The Glycome: Sweet Opportunities to Engineer Biomaterials

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Centennial Symposium, September 26, 2013

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The Secret Lives of Polysaccharides: A Story in 4 Acts

Act 1: (Prologue) The Importance of Polysaccharide Nanostructure

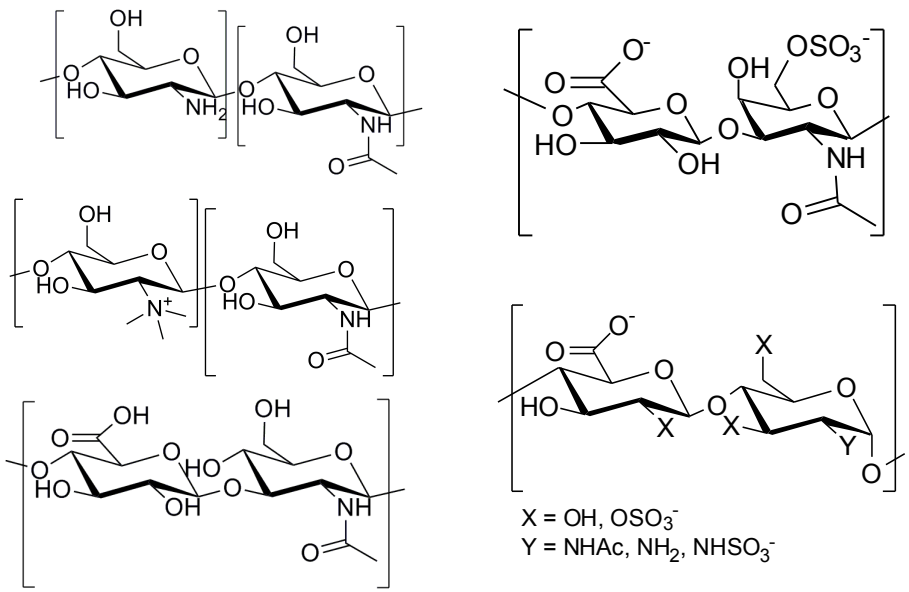
Act 2: Polysaccharide Nanostructures we can Engineer

Act 3: Engineered Bio-mimetic Function in Polysaccharide Nanoassemblies

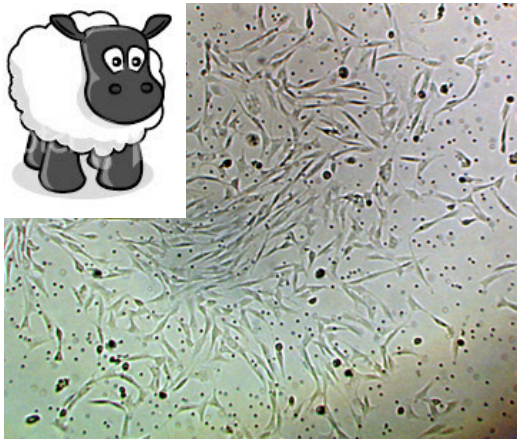
Act 4: A Tissue Engineering Application

Epilogue: Trailer for upcoming episodes?

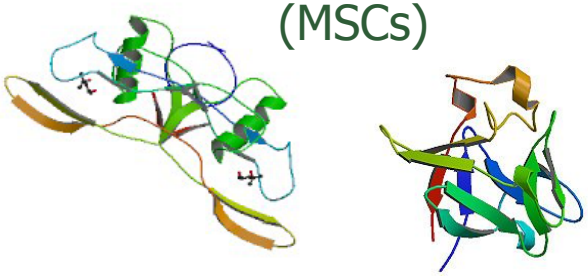
Cast of Characters



LOTS of DIVERSITY
~50 disaccharides in mammalian glycans
Cellular glycome – 100,000 to 500,000 species
Maybe several 1000's of glycan "determinants"
that interact with glycan binding proteins



Bone marrow-derived mesenchymal stem cells (MSCs)



Growth Factors
FGFs, BMPs

Saccharides Regulate Protein Function

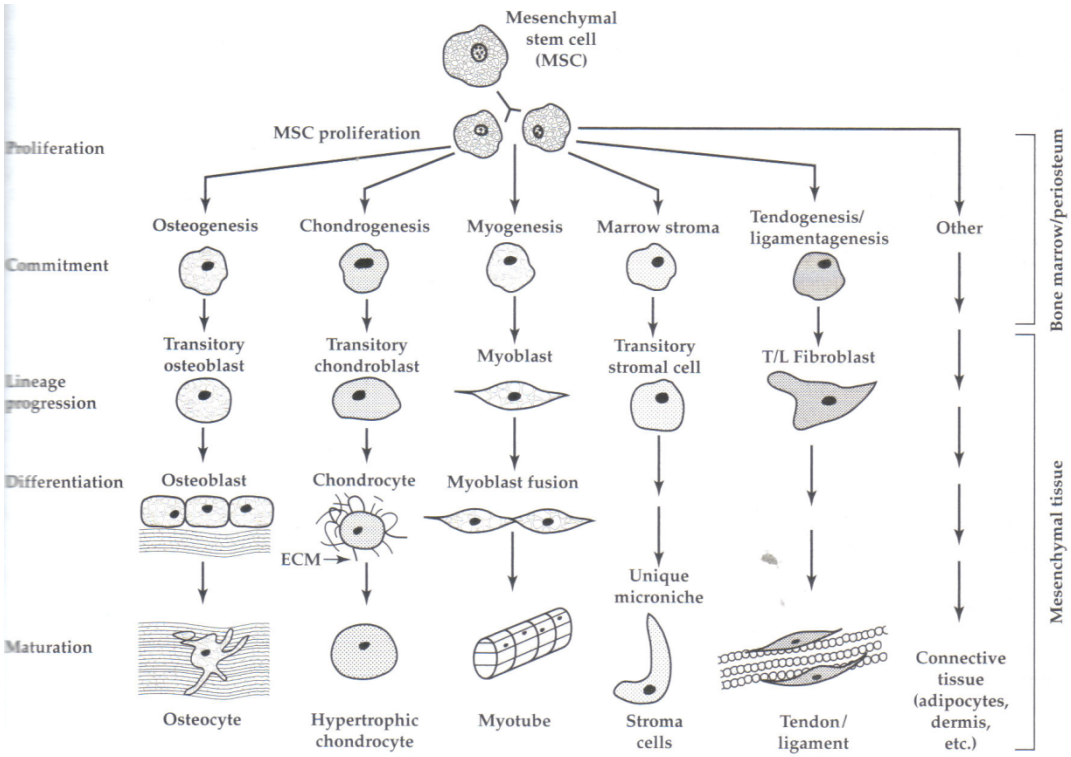
Emil Fischer, 1894:
"To give an illustration I will say that enzyme and glucoside must fit together like ***lock and key*** in order to be able to exercise a chemical action on each other."

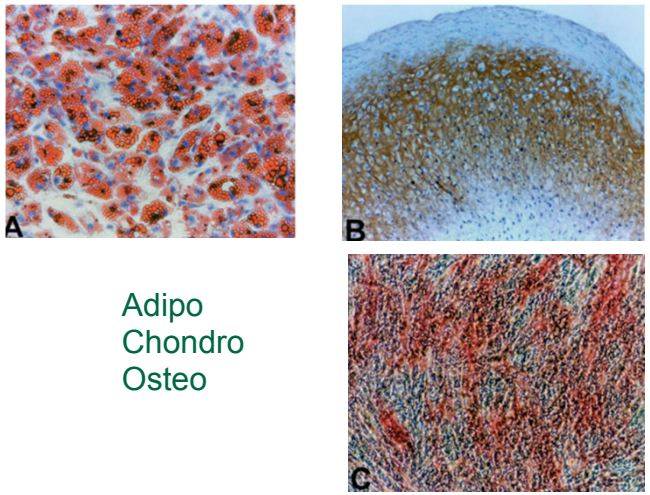
But… this is only part of the story:

1. Polysaccharides do *not* have template-determined sequences – they are more random than proteins and nucleic acids.
2. Many heparin-binding proteins (for example) interact with the same common motifs (e.g. sulfation patterns) but additional modifications within the motif are inconsequential. → Overlapping functional sequences that interact with multiple proteins → Promiscuity

Polysaccharides may be more aptly described as the *master keys* that can fit many locks.

MSCs





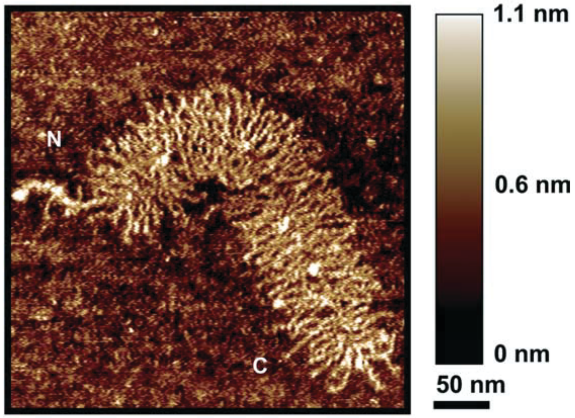
Adipo
Chondro
Osteo

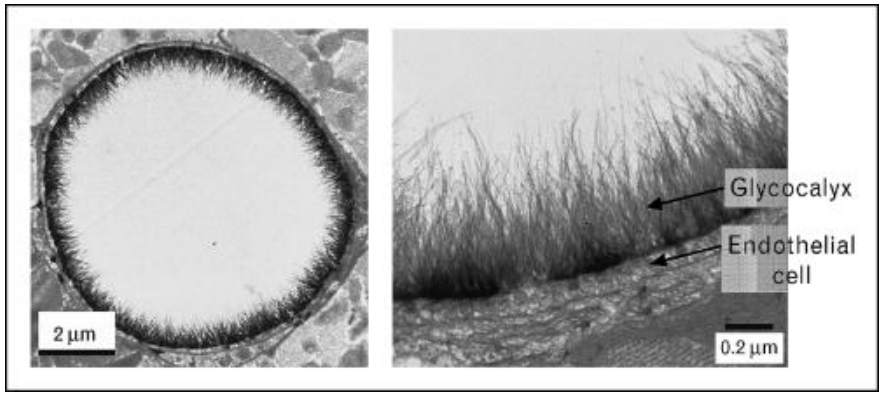
Pettinger, M.F. *et al.* "Multilineage Potential of Adult Mesenchymal Stem Cells." *Science* **284**:143-147 (1999).

Lanza, R.P.; Langer, R.; Vacanti, J., Principles of Tissue Engineering. Second ed. Academic Press: San Diego, 2000; p607

Act 1: Polysaccharide Nanostructure

TMAFM of aggrecan, Ng *et al. J Structural Biol.* **142**:242-257 (2003).





TEM of endothelial glycocalyx. Nieuwdorp M, et al., *Current Opinion in Lipidology*. 16:507-11. (2005).

