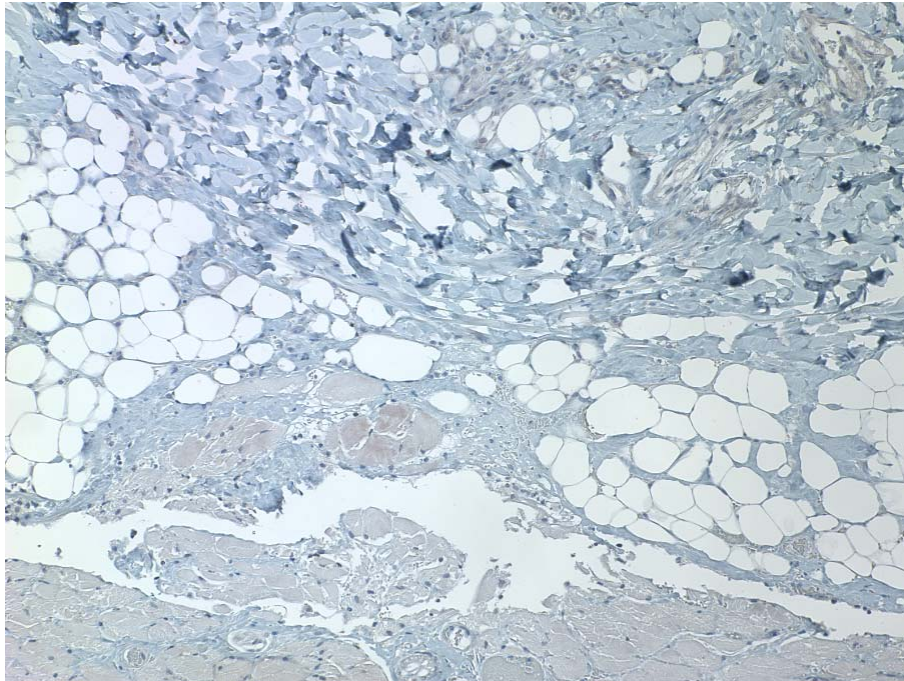
CD163/CCR7 Immunohistochemistry



- CD163 and CCR7 used as markers for macrophage phenotype
 - M1 phenotype tends to express CCR7
 - M2 phenotype stains positively by CD163
- No significant difference in the density of M2 phenotype macrophages between anti-IL1 β (+) and anti-IL1 β (-) samples
 - Significant fraction of M2 phenotype in mAb(+) and mAb(-) sites suggests that HA matrix influences phenotype
 - Significant fraction of M1 phenotype in saline-treated controls

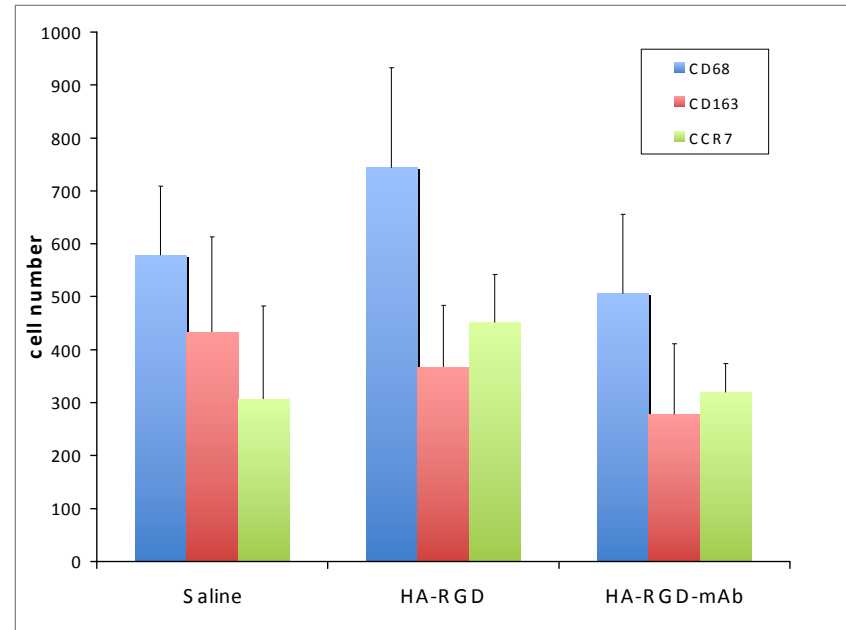
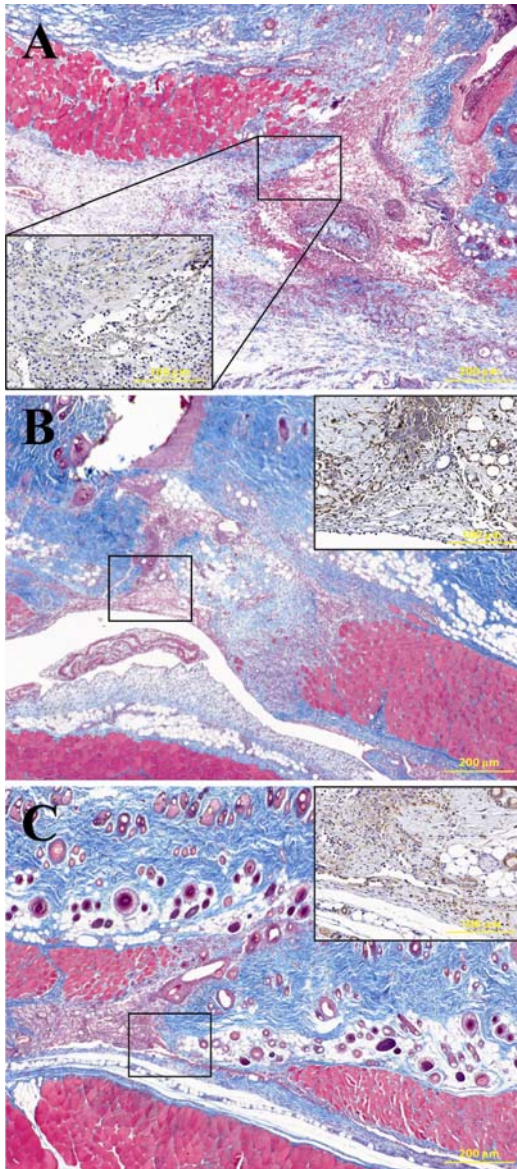


Effects of Neutralizing TNF- α and IL-1 β



- Immunohistochemistry data suggest that inhibiting multiple cytokines provides measurable biological response
 - Reduction in invasion of macrophages into wound sites
 - 30% fewer than control samples
 - Reduction in CCR7⁺ macrophages
 - 50% lower than HA-RGD alone
 - General inhibition of healing
 - Massive reduction in cytokine activities appears to prevent healing

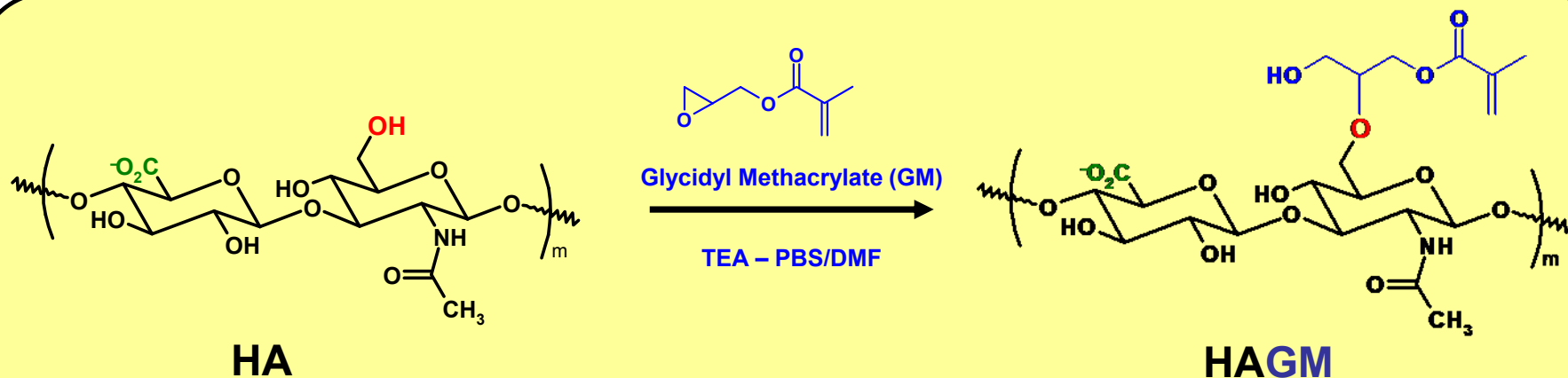
Quantification of Immunohistochemical Data



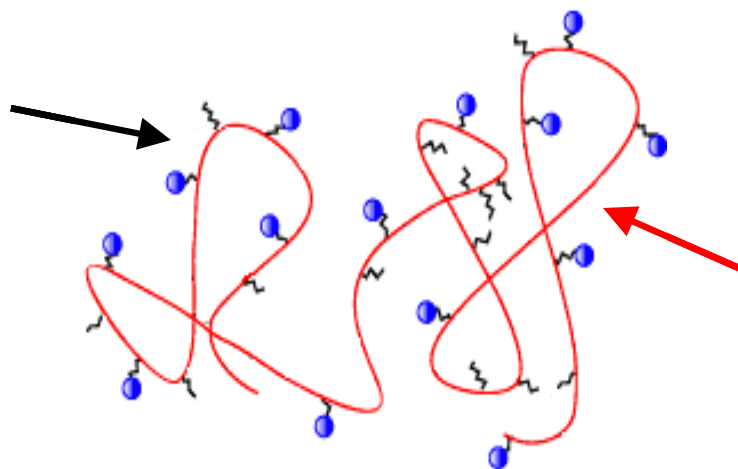
- HA-RGD treatment increases macrophage infiltration and ratio of M1:M2 phenotype relative to saline treatment
 - Consistent with understanding of HA as damage-associated molecular pattern
- Incorporation of anti-TNF- α /anti-IL-1 β in gels reduces both number of macrophages and number expressing M1 marker



Can we impart this activity into mechanically robust HA matrices?