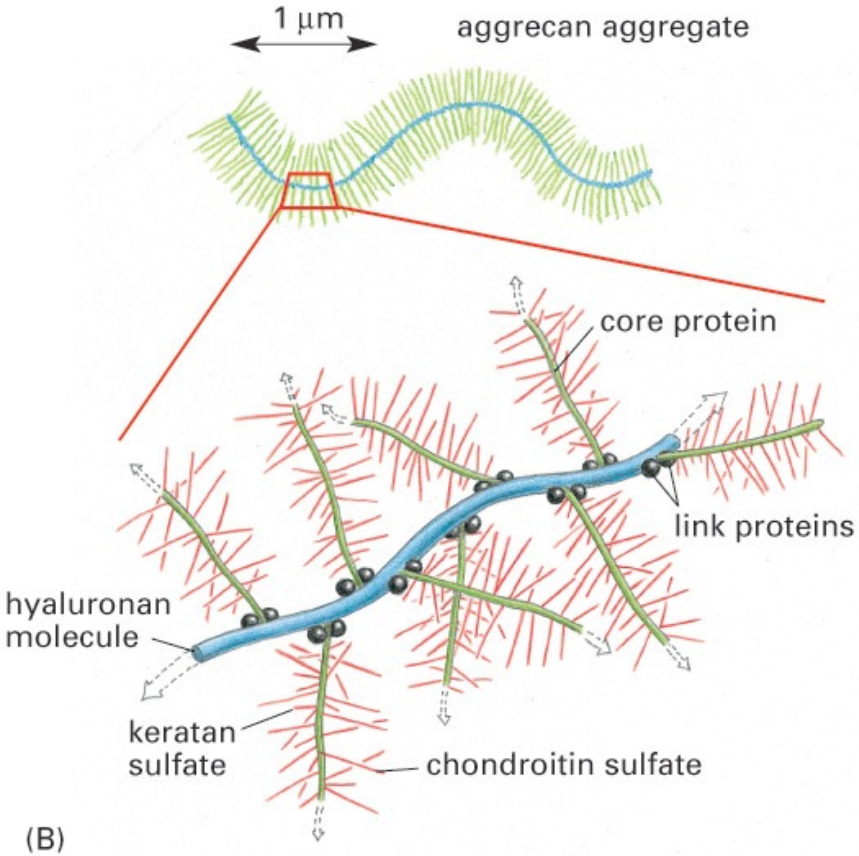
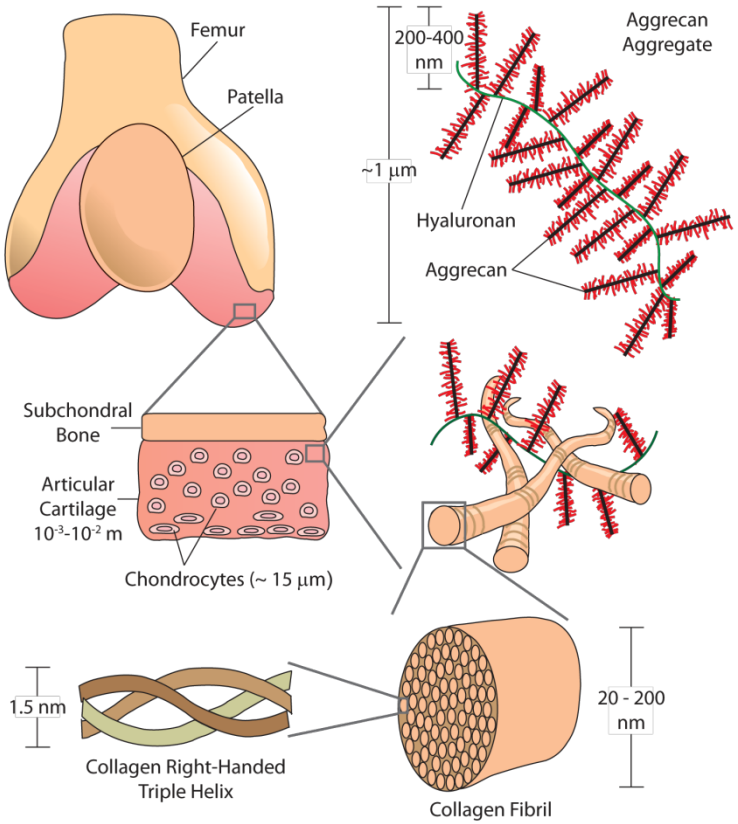
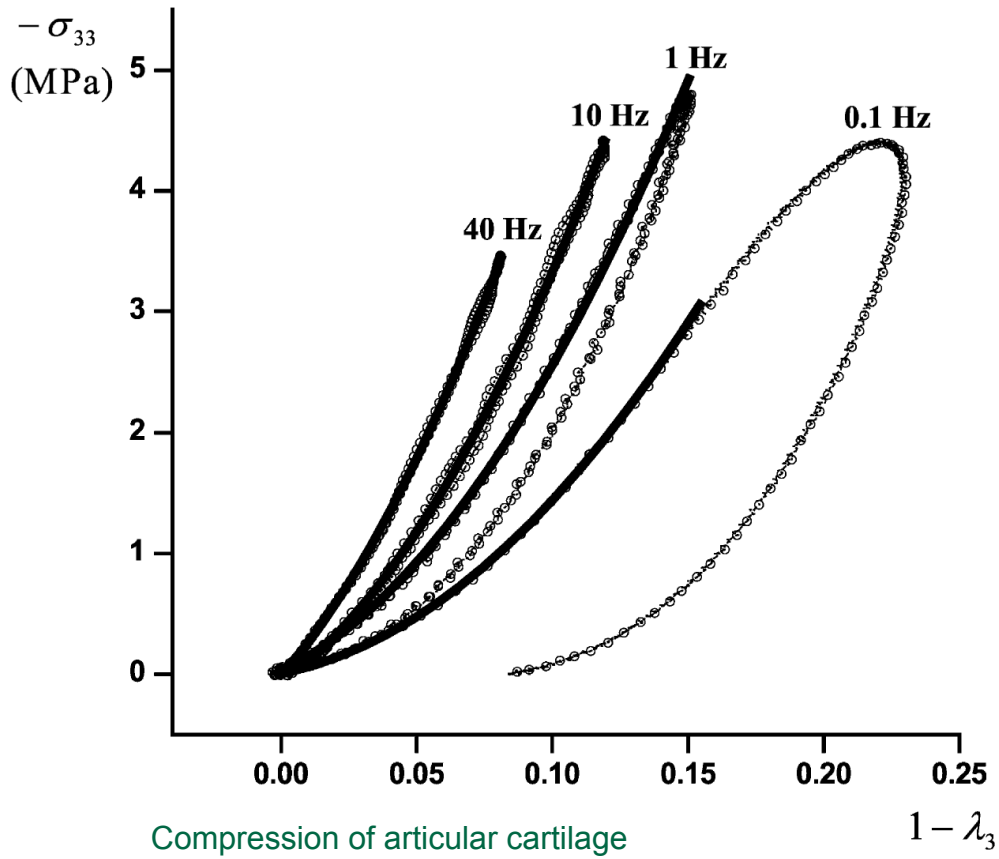


Figure 19–41 part 2 of 2. Molecular Biology of the Cell, 4th Edition.

Polysaccharide Nanostructure Controls Biochemistry and Biophysics

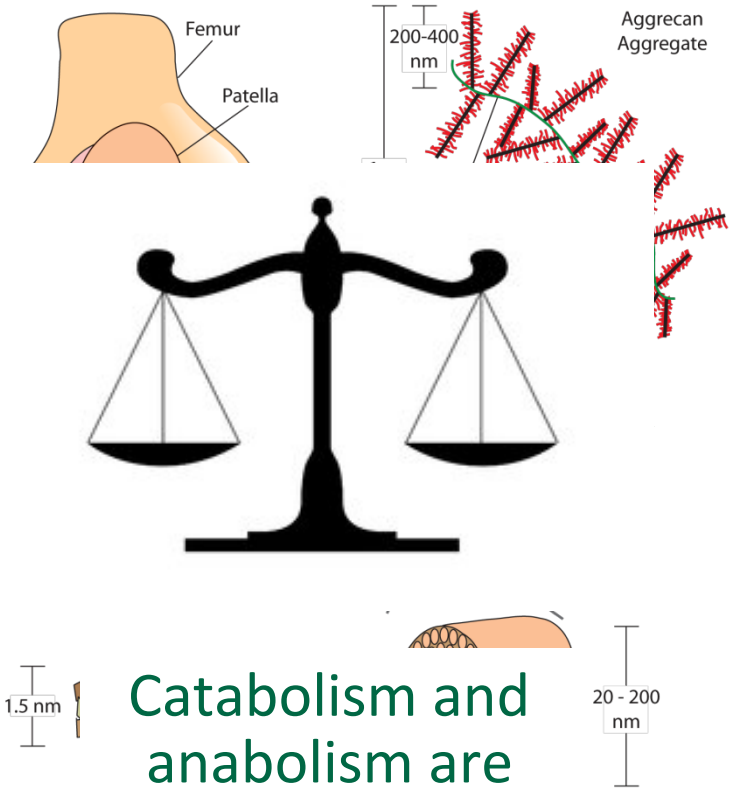


J. Almodóvar and M.J. Kipper, In *Nanotechnology for Tissue Engineering and Regenerative Medicine*, K.C. Popat, Ed., 2011 pp. 13-1 – 13-26.



Compression of articular cartilage
S. Park, C.T. Hung, G.A Ateshian, *Osteoarthritis and Cartilage*, **12**:165-73, 2004.

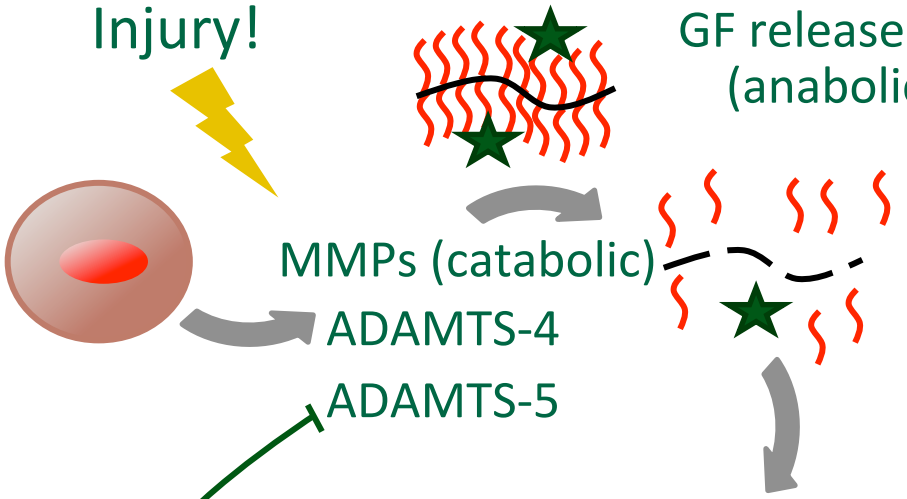
Polysaccharide Nanostructure Controls Biochemistry and Biophysics



Catabolism and anabolism are balanced

Normal function

Injury!



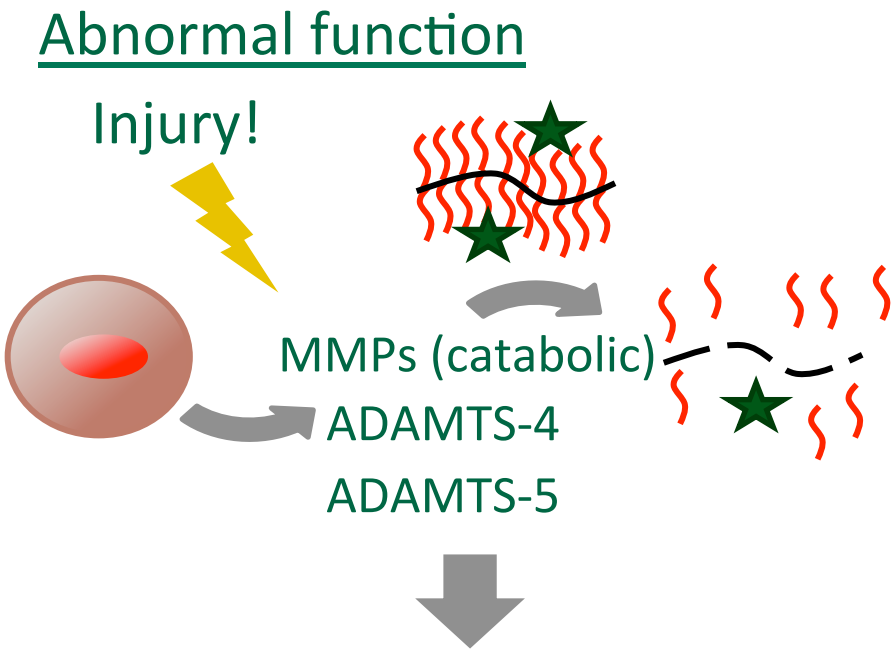
GF release (anabolic)

MMPs (catabolic)
ADAMTS-4
ADAMTS-5

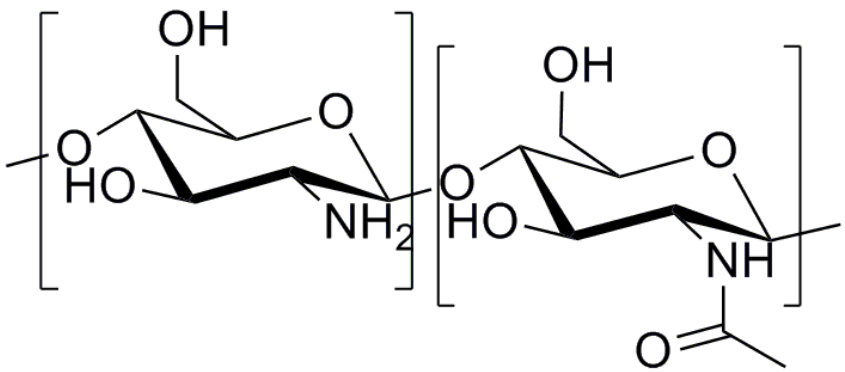
GF's do many things!
- Cell proliferation/differentiation
- ECM synthesis
- TIMP production

Polysaccharide Nanostructure Controls Biochemistry and Biophysics

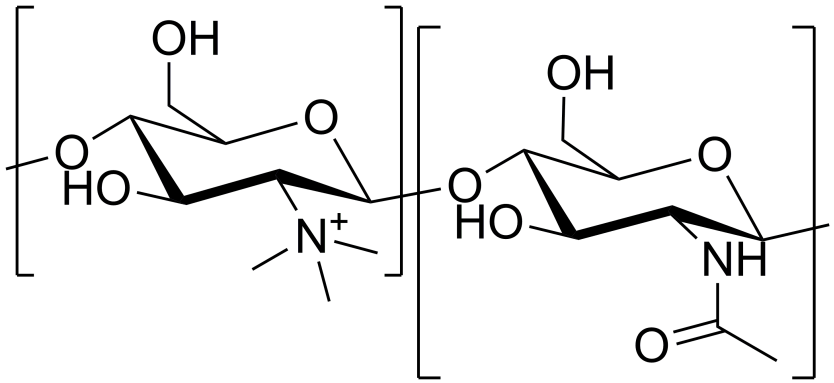
Catabolism and anabolism are *not* balanced



Polysaccharides have Useful Biological Functionality



Chitosan



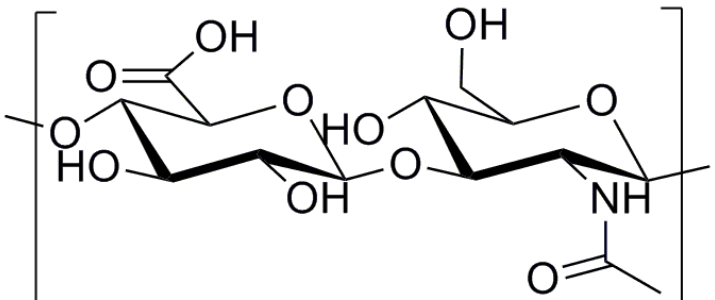
Quaternary Chitosan

- Antimicrobial
- Support mammalian cell growth
- Promotes wound healing, fibroblast migration, growth factor (PDGF, TGFb1) and interleukin (leukotriene B_4, IL-1, IL-8)
- Used for blood vessel, skin, bone, cartilage, and liver tissue engineering

Polysaccharides have Useful Biological Functionality

- Organizes other glycosaminoglycans in extra cellular matrix
- Soft materials have organization at multiple length scales, resulting in multiple relaxation time scales.

pK_a = 2.9



Hyaluronan

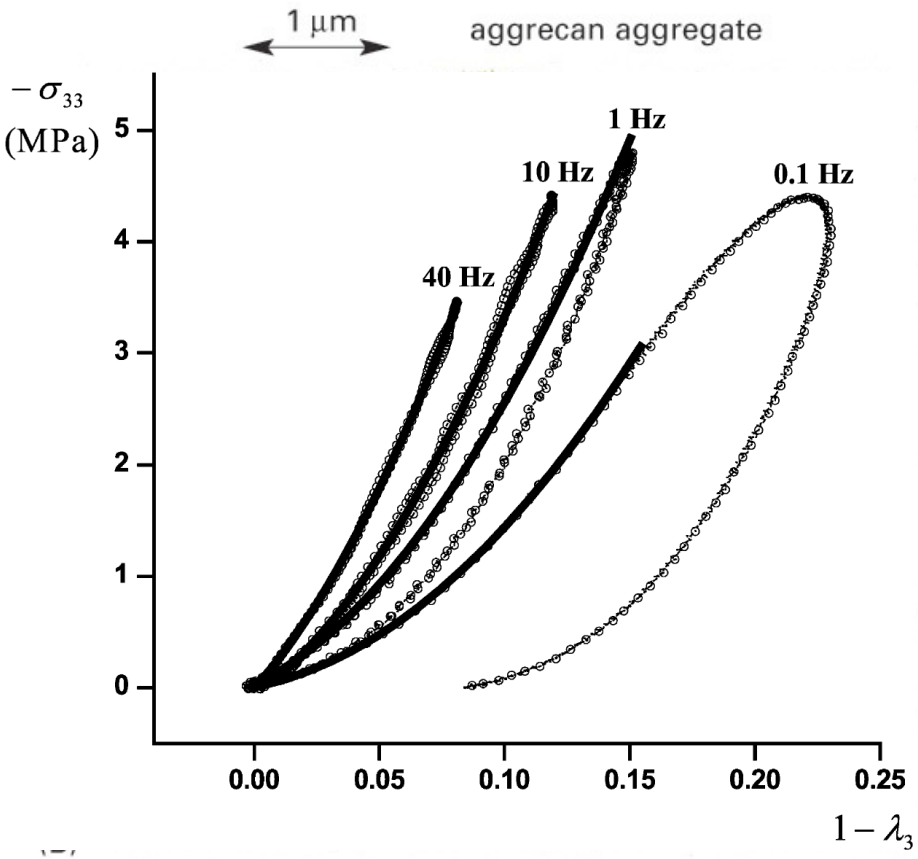
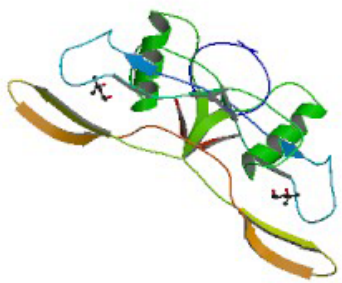
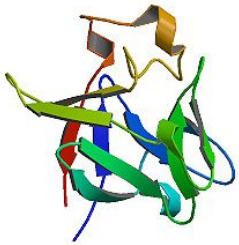


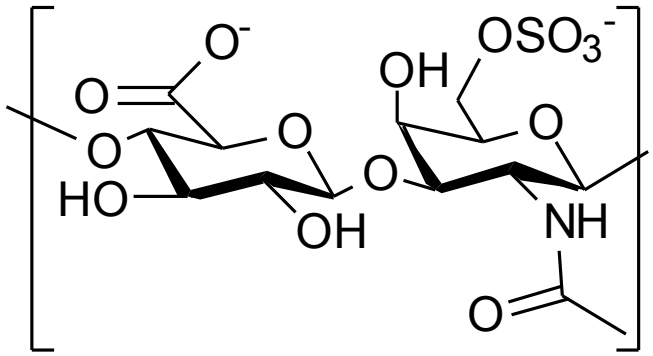
Figure 19–41 part 2 of 2. Molecular Biology of the Cell, 4th Edition.

Glycosaminoglycans have Important Biochemical Functionality

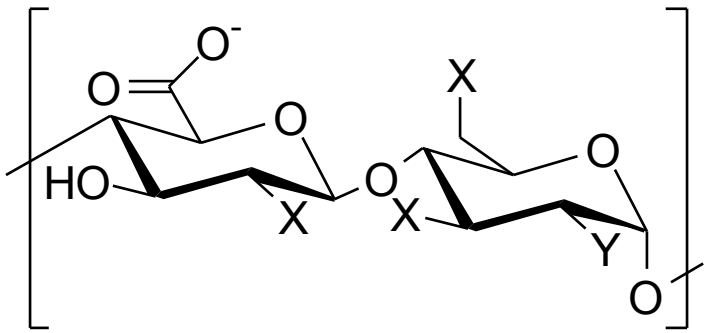
- Bind, stabilize, and activate many growth factors (TGF-β superfamily, FGF family).
- Potentiate other enzyme activity (e.g. inhibiting coagulation).







Chondroitin-6-Sulfate

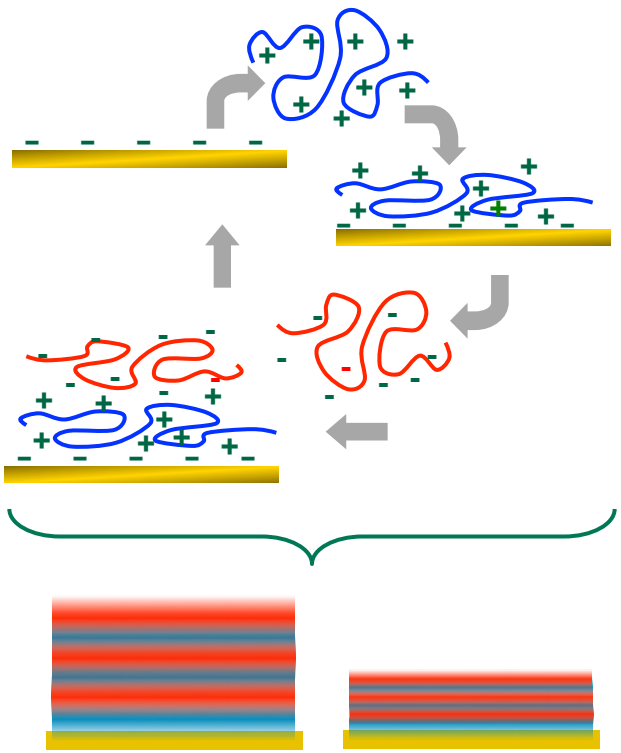


X = OH, OSO_3^-
Y = NHAc, NH_2, $NHSO_3^-$

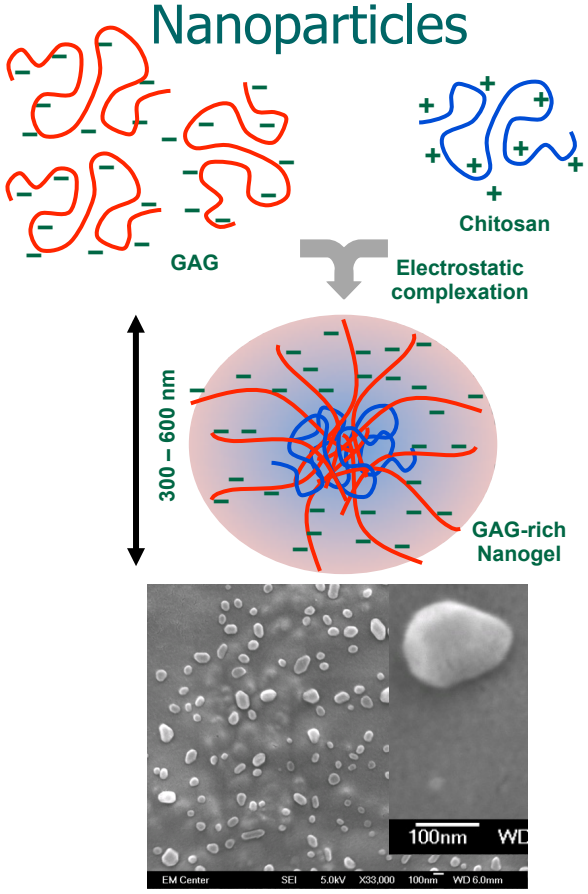
Heparin

Act 2: Polysaccharide Nanostructures we Can Engineer

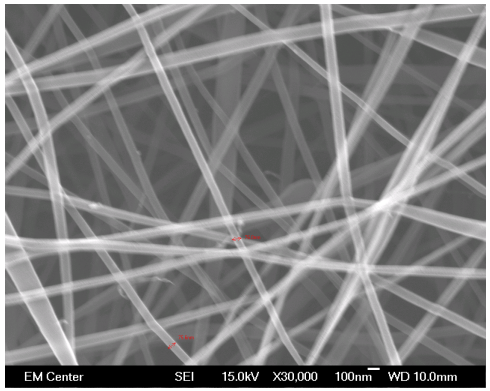
Polyelectrolyte Multilayers

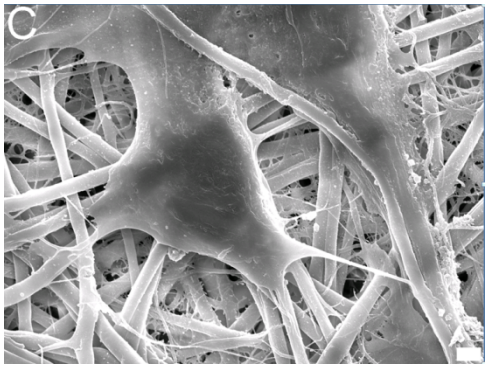


Polyelectrolyte Complex Nanoparticles

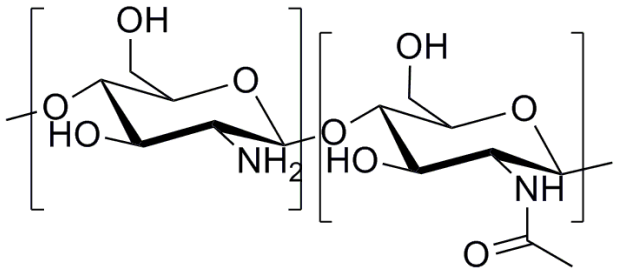


Electrospun Nanofibers

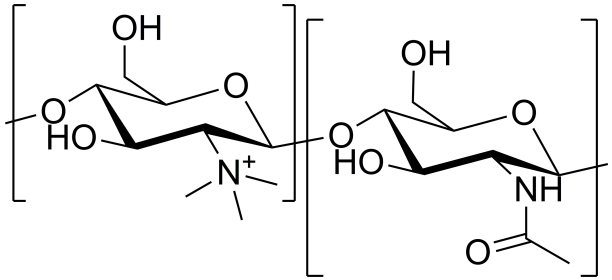




A Combination of Techniques Provides a Detailed Understanding of PEM Formation

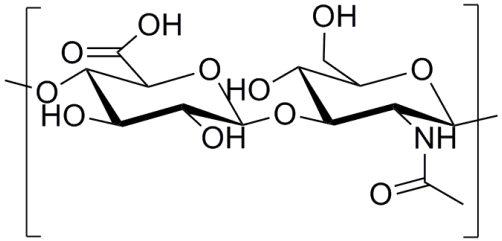


Chitosan (CHI)

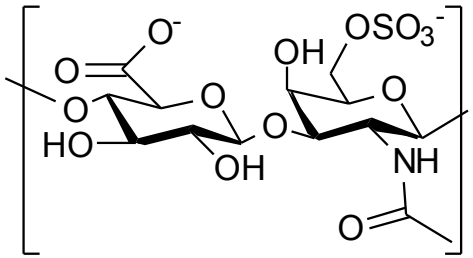


Trimethyl chitosan (TMC)