Methacrylated fragments



Hyaluronic Acid Chain

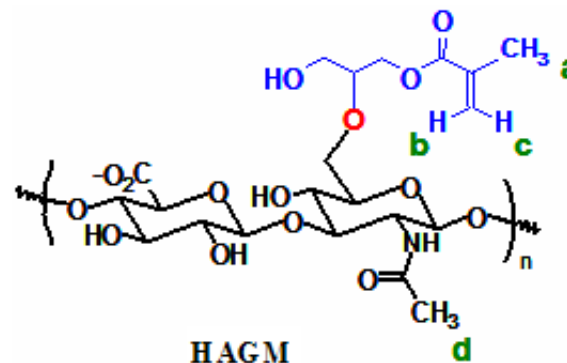
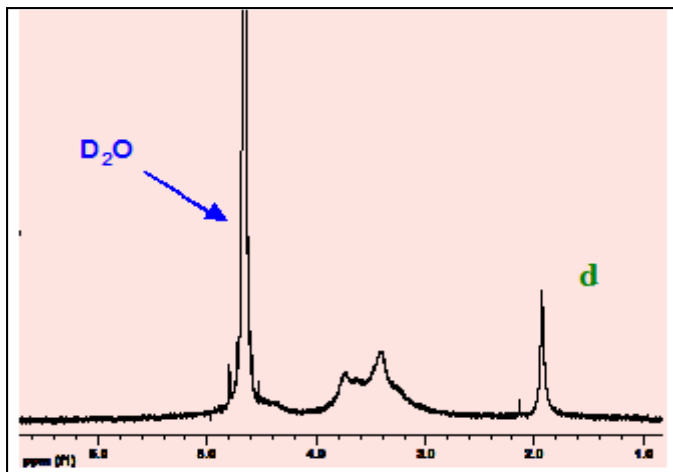
Mw ~ 1.6×10^6 Da

Methacrylated HA



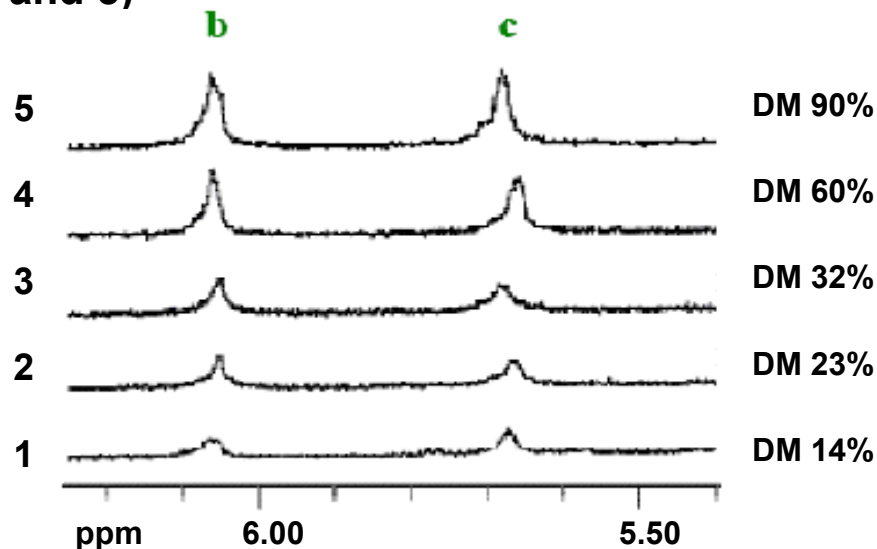
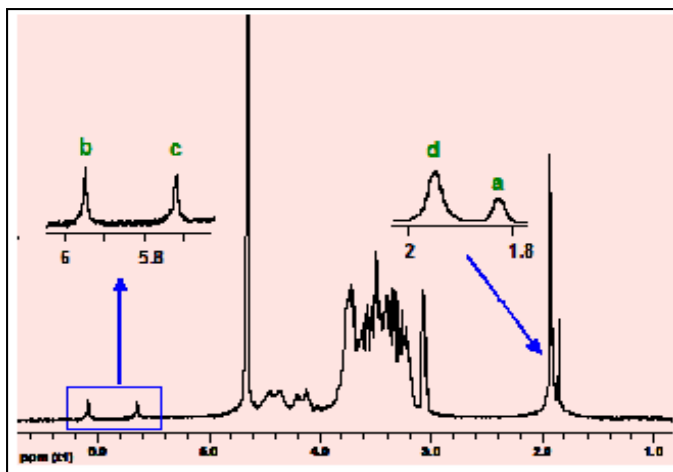
Characterization of HAGM Macromomers by ^1H NMR

- ^1H NMR of native HA (Mw ~ 1.7 MDa)



- Normalization of acrylate proton integration picks of HAGM (reactions 1, 2, 3, 4, and 5)

- ^1H NMR of HAGM (reaction 3)





Preparation of HAGM Hydrogels

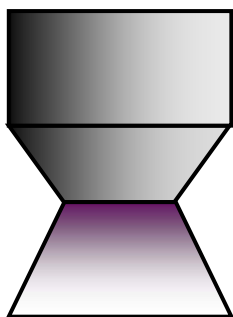
Hydrogel Composition (5 wt./v %)

HAGM	:	50	mg
d-H ₂ O	:	1000	mg
Initiator (I ₂₉₅₉)	:	1	mg

UV lamp

$\lambda = 360 \text{ nm}$

HA-based
Hydrogel



Curing time: 10 min

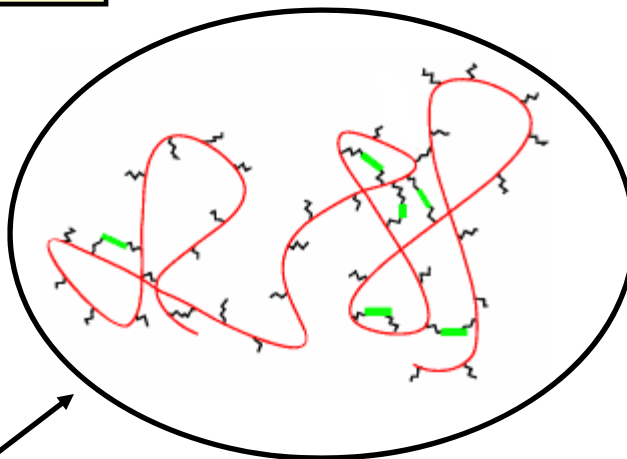


Photo-crosslinked HA

HA in water



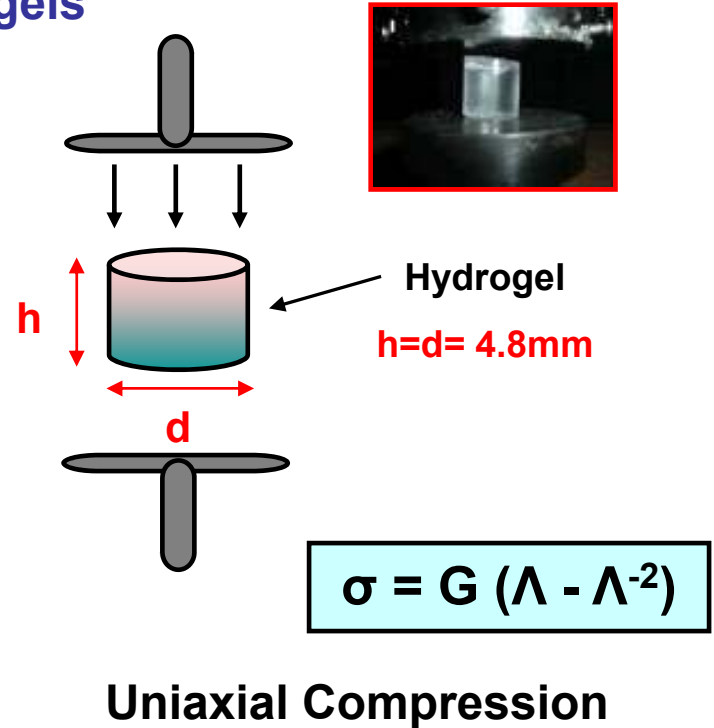
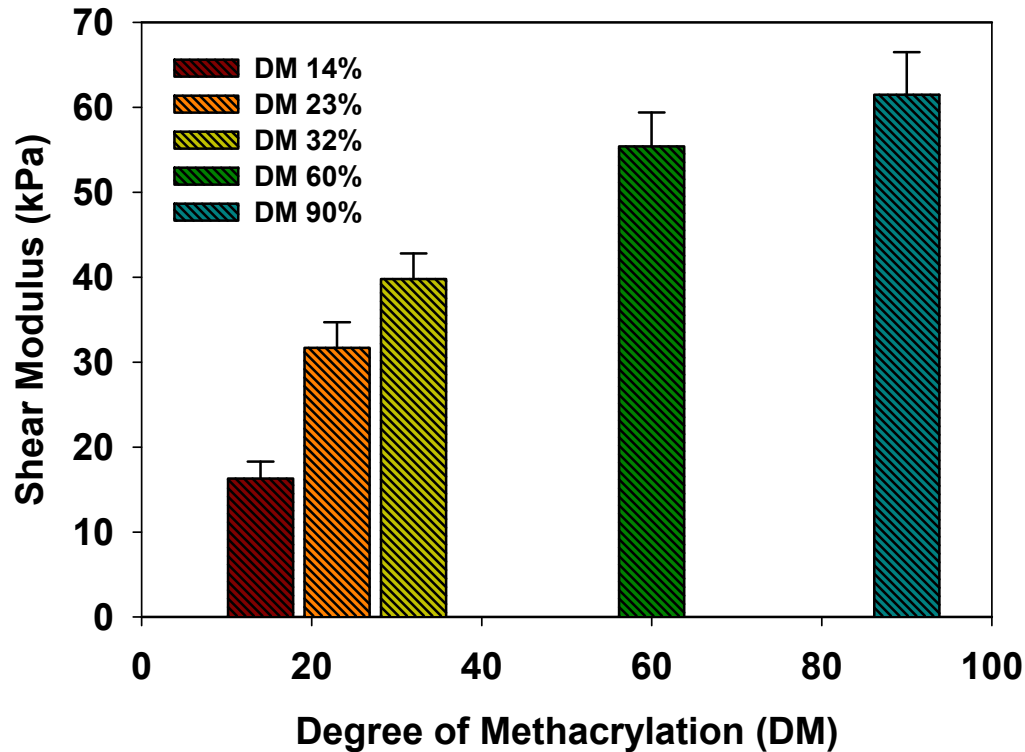
HA Hydrogel





Mechanical Properties of Hyaluronic Acid-GM hydrogels

■ Mechanical Testing on 5 wt/v % HAGM Hydrogels

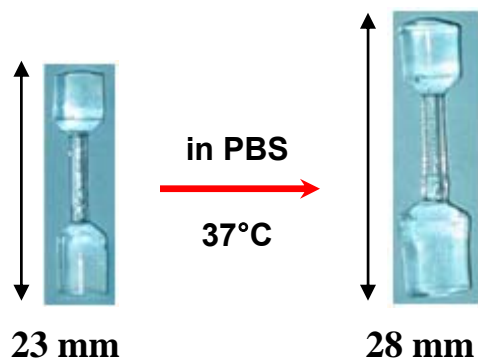


- DM affects tremendously the mechanical strengths
- Broad range of Shear Modulus values (16-65 kPa)