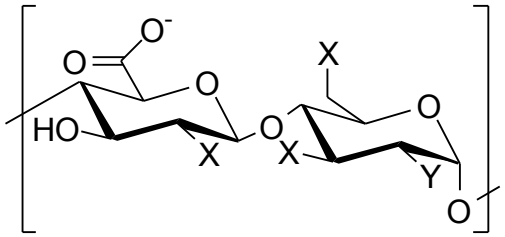
Increasing polyelectrolyte strength



Hyaluronan (HA)



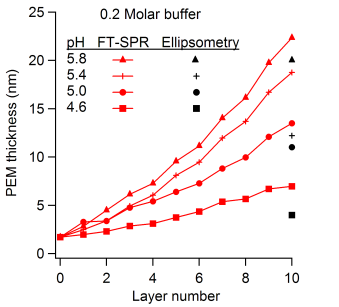
Chondroitin Sulfate (CS)

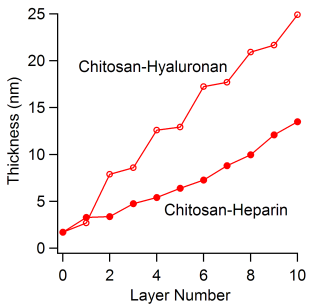


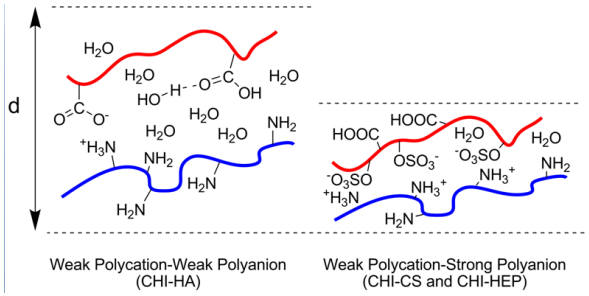
X = OH, OSO_3^-
Y = NHAc, NH_2, $NHSO_3^-$

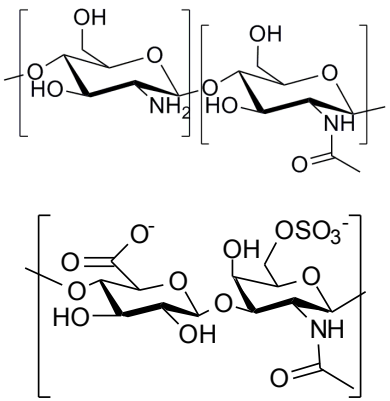
Heparin (HEP)

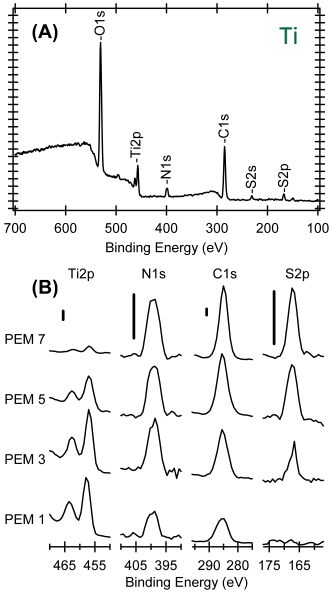
Controlling Polyelectrolyte Multilayer Assembly

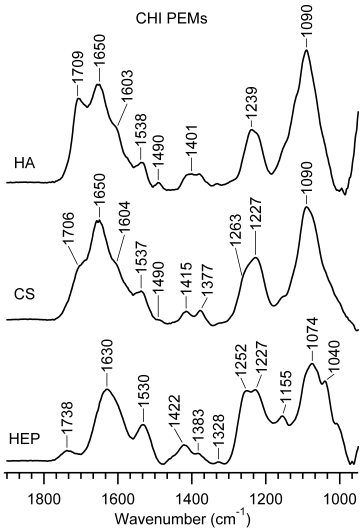


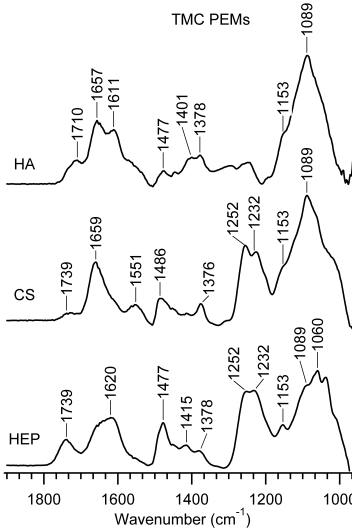


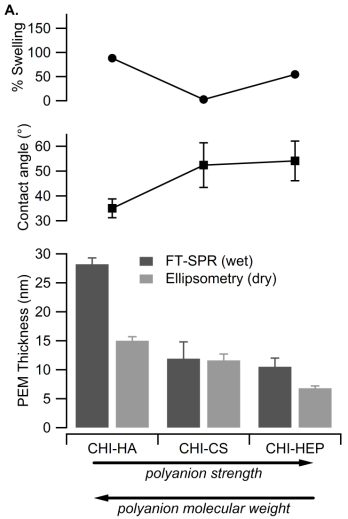


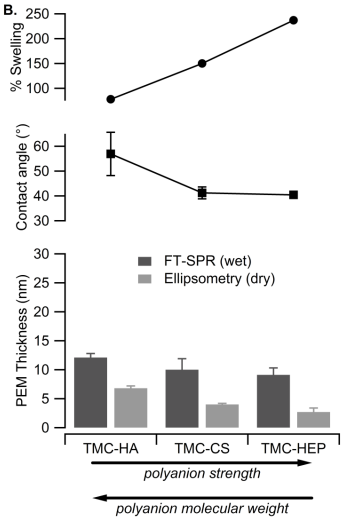








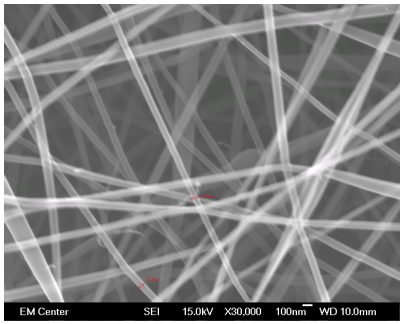


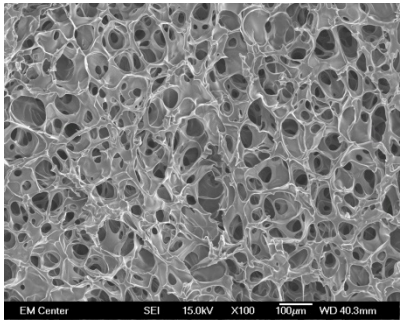


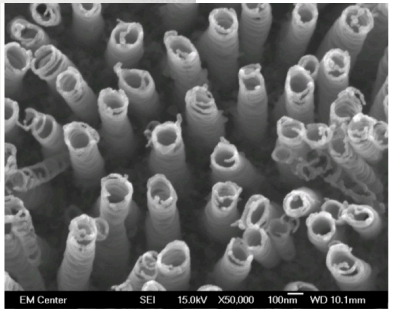
Rules Governing PEM Assembly

1. The stronger polyelectrolyte governs the charge density of the weaker polyelectrolyte
2. Relative degree of ion pairing controls the swelling of PEMs in water

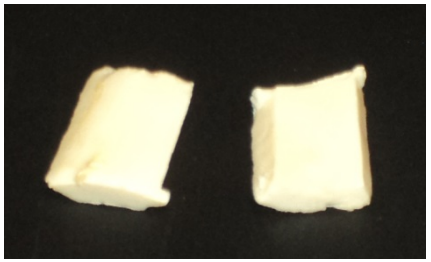
PEM Construction on Lots of Things

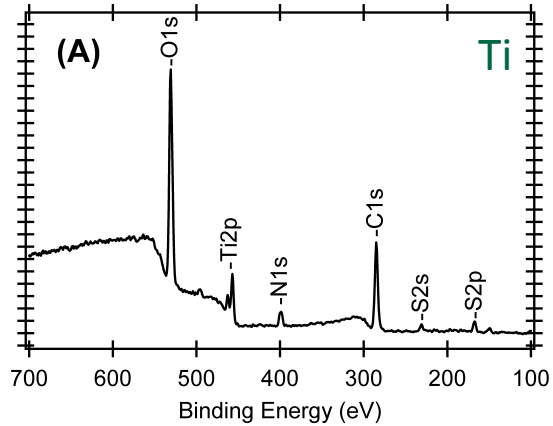


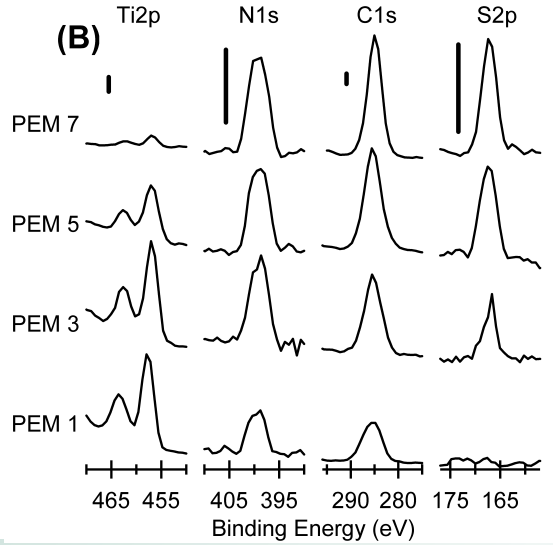




PEM coatings translated to lots of surface types

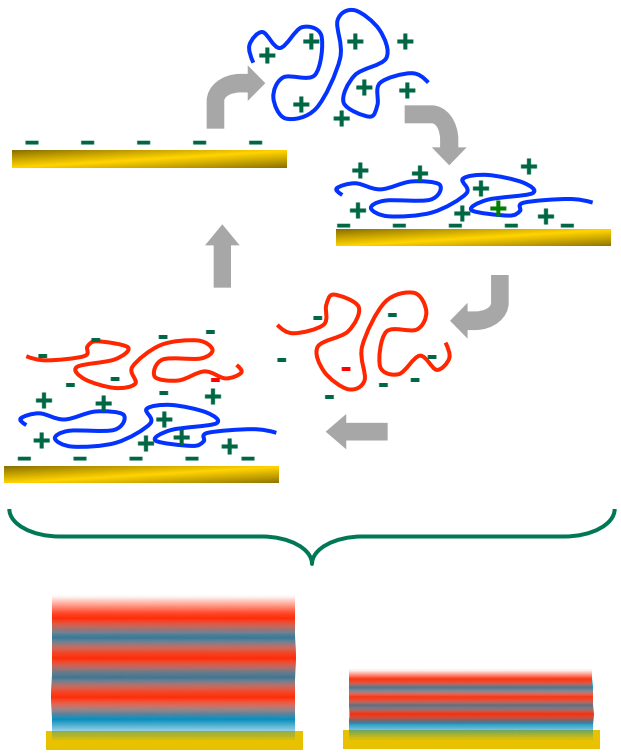




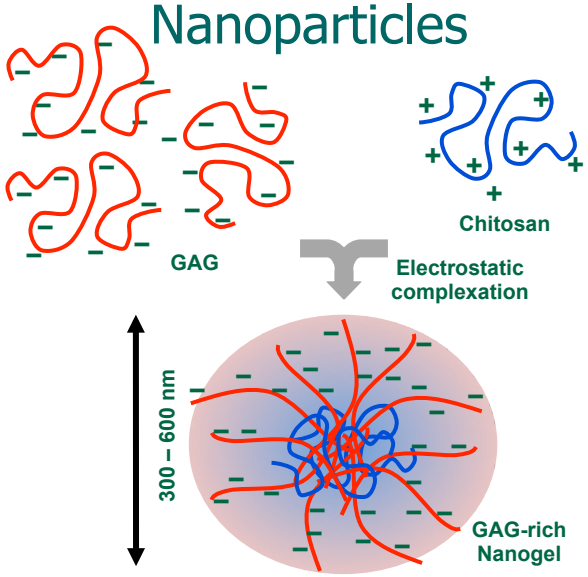


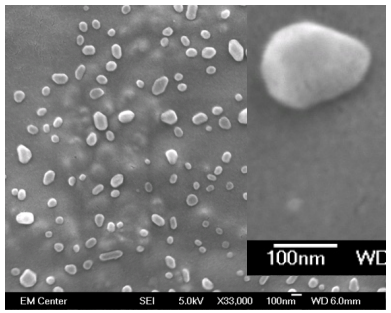
Act 2: Polysaccharide Nanostructures we can Engineer