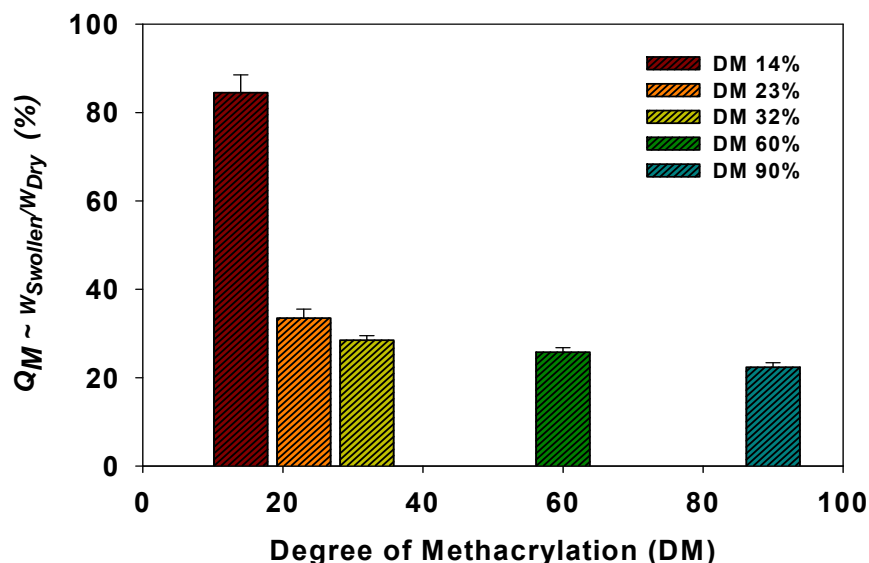


Effect of DM on the Swelling Behavior of HAGM hydrogels

■ Swelling ration of 5 wt/v % HAGM Hydrogels



	Degree of Methacrylation (%)				
	14	23	32	60	90
W_{Swollen} (mg)	270.5	110.6	119.3	77.4	55.9
W_{Dry} (mg)	3.2	3.3	4.4	3.0	2.5
Q_M	84.5	33.5	27.1	25.8	22.4
M_c (10^5 g/mol)	18.72	3.98	2.79	2.57	2.03
ξ (nm)	1123	380	296	280	237



■ Flory-Rhener Equations:

$$Q_v = 1 + \frac{\rho_p}{\rho_s}(Q_M - 1)$$

$$Q_v^{5/3} \cong \frac{\bar{v}\bar{M}_c}{V_1} \left(\frac{1}{2} - \chi \right)$$

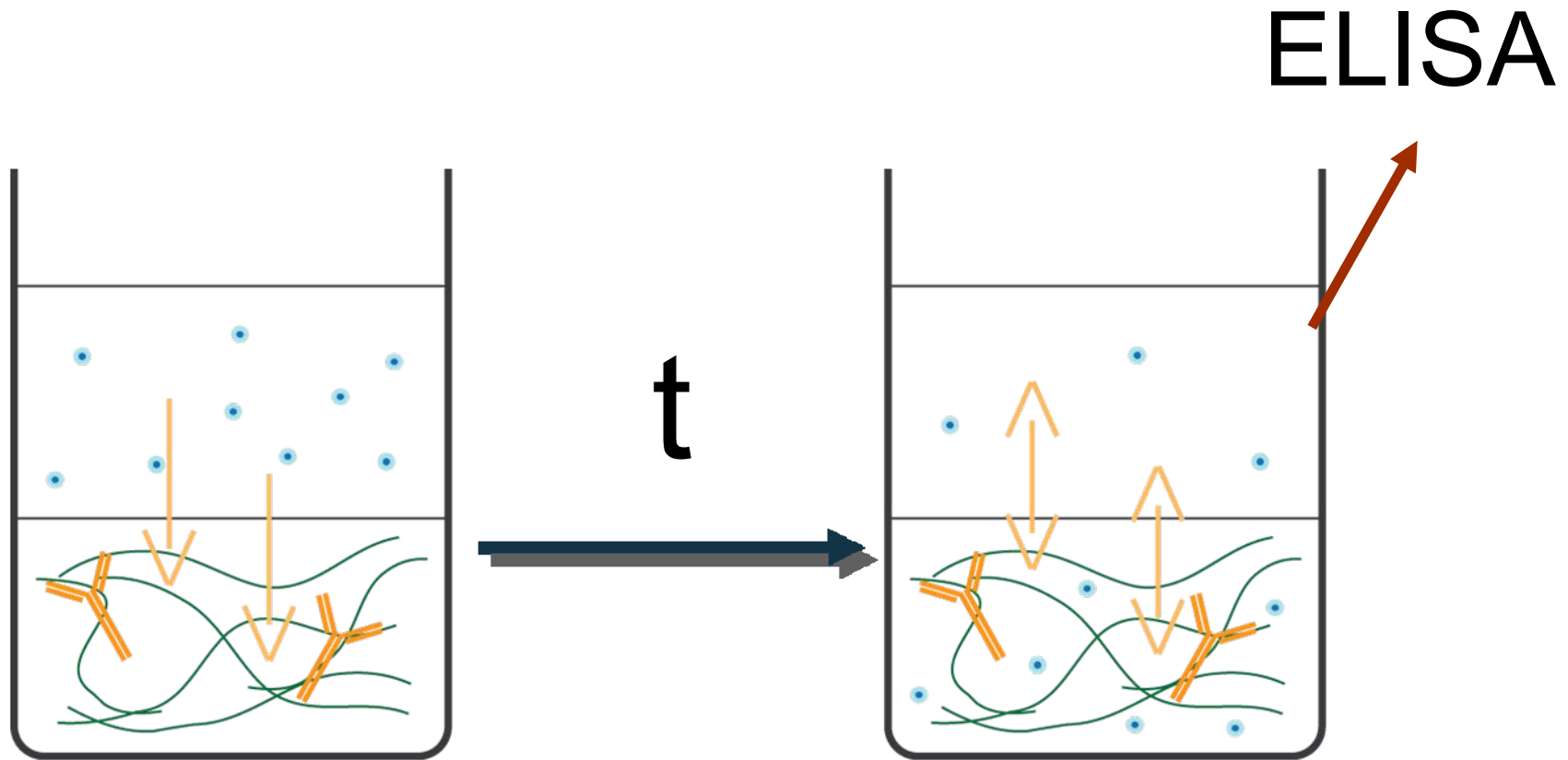
$$\xi = 0.1748 \sqrt{\bar{M}_c} Q_v^{1/3} \text{ (nm)}$$