Polyelectrolyte Multilayers

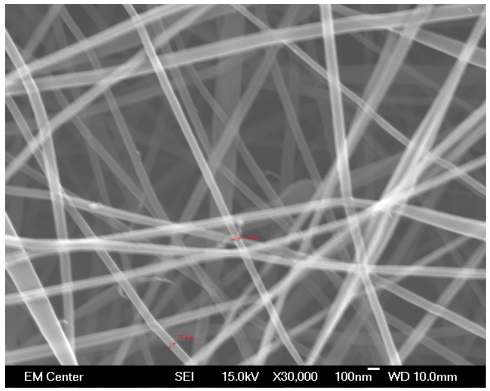


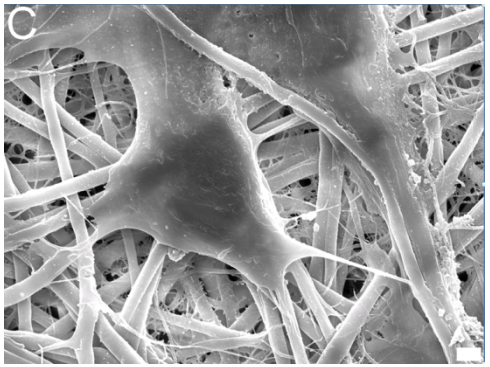
Polyelectrolyte Complex Nanoparticles



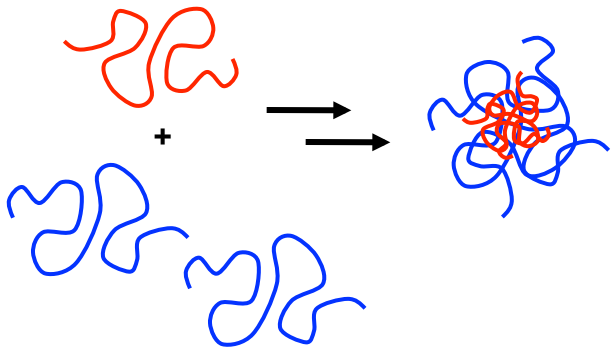


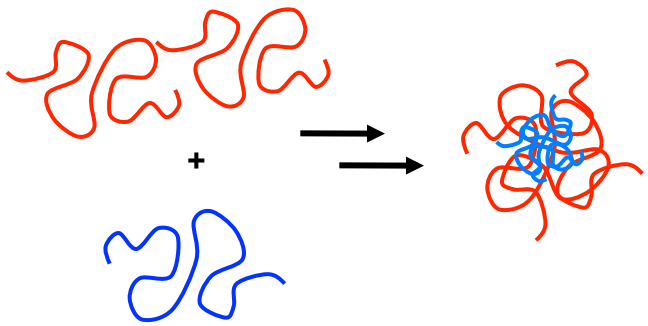
Electrospun Nanofibers





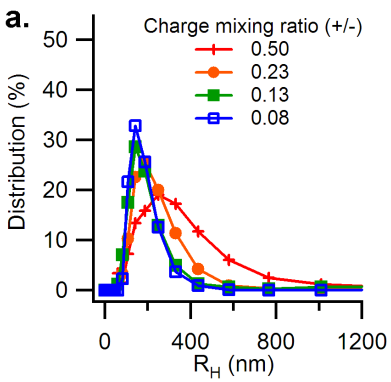
PCN Fabrication

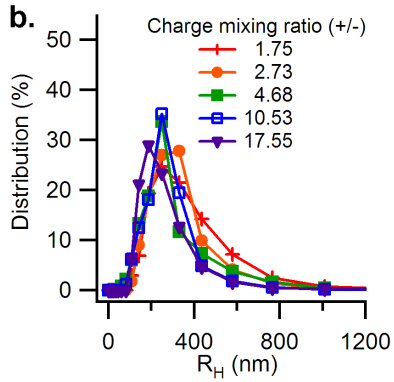


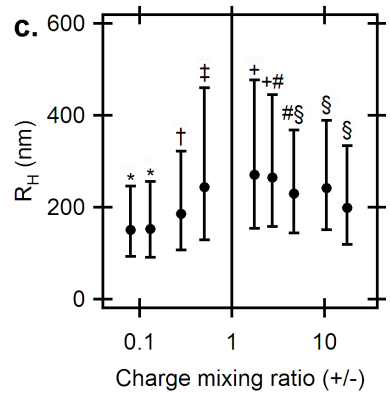


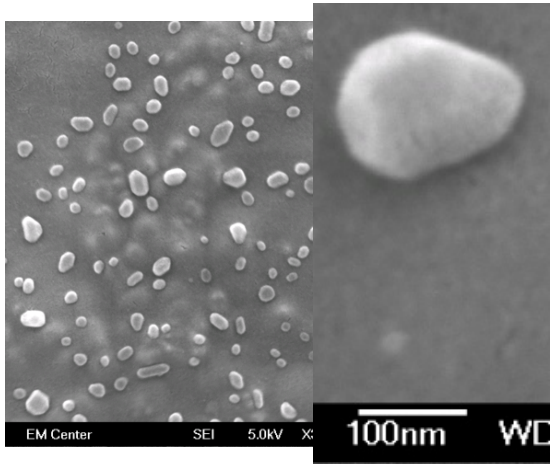
- Combine solutions of polyelectrolytes
- Stir
- Settle (to remove aggregates)
- Centrifuge
- Size (Photon correlation spectroscopy), composition (FTIR), image (SEM)

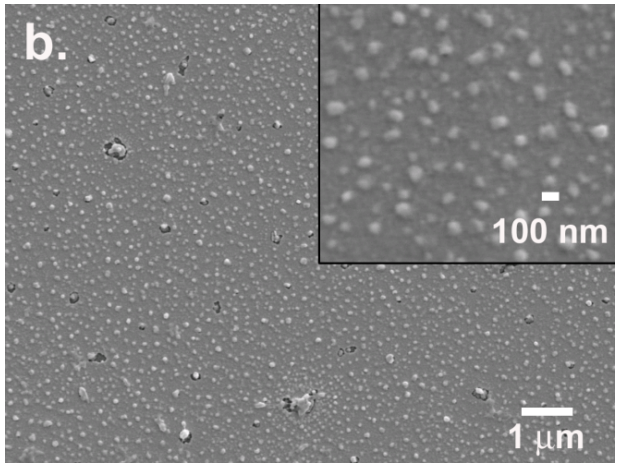
PCN Sizes Depend Upon Stoichiometry

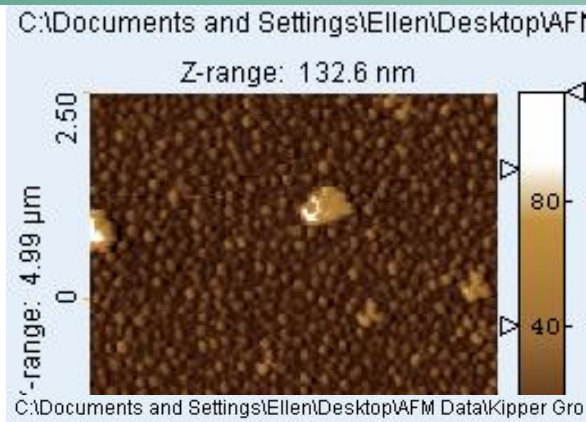


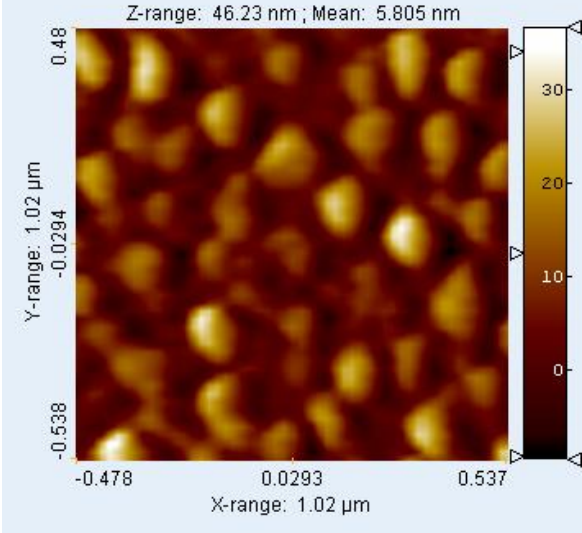






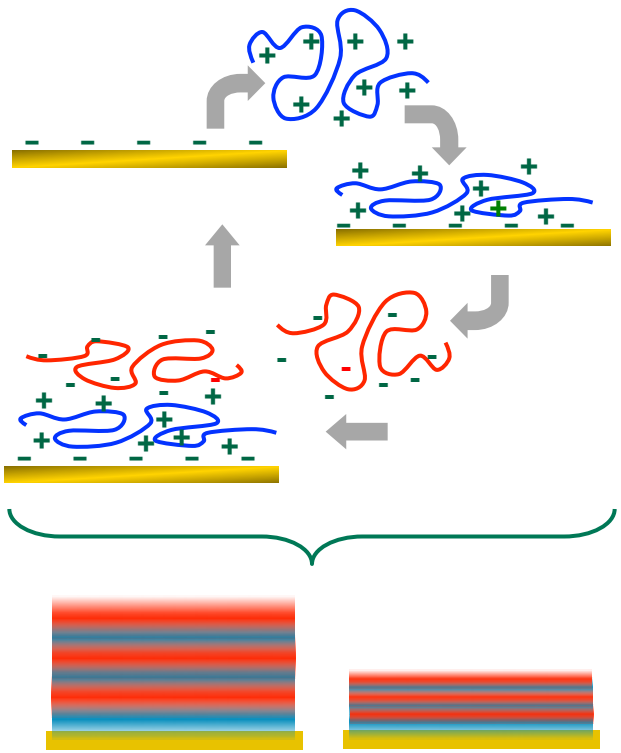




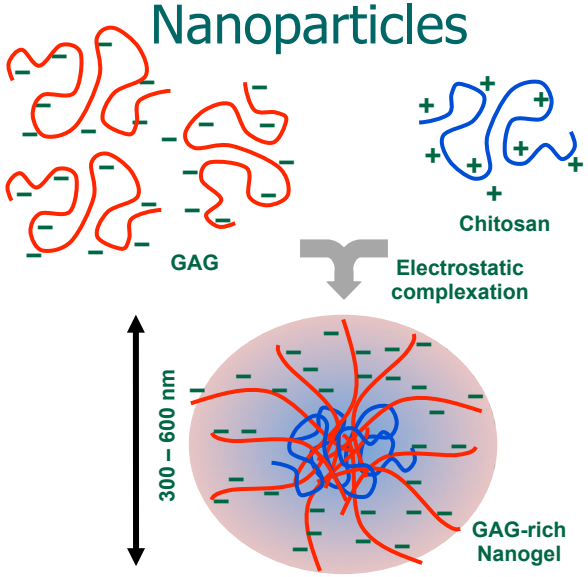


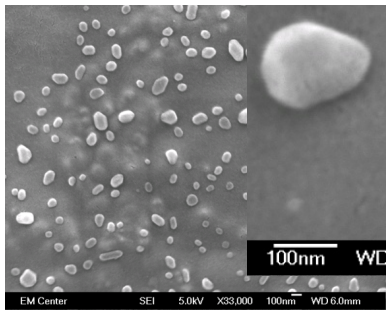
Act 2: Polysaccharide Nanostructures we can Engineer