Large mesh size large enough to allow protein diffusion into gels

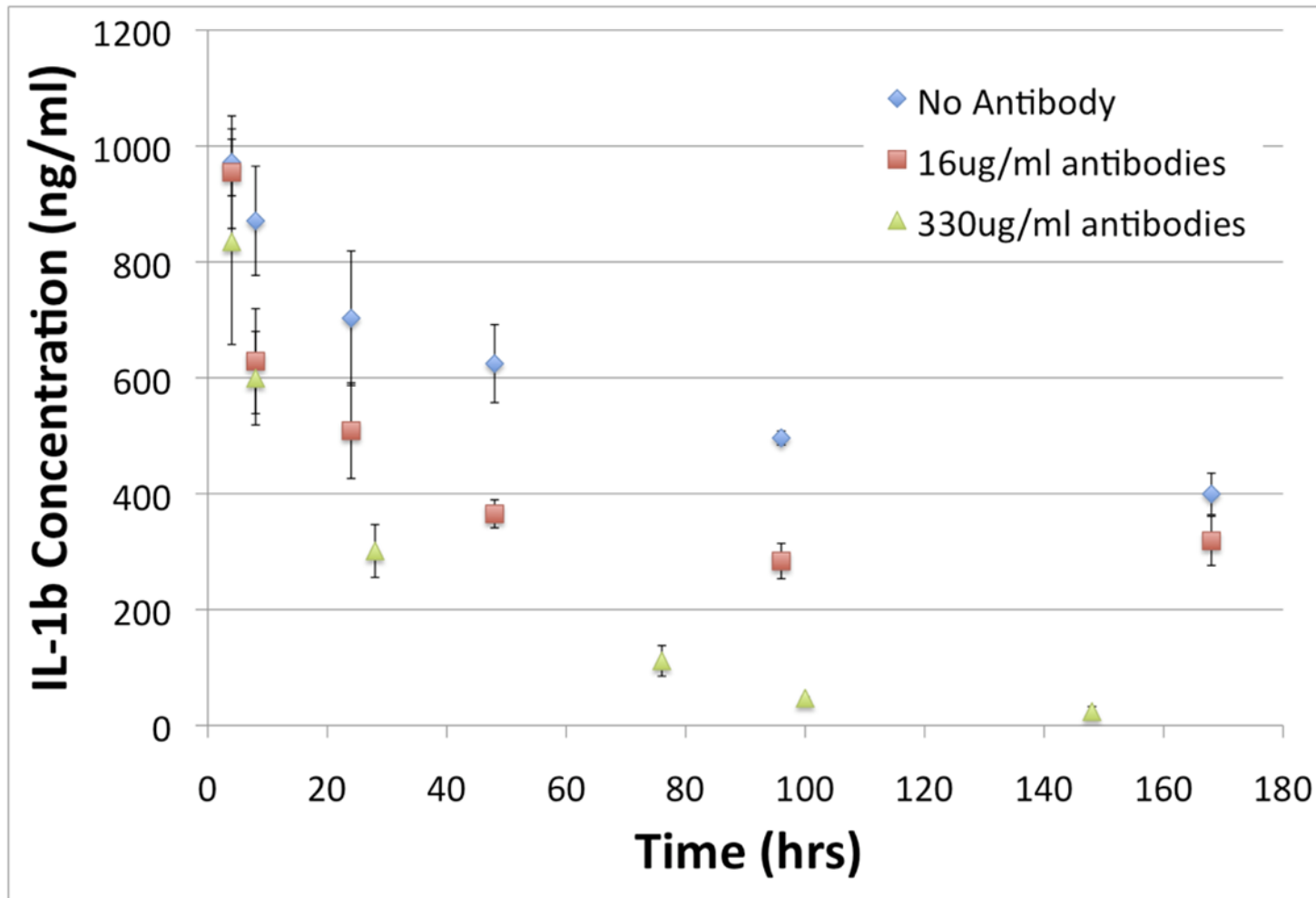


Verifying antibody activity in crosslinked gel





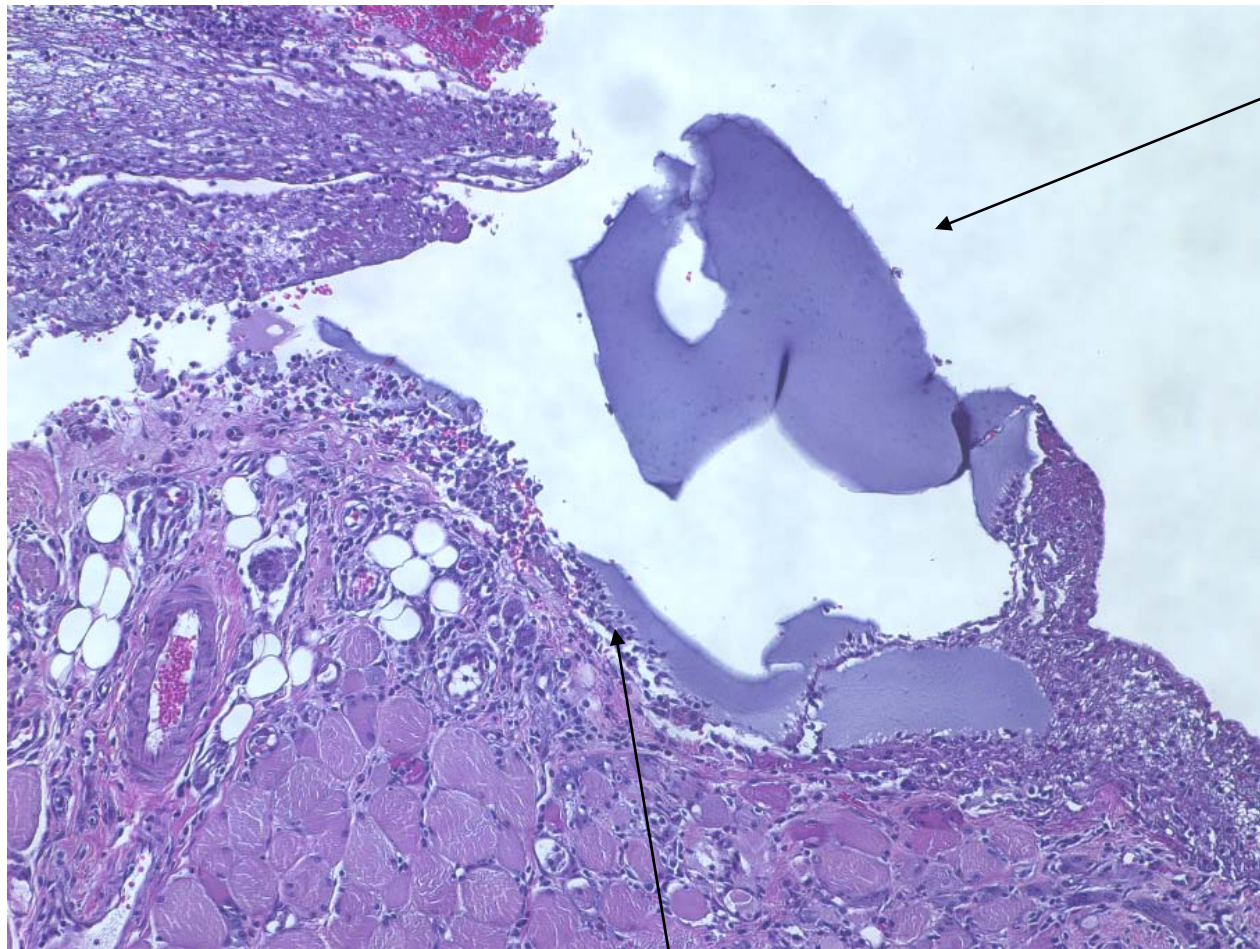
Results from experiments measuring cytokine diffusion into antibody-functionalized gels



*Antibody is still active after cross-linking but protein diffusion is slow.
Is the diffusion too slow to be effective at neutralizing cytokines in vivo?*



Unfortunately... yes



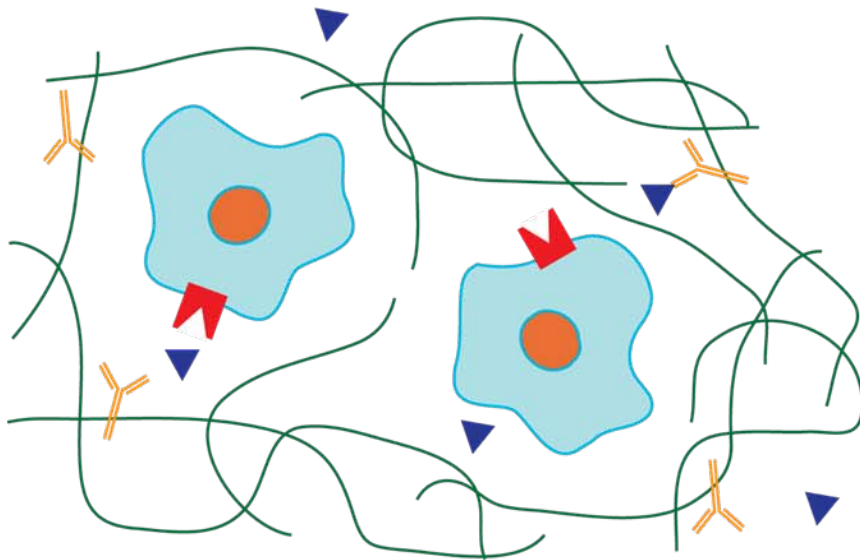
gel

Invading monocytes

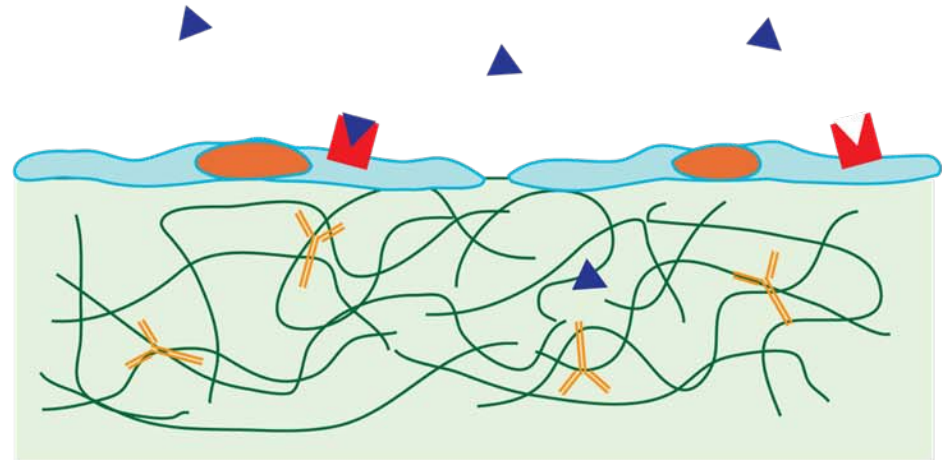


Matrix Geometry: Question of Diffusion and Release for Control of Inflammation

Uncrosslinked Network



Crosslinked Network



Uncrosslinked network mixed with cells

- Antibodies compete directly with cell receptors for cytokines
- Effective neutralization of inflammation in vivo

Cells excluded from crosslinked network

- Cytokines can diffuse into gel and are effectively sequestered
- Slow diffusion makes it more likely that cytokines will target cell receptor



Conclusions and Next Steps

- Covalent attachment of cytokine-neutralizing antibodies to hyaluronic acid retains antibody activity
- Implanting gels provides control over inflammation
 - Neutralization of $\text{TNF-}\alpha$ and $\text{IL-1}\beta$ resulted in significant decreases in number of invading macrophages and their dominant phenotype
 - Biomaterial geometry critical for local control of inflammation
 - What are the effects of neutralizing $\text{TNF-}\alpha$ only?
 - Effects of biological activities of HA?
 - Pharmacokinetics?
- Can biomaterials help biotech?
 - Raptiva
 - Etanercept
- Can biotech help biomaterials?
 - HA materials for tissue repair
- Can we commercialize the results of this research?
 - Local control of inflammation



Toward Commercialization

- Market for gel that inhibits healing is probably small
- Market for therapeutics capable of controlling inflammation locally is quite large
 - Partial-thickness burns
 - Diabetic foot ulcers
 - Pressure ulcers
 - Psoriasis
 - Rheumatoid arthritis
- How do we take this technology forward?



Bob Langer's 4-step program

- 1. **A huge idea** conceived by recognizing a critical societal need that could be met by inventing a platform product.
- 2. **A seminal paper** based on research to establish the science underlying the product concept and its efficacy.
- 3. **A blocking patent** derived from patent disclosures written in parallel with the research process, the goal being to have patents filed before the research paper's publication.
- 4. **Preliminary in vivo studies** in animals that demonstrated the efficacy of the research.



Prof. Robert Langer

The Langer Lab: Commercializing Science, Harvard Business Online (March 5, 2005)



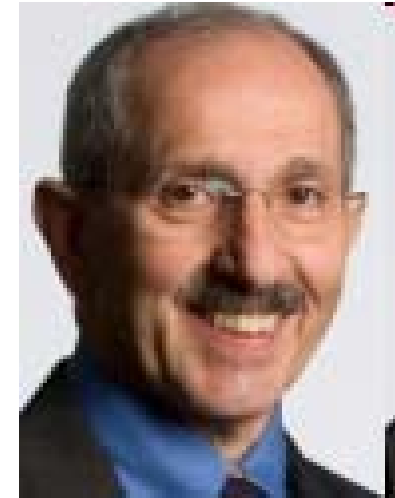
How am I doing?

- 1. **A huge idea** conceived by recognizing a critical societal need that could be met by inventing a platform product.
 - Remains to be seen
- 2. **A seminal paper** based on research to establish the science underlying the product concept and its efficacy
 - Working on it (patenting came first)
- 3. **A blocking patent** derived from patent disclosures written in parallel with the research process, the goal being to have patents filed before the research paper's publication
 - Yes!
- 4. **Preliminary in vivo studies** in animals that demonstrated the efficacy of the research
 - Yes, but need to think about relevant pre-clinical models
- 5. **Incorporate** Washburn Therapeutics, Inc.
 - Explain the name
 - Look for help...