Polyelectrolyte Multilayers

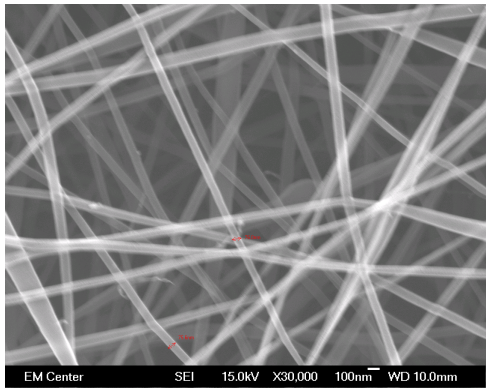


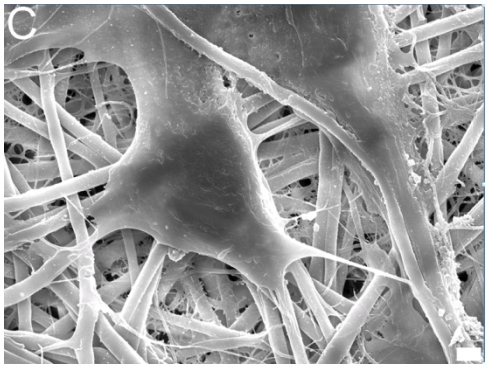
Polyelectrolyte Complex Nanoparticles



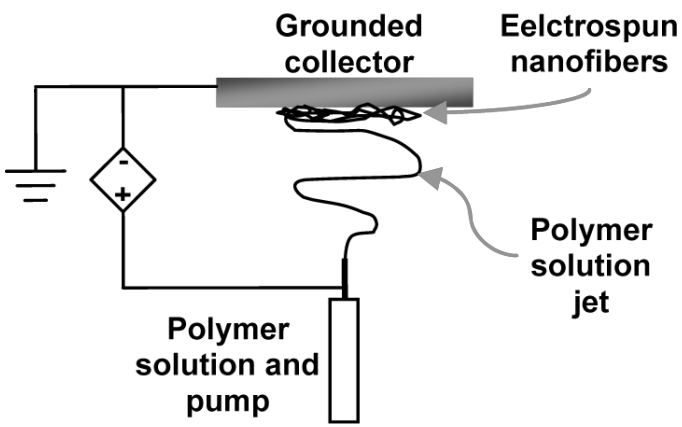


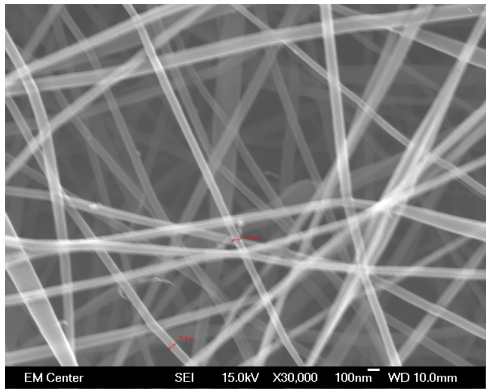
Electrospun Nanofibers



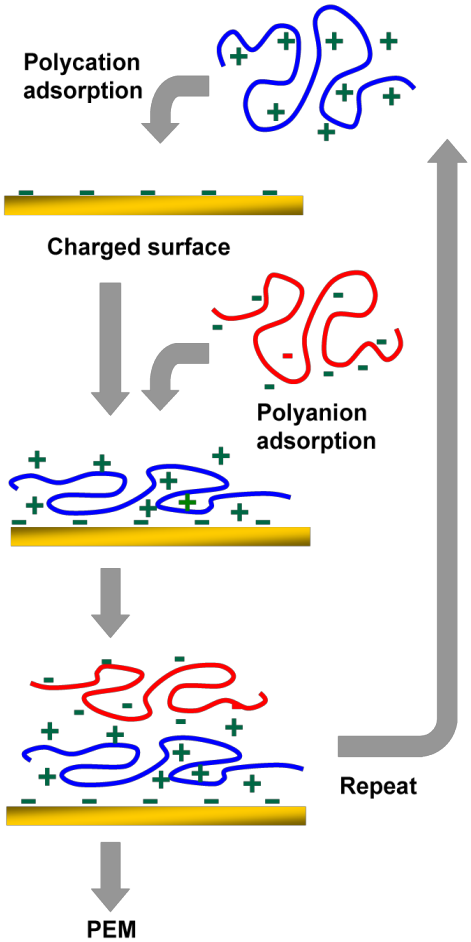


Electrospinning Nanofibers and Modifying with Multilayers



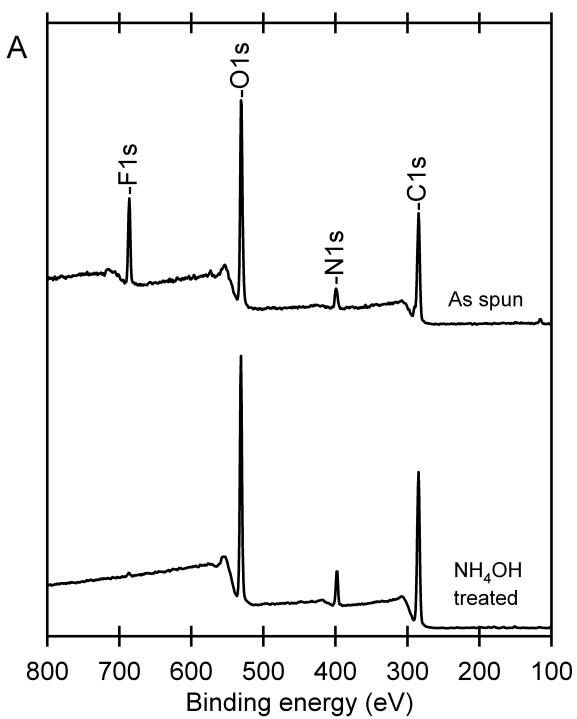


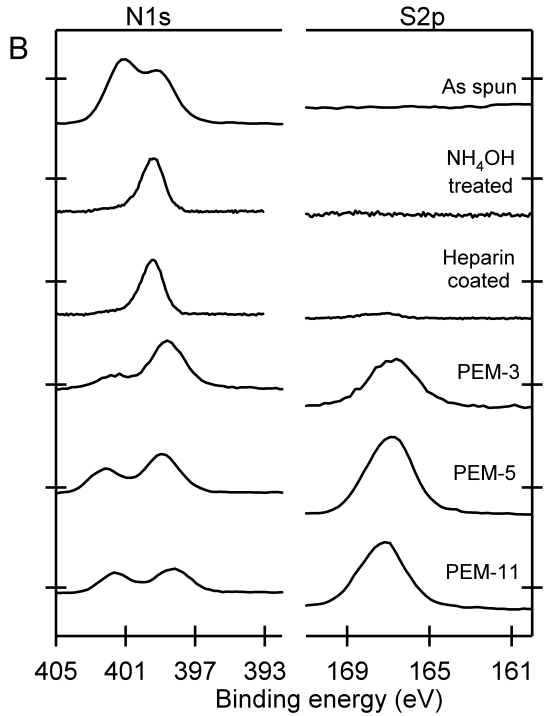
Chitosan Nanofibers

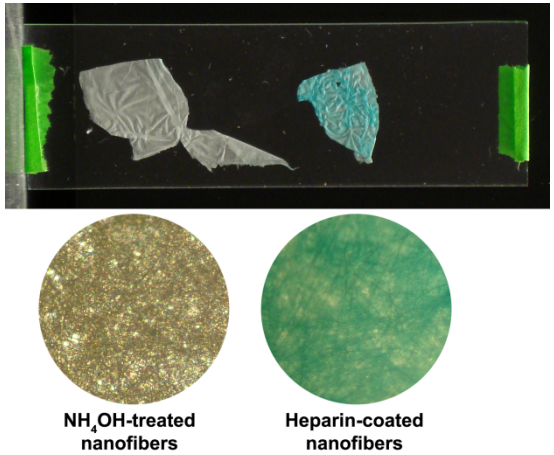


Processing with heparin-chitosan multilayer formation leads to fiber dissolution

Confirmation of Multilayer Formation by XPS and Alcian Staining

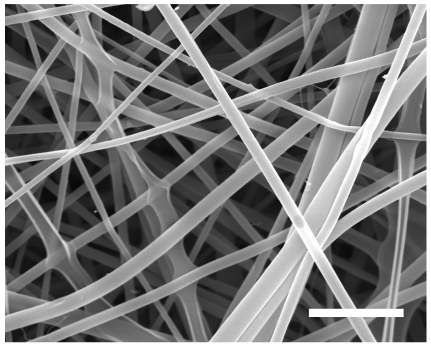




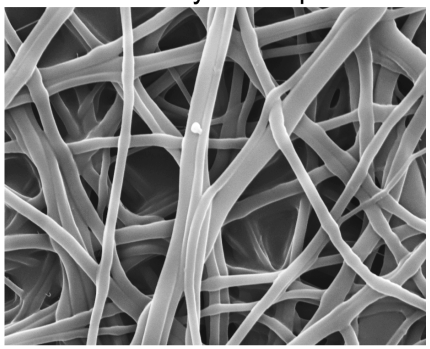


Electrospinning Nanofibers and Modifying with Multilayers

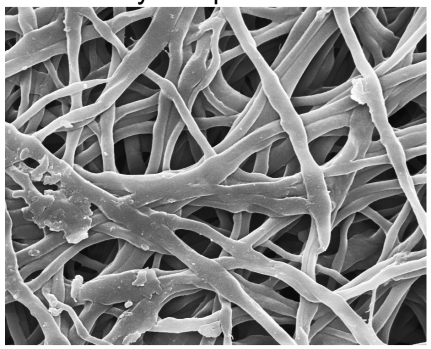
As spun chitosan nanofibers



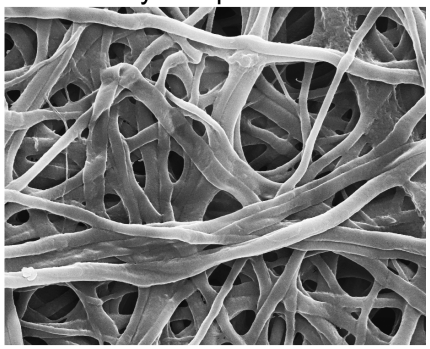
Nanofibers treated with NH_4OH and one layer of heparin

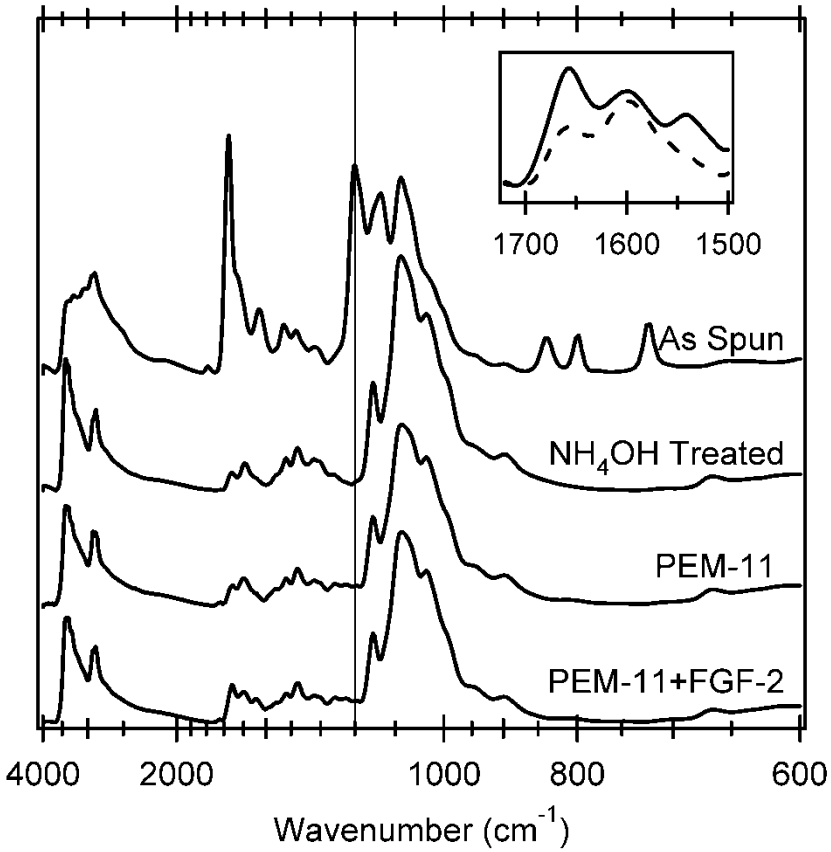


Nanofibers treated with NH_4OH and a 3-layer heparin-TMC PEM



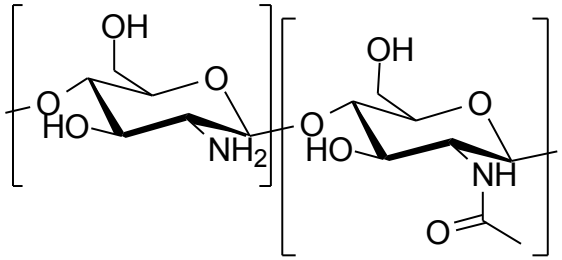
Nanofibers treated with NH_4OH and a 5-layer heparin-TMC PEM

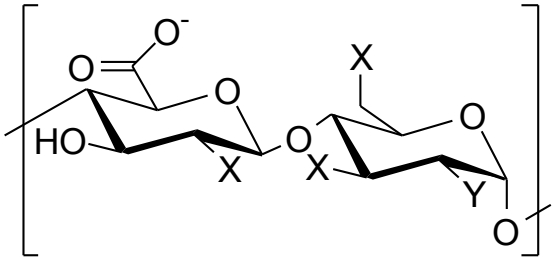




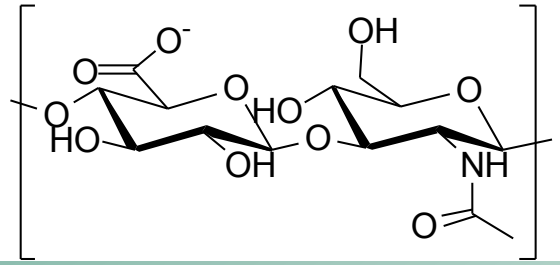
Act 3: MSC Responses to FGF-2 Delivered from Polysaccharide Nanoassemblies

Polysaccharides

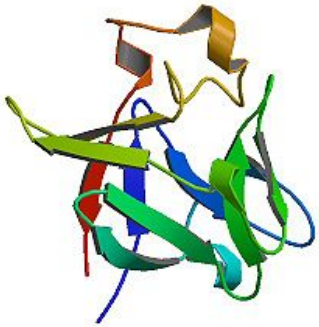




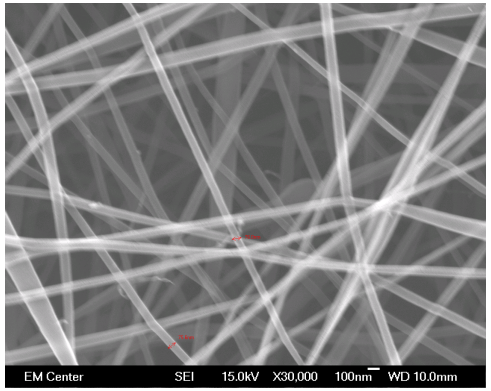
X = OH, OSO_3^-
Y = NHAc, NH_2, $NHSO_3^-$