Interactions with CMU's b-school

- CMU's Tepper School of Business currently ranked #3 in Entrepreneurship
- Prof. Art Boni is an expert in biotech commercialization and entrepreneurship
- Teaches a course on technology entrepreneurship to MBA students
 - “Technology Commercialization Business Development Strategy”
- Team-based project that uses CMU start-ups as case studies
 - Market research
 - Business plan development



Art Boni



Chris, Pamela, and Arijith

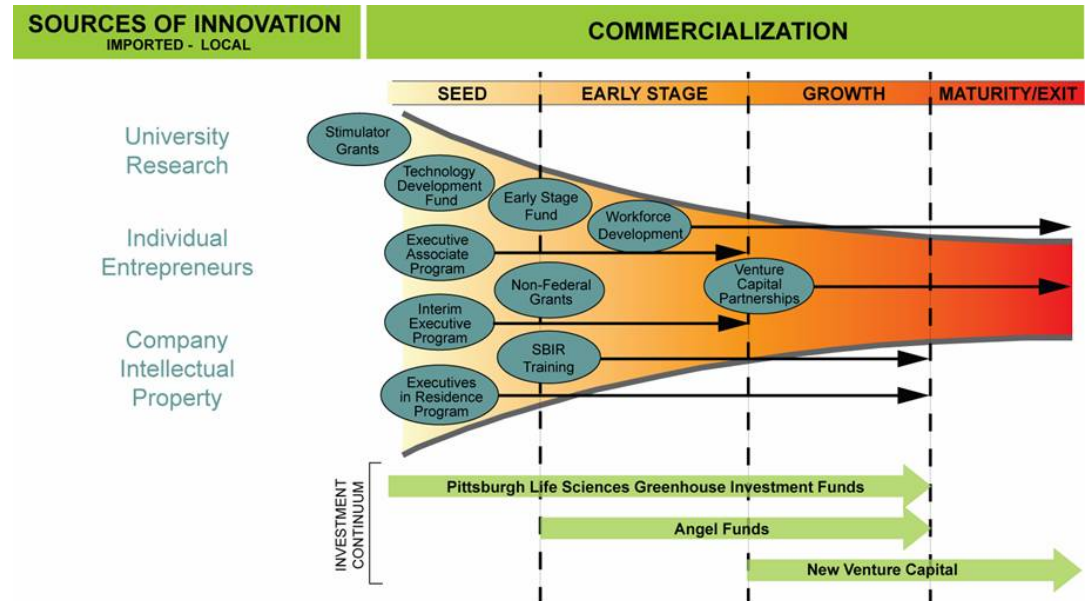


Pittsburgh Life Science Greenhouse

Mission:

To help foster a robust and self-sustaining research and economic community based on the life sciences. The PLSG will create, nurture and help establish a globally dominant life sciences industry in the Pittsburgh region.

- Founded on funds from State of Pennsylvania and local foundations
- Provides assistance in writing SBIR proposals, developing business plans, Executives in Residence program, and early-stage funding



Watch out, Boston!



Impact of Inflammatory Conditions in the United States



Diabetic foot ulcers
>1,000,000/year



Burns
80,000/year



Rheumatoid arthritis
1.3 million



Psoriasis
7.5 million



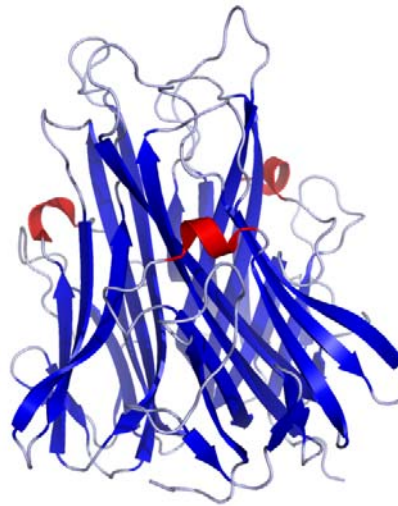
Impact of Inflammatory Conditions in the United States



Diabetic foot ulcers
>1,000,000/year



Burns
80,000/year



TNF- α



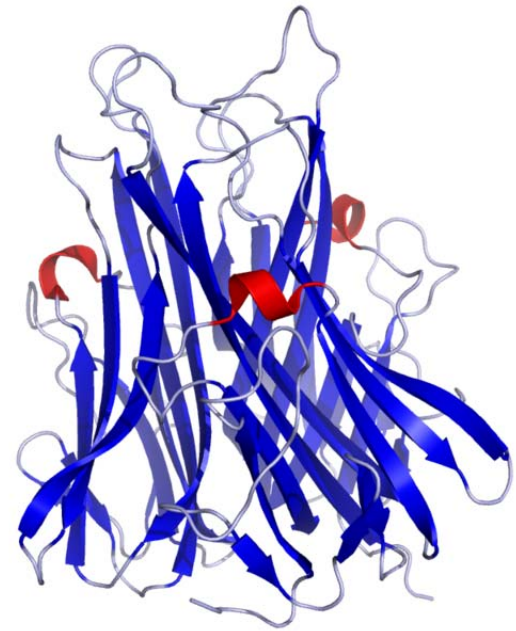
Rheumatoid arthritis
1.3 million



Psoriasis
7.5 million



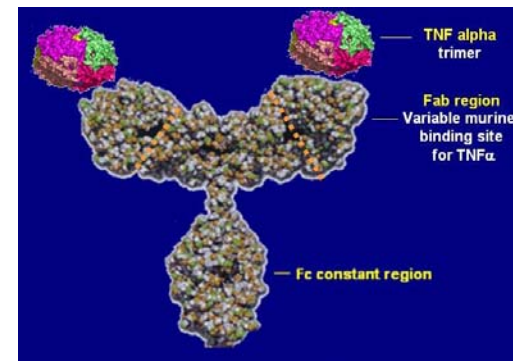
- Tumor necrosis factor- α is a pro-inflammatory cytokine that plays a central role in inflammatory responses
- Established therapeutic target in psoriasis, rheumatoid arthritis, Crohn's disease, ankylosing spondylitis
- Known to be important in the progression of chronic wounds and burns



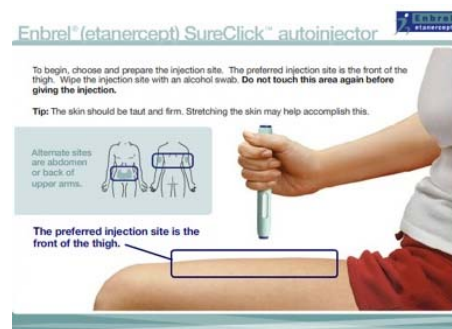
TNF- α



- Several protein inhibitors have been developed
 - Infliximab (Centocor)
 - Etanercept (Amgen/Wyeth)
 - Adalimumab (Abbott)
 - Certolizumab pegol (UCB)
- Global sales > \$13.5b/year
- FDA has issued black box warnings for use in psoriasis
 - Complications due to systemic immunosuppression
- Need technology to localize activities of TNF- α inhibitors

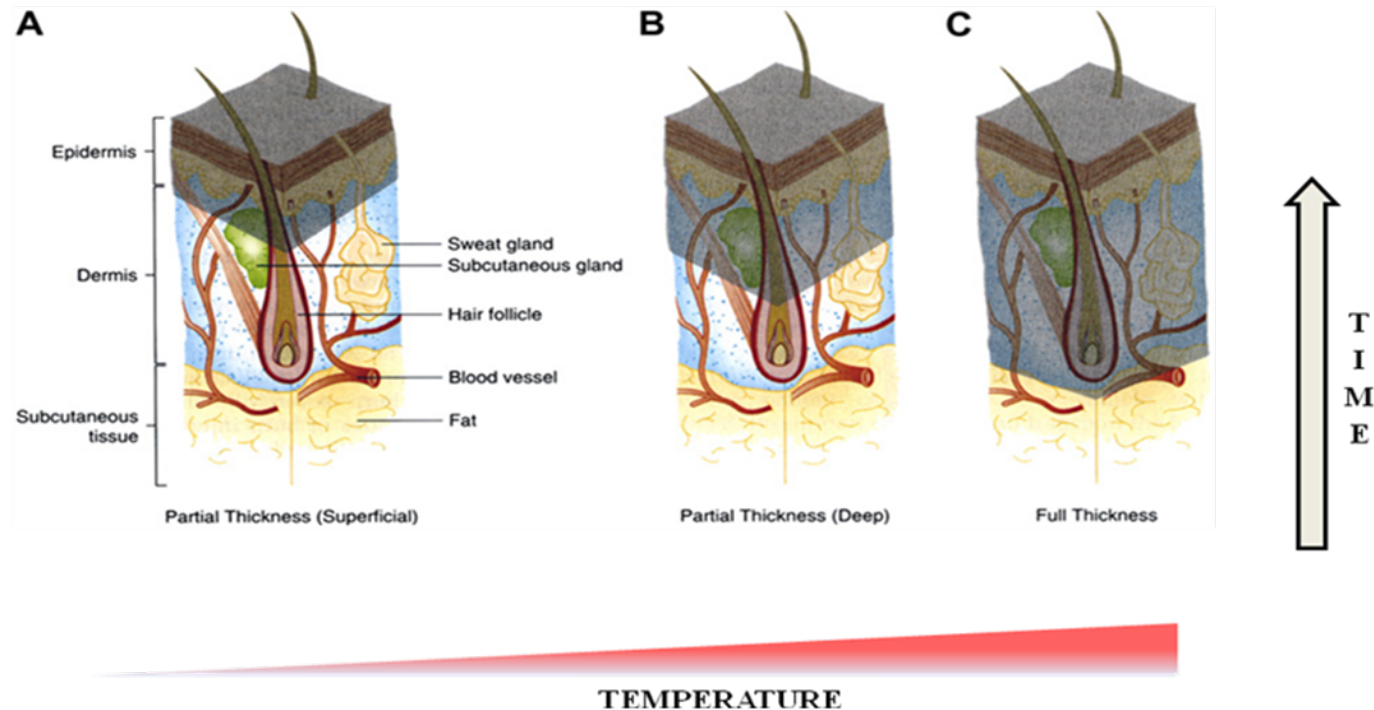


Infliximab (Centocor)





Tissue Responses to Thermal Injuries

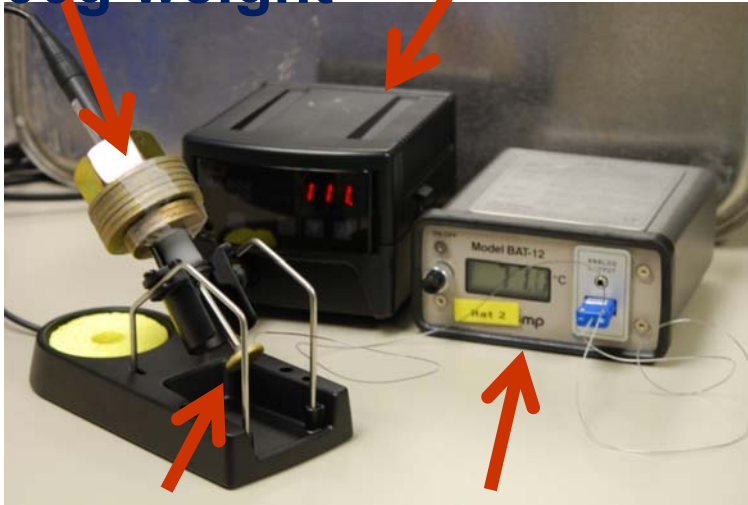


- Roughly 80,000 burns/year require hospitalization
 - Significant contributing factor to combat injuries
- Intense inflammatory response (hyperinflammation) immediately following burn
 - Results in continued tissue death for days after initial injury
- Current treatment involves removal of dead tissue and application of saline solution and antibiotic ointments

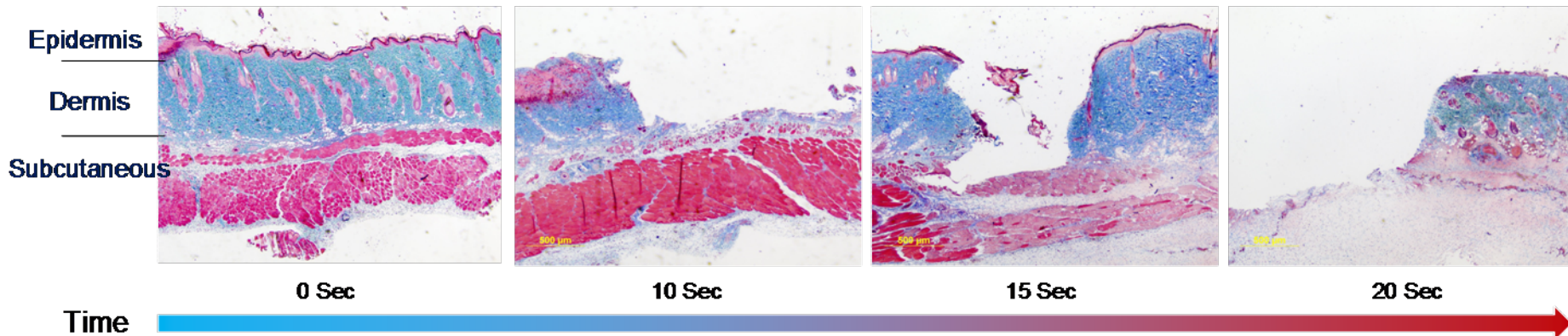


Rat Model of Thermal Injury

500g weight **Heat Source (soldering unit)**



Brass Disk **Thermometer**





Improving Burn Closure with Cytokine-Neutralizing Gels



Improving Burn Outcomes with Cytokine-Neutralizing Gels

- Hyperinflammation following thermal injury results in increased tissue necrosis
- Partial-thickness burns in rats were treated with saline or HA-mAb conjugate
 - Eschar removed after 1 day
 - Sites then treated and covered with Tegaderm bandage
- Saline-treated sites displayed cracked, necrotic tissue at day 4
- HA-mAb-treated sites displayed granulation tissue at day 4 and showed signs of faster closure by day 7





Development Pathway for Cytokine-Neutralizing Gels as Burn Therapy

- Validate in rat model
 - Need biochemical data
- Validate in pig model
 - Healing through re-epithelialization
 - Scar-forming model
 - Need antibody fragment
- FDA approval before clinical trials
 - Pharmacokinetics data
 - Biocompatibility data
 - Partner with manufacturer of approved TNF- α inhibitor
 - Possible 505(b)(2) mechanism



Innovation in the engineering curriculum at CMU

- Responsive to tectonic shifts that have been occurring in the business environment and their impact on the engineers we graduate and their education.
- The vision put forth is that the engineer of the future should be able to "enable, manage, and deploy innovation in multicultural, multilingual, distributed environments".
- Two-step process:
 - (1) Focus on Gen Ed requirements and in developing a strategy for incorporating innovation
 - (2) every department would focus on incorporating "innovation across the curriculum" in their courses/curriculum.



Dean Pradeep Khosla



Entrepreneurship project in the classroom: Polymeric Biomaterials

- Polymeric Biomaterials is a senior-level elective offered in MSE and BME
- As part of the course, students used to write term papers on subjects related to polymers in medicine
 - Unstructured
 - Difficult to grade
 - Very few students appeared to get anything out of this
- Last year I replaced it with an project in entrepreneurship
 - Team-based
 - Students propose technologies and commercialization
 - Focus on wound healing/cutaneous drug delivery



Implementing Entrepreneurship in Polymeric Biomaterials

- In addition to covering course material students learned to:
 - Read patents
 - Understand the patent process
 - Identify opportunities for new technologies
 - Unmet needs
- Guest lectures
 - Prof. Tom Emerson (CMU School of Business)
 - Can entrepreneurship be taught?
 - Mr. Alan West (PLSG EIR)
 - Survey of current technologies for wound healing
 - Experiences in medical device companies





Milestones

- 1. Leads e-mail me improved team name and e-mails of team members
- 2. Survey competing products/companies
- 3. Survey patent literature
- 4. Summarize clinical understanding of target application
- 5. Propose novel technology and explain rationale for its efficacy
- 6. Propose product name; identify regulatory testing pathway (510(k) vs. PMA); suggest method for production
- 7. Present product to class; submit final report



Products of Entrepreneurship Project

Team and/or Product Name

Kancer Killers (WashDerm)
Team "Insert Creative Name Here"
NewellStitch
DermaAdvantage
PricklyFix
Team Mufasa (Scar beta)

Application

Basal cell carcinoma
Diabetic foot ulcers
Self-anchoring sutures
Eczema
Diabetic foot ulcers
Scarless wound healing

- Projects tended to focus on cutaneous drug delivery
- Broad range of applications
 - Much broader than I originally suggested
- The best projects didn't necessarily come from the students with the highest grades in the course



Protects the affected site from the sun
Effective cycle therapy allows for less irritation, redness, and swelling
Dual system increases the response rate

WashDerm

(Fluorouracil) Patches

WashDerm presents a dual patch system that moves to try and reduce painful symptoms associated with standard topical chemotherapy. The treatment starts with stage 1, where a tretinoin based patch is applied to the affected area. The tretinoin acts by removing parts of the stratum corneum, giving way to a faster diffusion rate of the second stage drug - fluorouracil. In the aforementioned stage 2, a fluorouracil (5-FU) patch is applied to start the attack and removal of cancerous cells. 5-FU is a thymidylate synthase inhibitor, which when interrupting the enzyme, blocks the synthesis of pyrimidine thymidine; a nucleotide required in the DNA replication of the cell. What makes WashDerm so advanced is its unique double drug layer scheme with two individual cellulosephane central membranes. These two membranes delicately control the diffusion of 5-FU out of the matrix and onto the skin. This improved regulation of drug release presents a solution to more efficient cycle therapy. Rather than normal topical creams which are simply applied with no real limit, WashDerm carefully curbs the amount of fluorouracil released over longer periods of time. This allows a potentially large decrease in skin irritation, redness, swelling and painful sores; overall leading to a much more comfortable healing process. Another added advantage is WashDerm's opaque backing which protects the injury site from the sun's harmful rays - permitting the patient to freely carry on with everyday outdoor activities without the hassle of worry.

Kelly Nakamura, Hilary McWilliams, Jennifer Shih, Reva Street





Some thoughts on integrating research, education and entrepreneurship

- Exciting synergies between these three areas
 - Slows progress but hopefully the final product will be worth it
- Potential benefits are appealing
 - Critical experience for science and engineering students
 - Access to broader range of funding sources
 - Direct support of regional and national economic development
- Requires university support
 - Translational research
 - Curriculum development
 - Start-up incubation
 - Patience
- Perhaps the best way to develop new technologies?
 - Research and education are valuable even if technology flops
 - Dissemination of ideas could spawn new ideas



Acknowledgments

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- Prof. Gustavo Rohde

CMU Chemistry

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- Prof. Bruce Armitage
- Prof. Hyung Kim

CMU Chemical Engineering

- Annette Jacobsen/CPS Lab
- Lynn Walker





Research Conclusions

Accomplishments

- Developed new class of biomaterials that locally regulate cytokines and control inflammation
- Validated technology in vitro and in vivo
- Identified design principles for 2nd generation materials

Future Directions

- Down-stream effects on healing
- Dose-response curves for neutralizing IL-1 β and TNF- α
- Neutralization of other pro-inflammatory cytokines
 - Interferons
 - Interleukin-6
- Increasing the activities of anti-inflammatory cytokines
 - Interleukin-10
- Testing in scar-forming animal models
 - Full-thickness wounds
 - Burns
- Testing as cell-delivery vehicle
 - Autologous cells
 - Stem cells



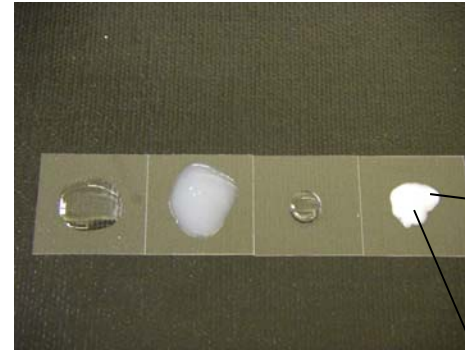
Next steps

- Develop porous, multi-layer bioscaffolds that can be used in full-thickness wounds
 - Incorporate other therapeutics in constructs
- Test constructs in scar-forming animal models
 - Can altering inflammatory phase influence healing outcomes?