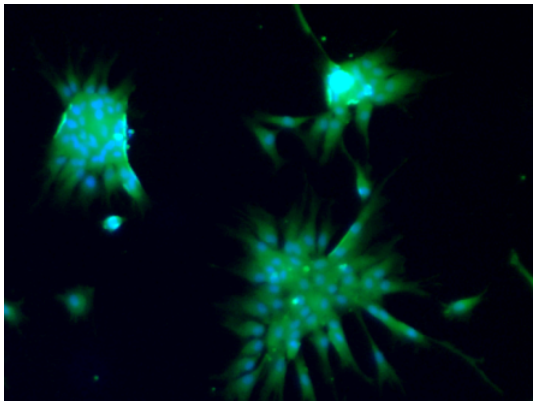
FGF-2

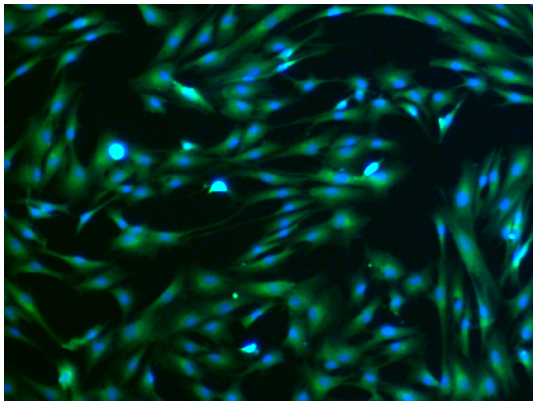


Nanostructures

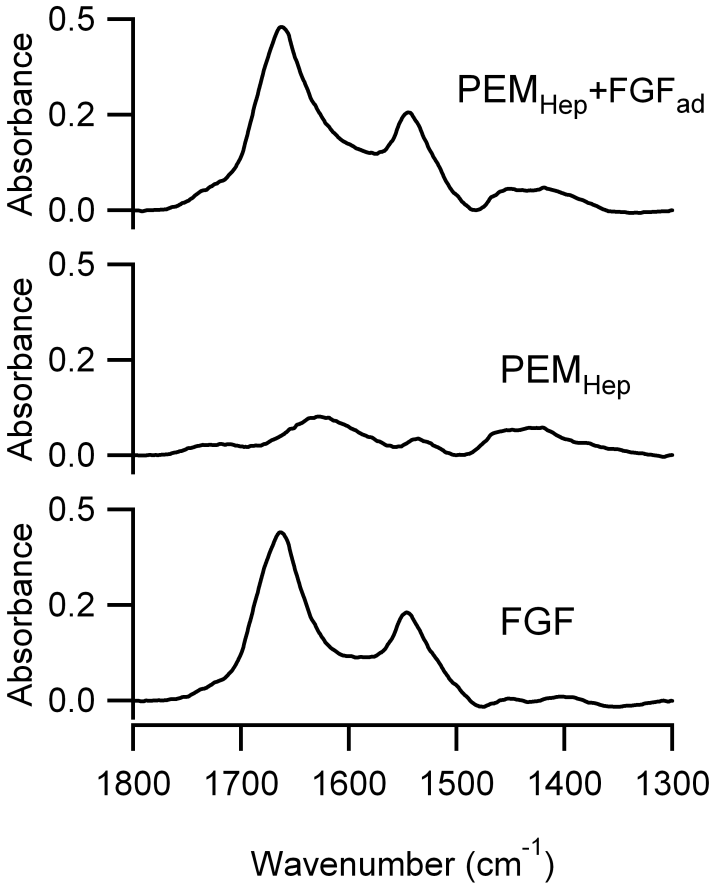


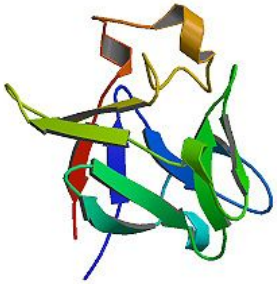
Cell Response?

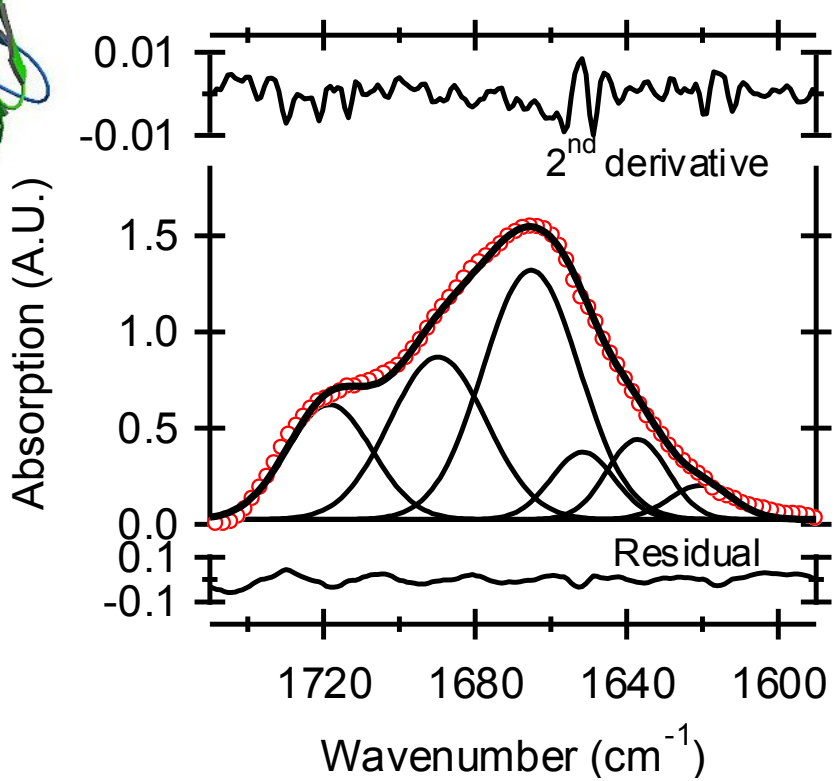




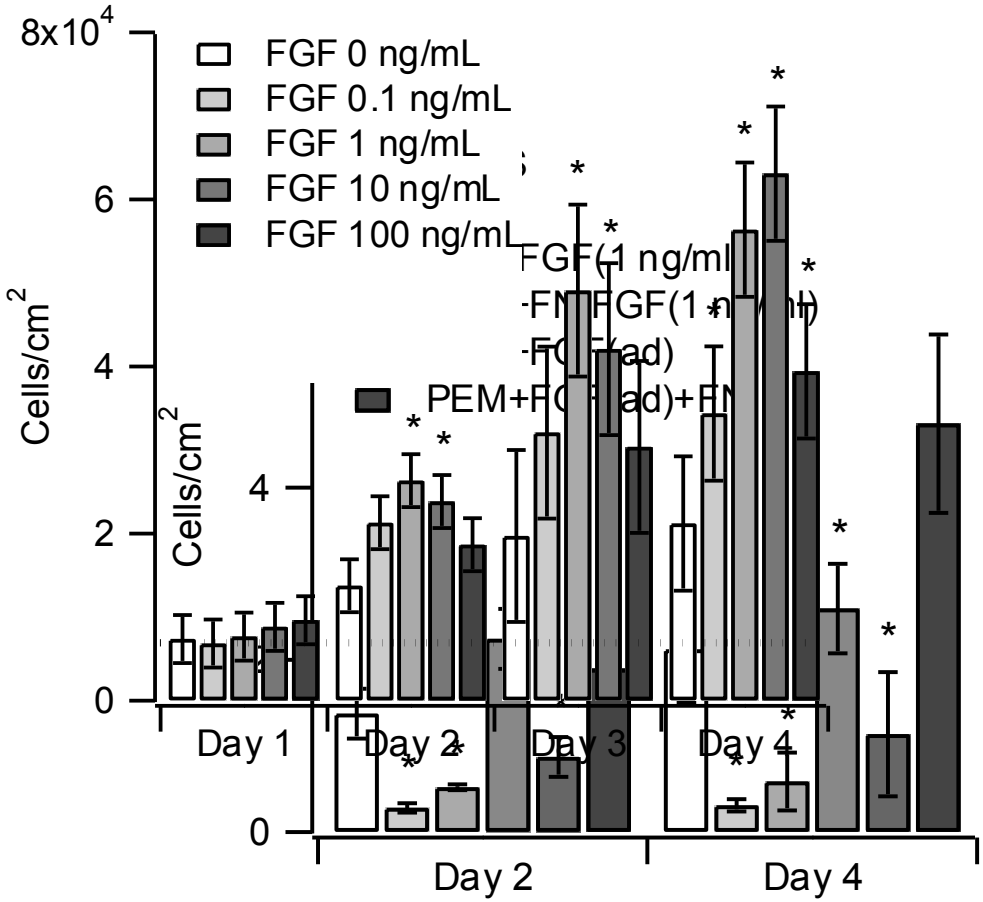
PM-IRRAS Confirms Protein Stability

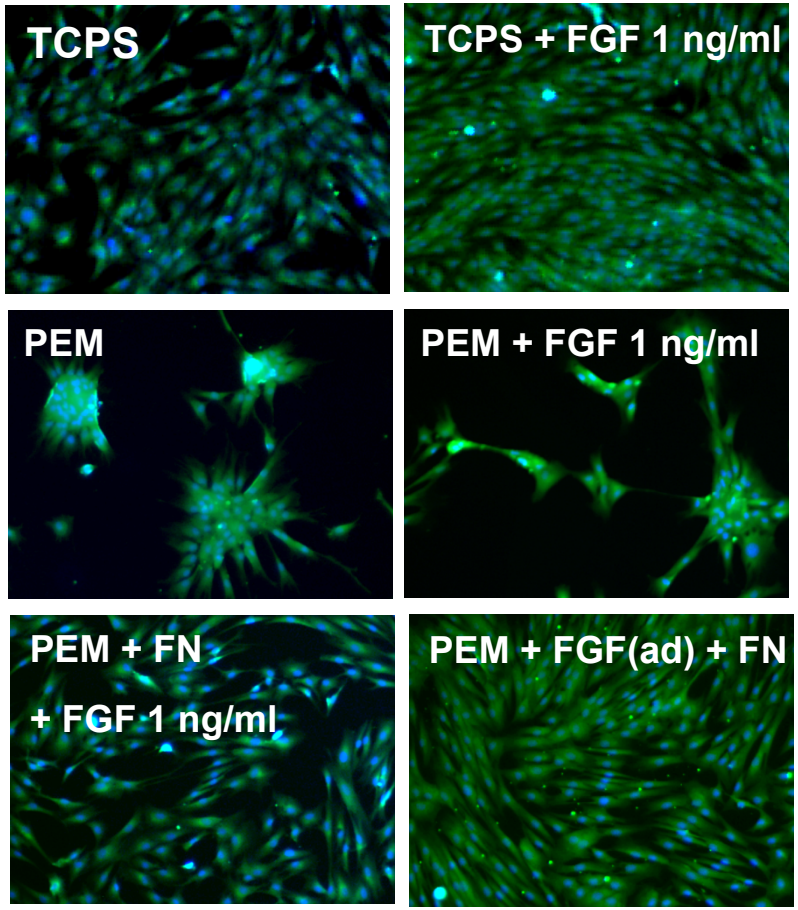




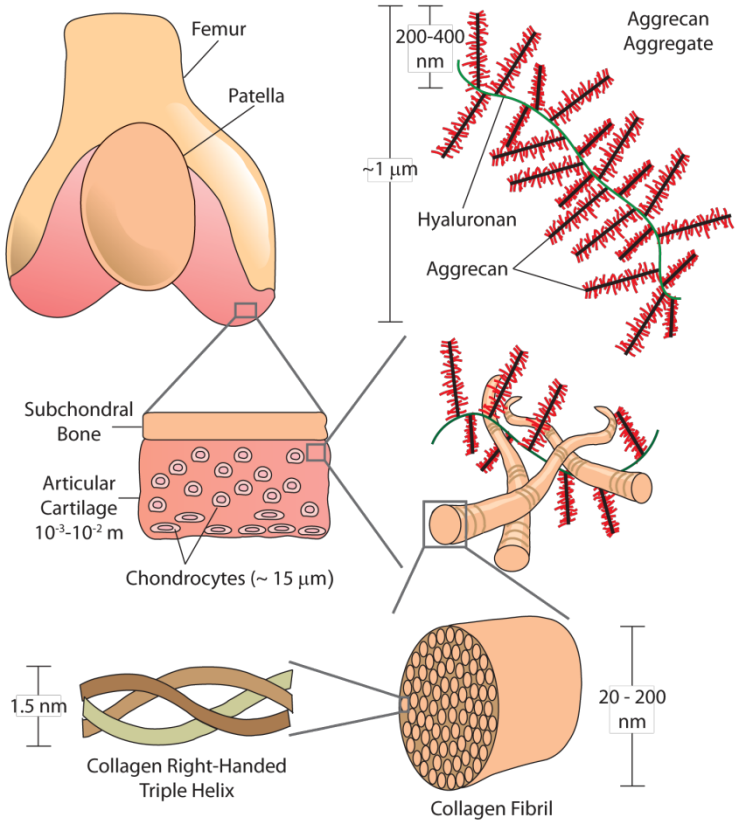


FGF-2 Adsorption and Release from PEMs





Can We Design Polysaccharide Nanoassemblies with Biological Function?