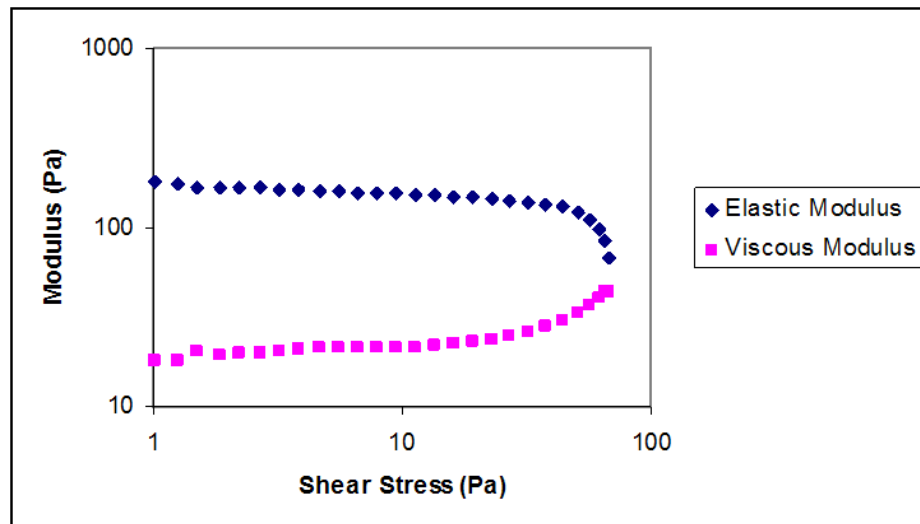


Optimizing Mechanical and Transport Properties in Microstructured HA-RGD-mAb Gels

- Microstructured HA-based gels have been prepared that display complex viscoelastic behavior
 - Similar to Bingham Plastic
 - Lotion, toothpaste, etc.
- Gels are composed of strongly interacting colloidal dispersion that can be easily delivered to an injury site but resists deformation once shear stress is removed
- Diffusion of cytokines to mAb embedded in microstructured gels is expected to be efficient allowing for rapid neutralization



Joseph Prata





Some Lead Applications

- Chronic wounds
 - Phase I SBIR research to begin in January
- Burns
 - Testing animal models at the Institute for Surgical Research
- Surgical adhesions
 - Initiating collaborative research with Genzyme
- Psoriasis
 - Working with PLSG to raise capital for clinical trial
- Rheumatoid arthritis/osteoarthritis
 - Looking for the right collaborator!



Hank Safferstein (PLSG)

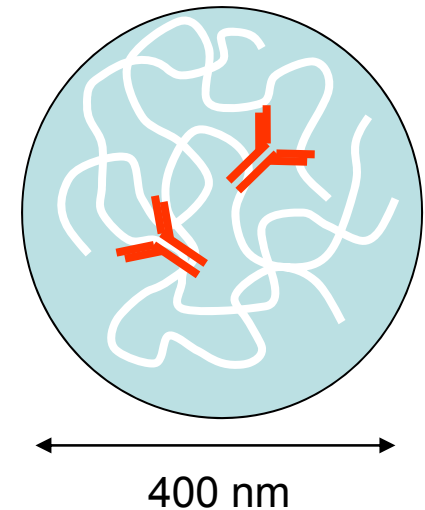


Toward HA-mAb Nanogels

- Nanometer-size gels based on HA-mAb could provide enhanced sequestration of cytokines with fast diffusion times
 - Cytokine roach motels
- Nanogels would prevent direct interactions between antibodies and effector cells
- Tuning interparticle interactions would create viscoelastic suspensions suitable for a broad range of applications



Joseph Prata

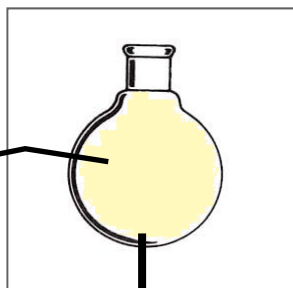




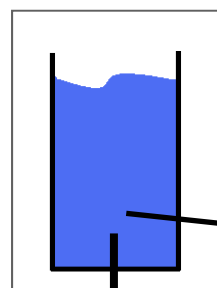
Emulsion Polymerization of Hyaluronic Acid

Reaction is prepared separately in the hexane and aqueous phases.

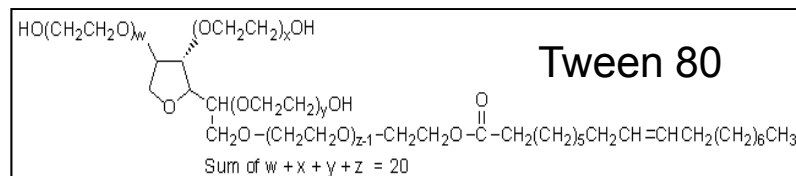
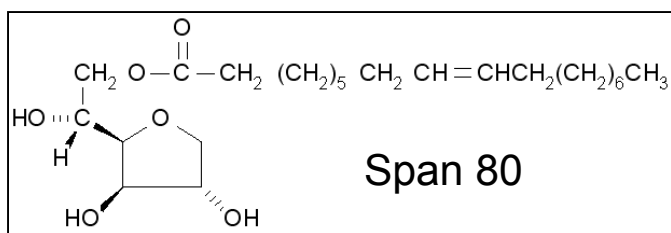
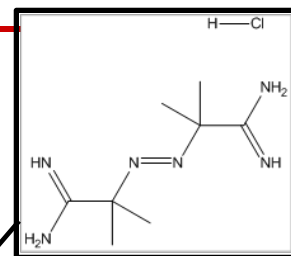
•Hexanes
•Span 80 /Tween 80



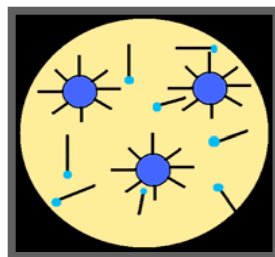
•HAGM
•Thermal Initiator



Initiator degradation temperature is 53°C.



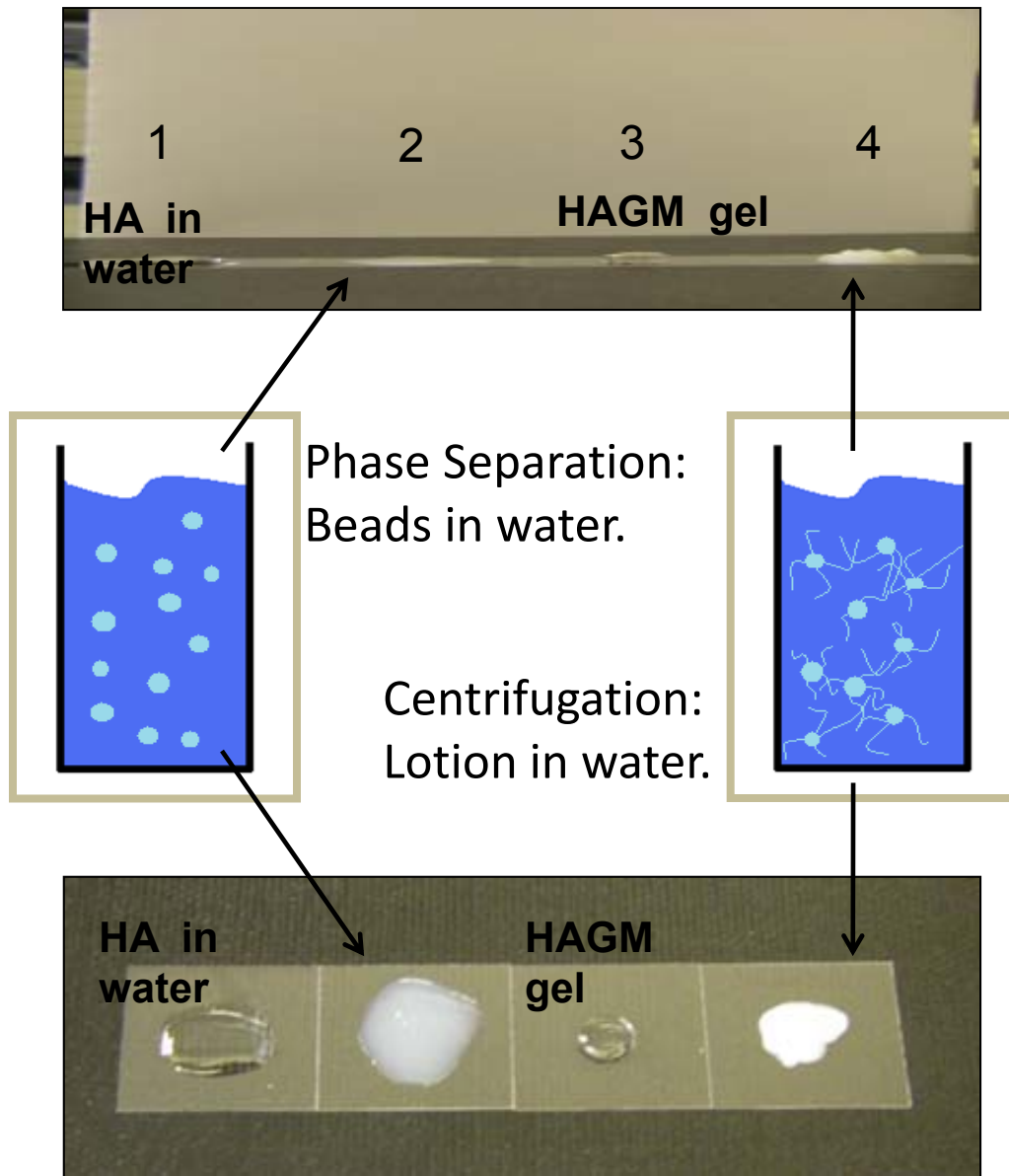
High frequency sonication is used to induce emulsification.



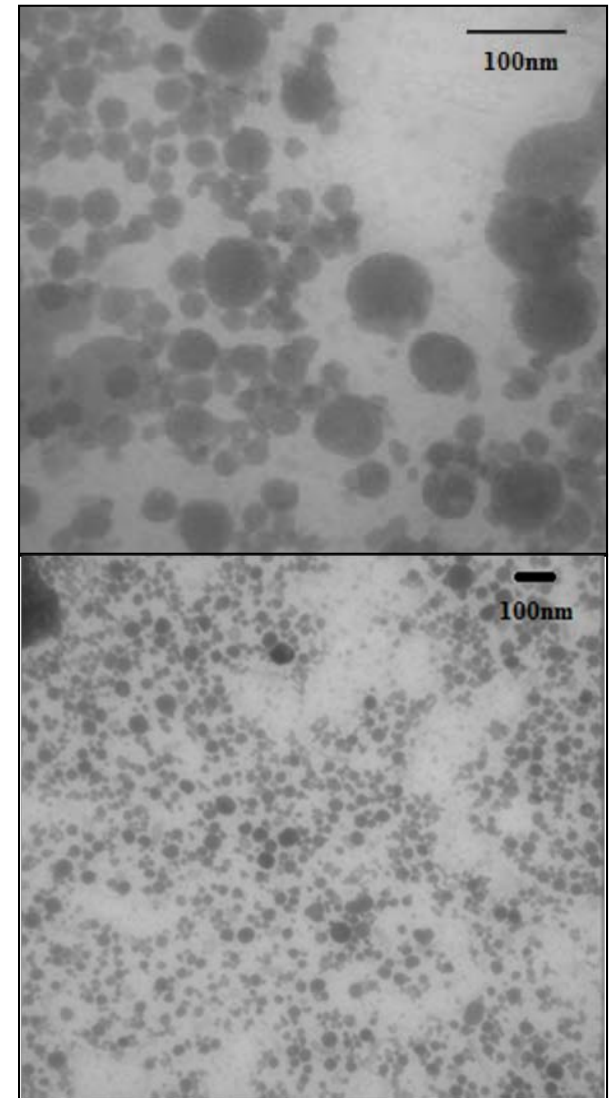
Micelles in hexanes are on the order of 150nm, but swell to 400nm in water.



Viscoelastic Formulations of Hyaluronic Acid

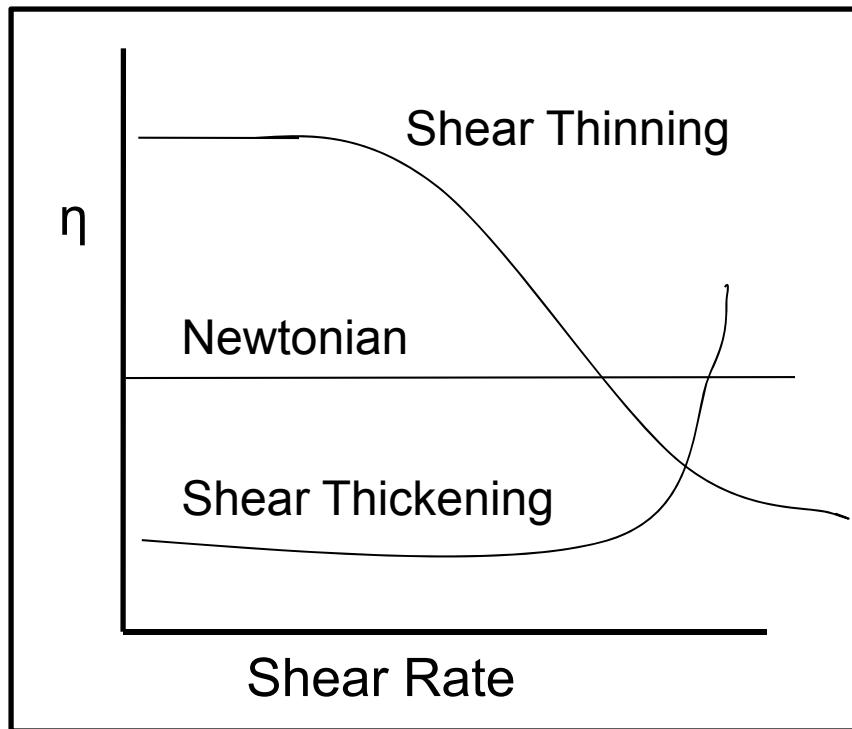


TEM Images - DM 14% - Centrifuged

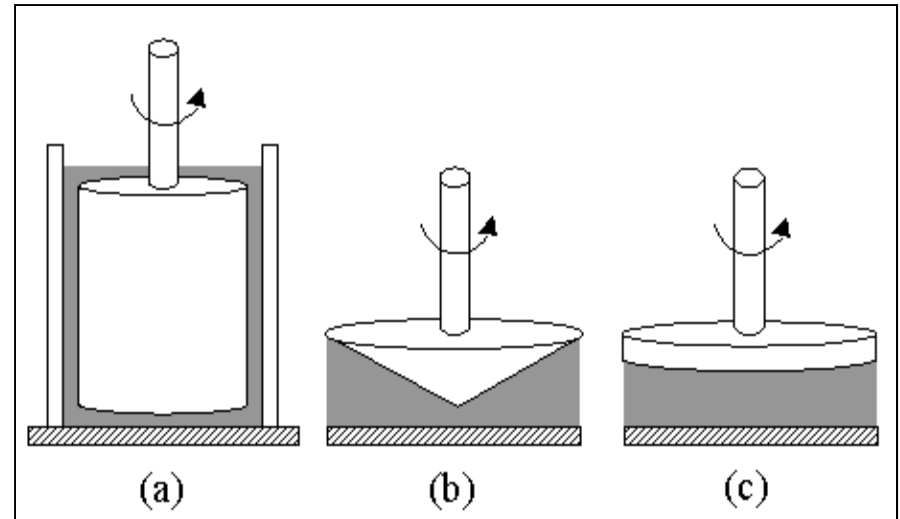




Measuring Viscoelastic Properties of Polymers

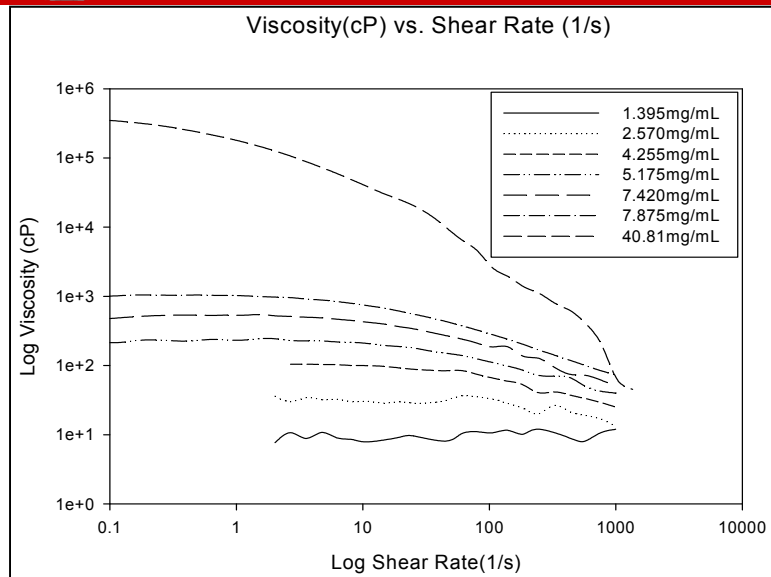


- Measurement of strain or strain rate as a function of stress provides information on elastic or viscous responses of materials





Viscosity of Hyaluronic Acid Solutions



- Consistent with previous results, hyaluronic acid in water behaves like an entangled solution

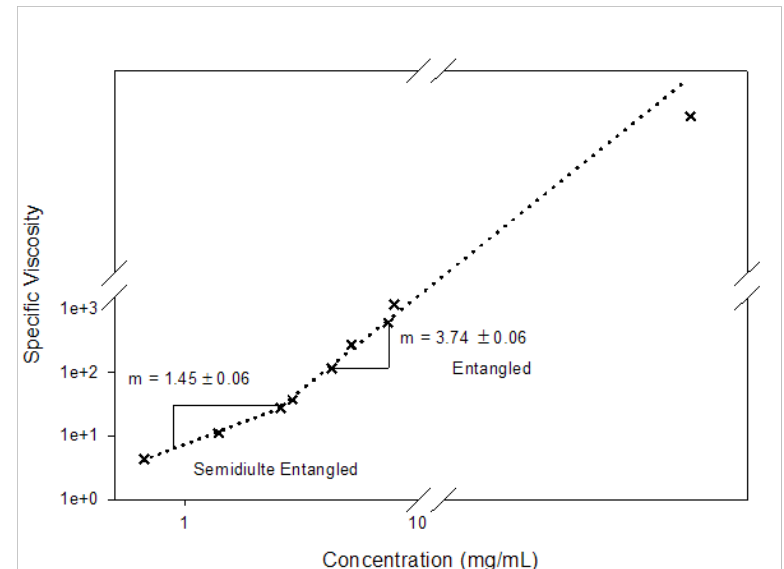
- Identical trends were observed in viscosity as well as entanglement regions.

Semidilute entangled: 0.70mg/mL

Literature: 0.59mg/mL

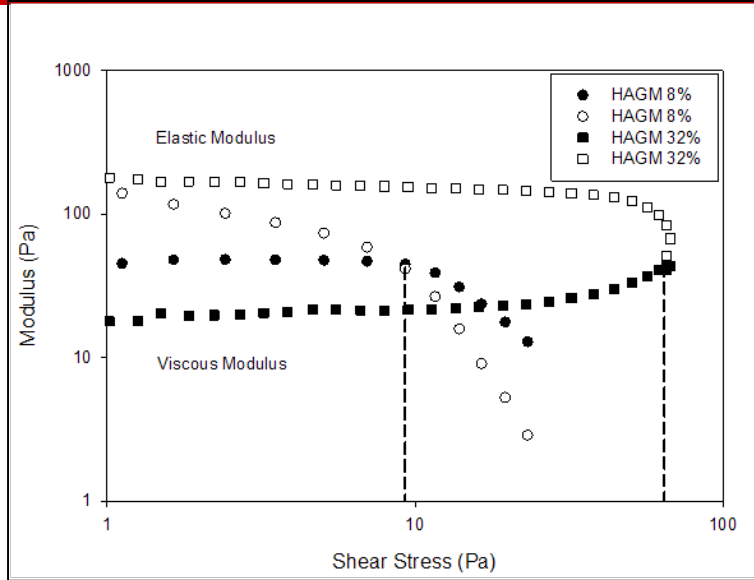
Entangled: 2.5mg/mL

Literature: 2.4mg/mL



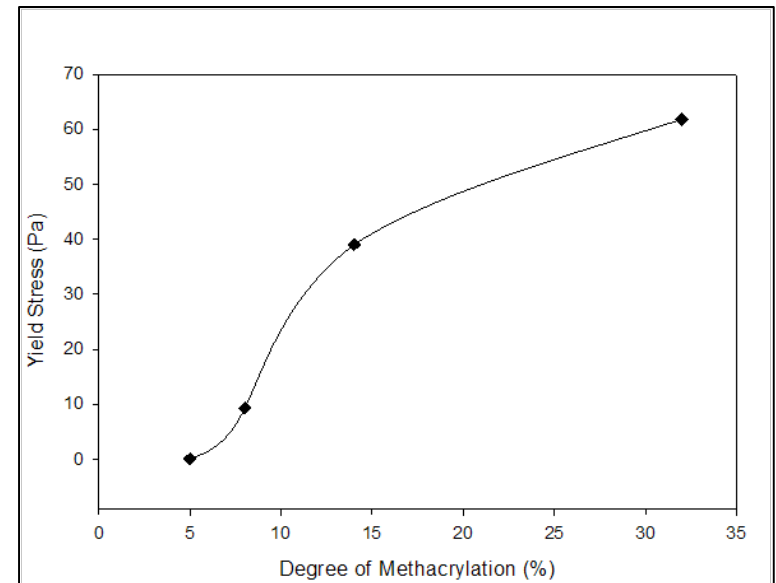


Viscoelastic Responses of Hyaluronic Acid Nanogels



- Upon increasing methacrylation degree, yield stress increased. This trend is similar to macroscopic HAGM gels.
- The stress at which the elastic and viscous moduli cross is the yield stress.
- Bingham plastic shows Newtonian behavior after yield point.

- Cross-linking and physical entanglement cause the texture of the lotion and high elastic modulus.
- Yield stress increases, but at a decreasing rate due to brittleness of the HAGM at high degree of methacrylation.



Next: Incorporation of antibodies in nanogels