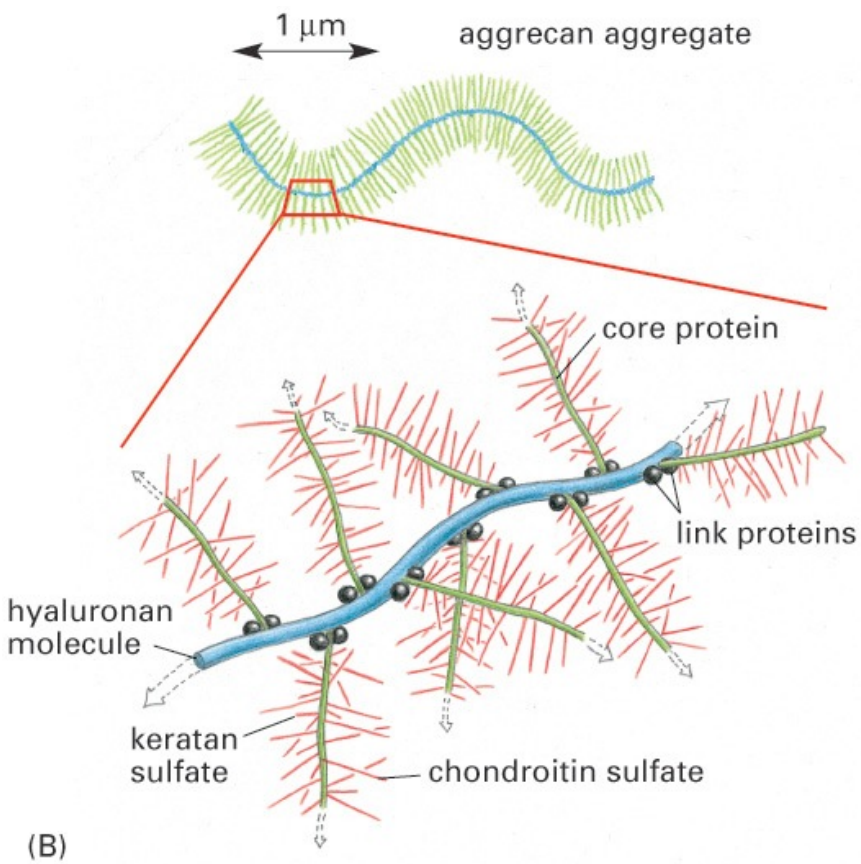
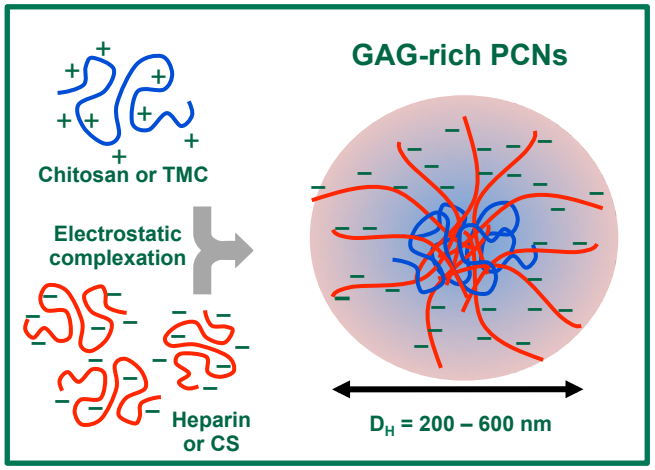
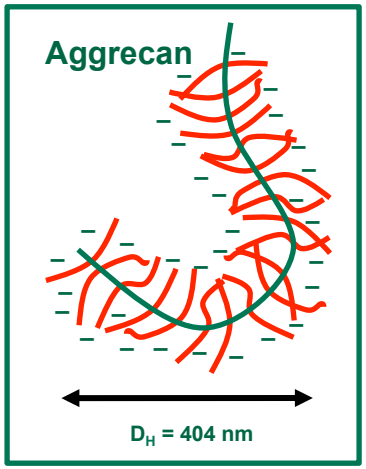
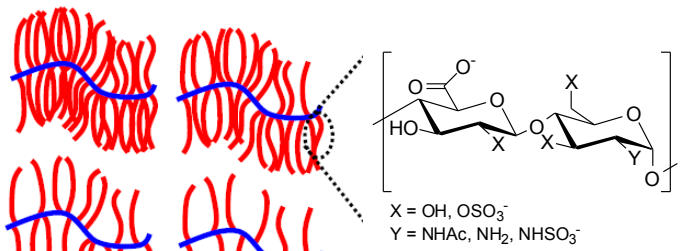


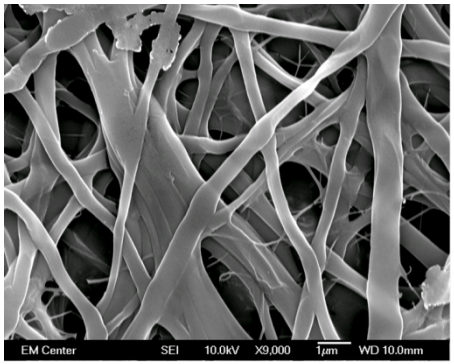
Figure 19–41 part 2 of 2. Molecular Biology of the Cell, 4th Edition.

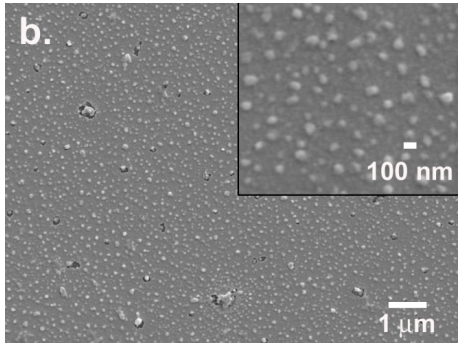
ECM and Proteoglycan Mimetic Structures

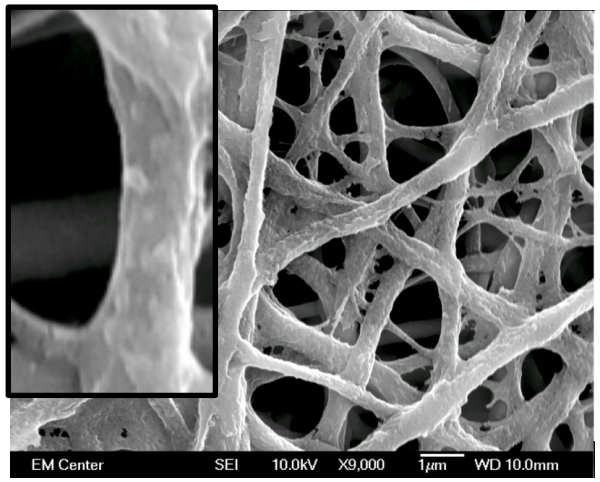




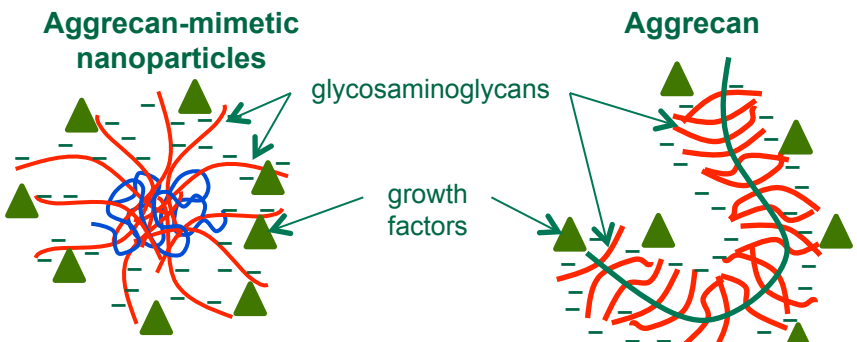


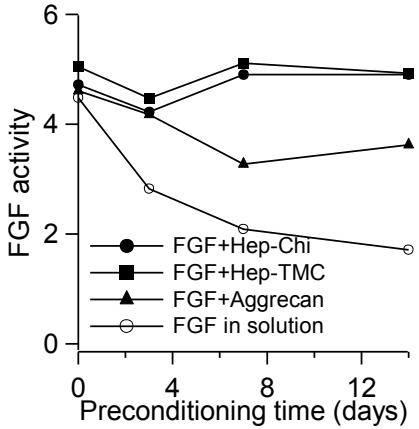


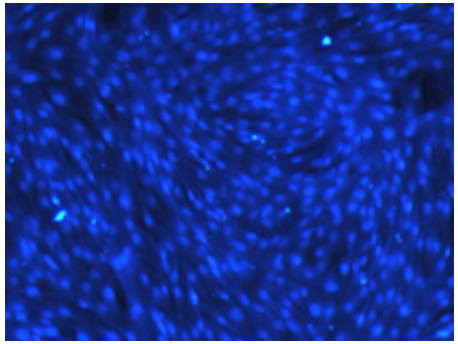


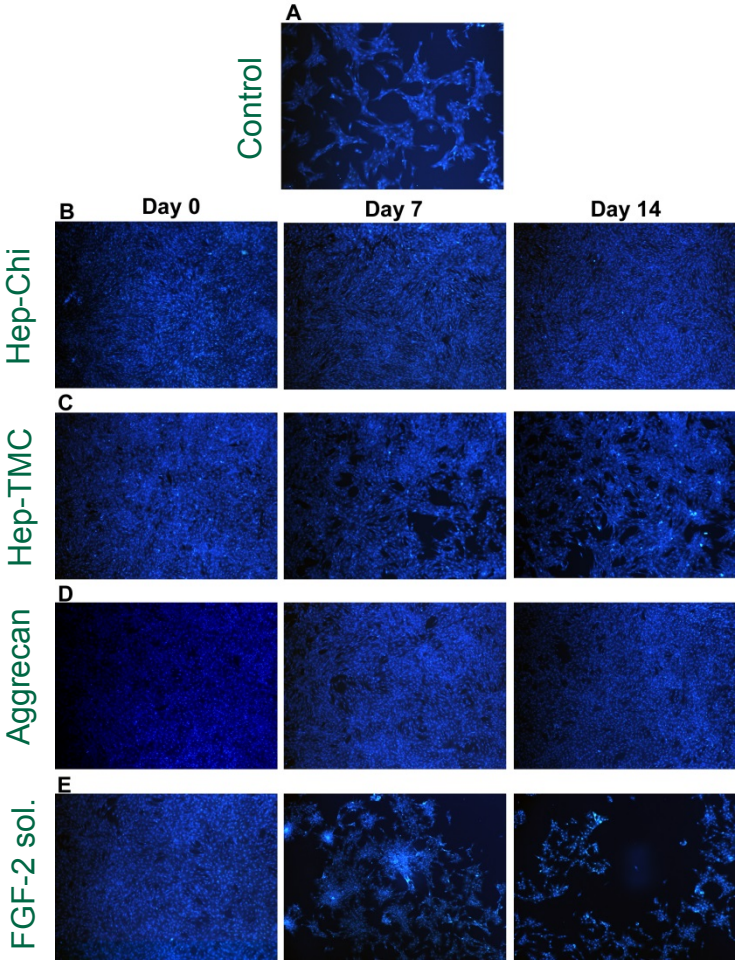


ECM and Proteoglycan Mimetic Structures

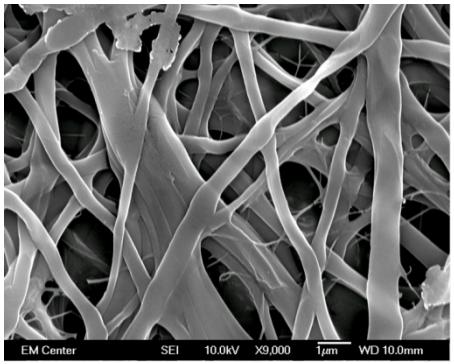


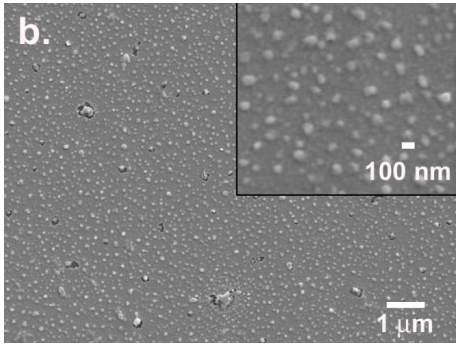


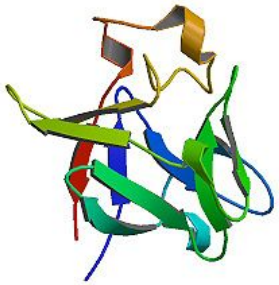


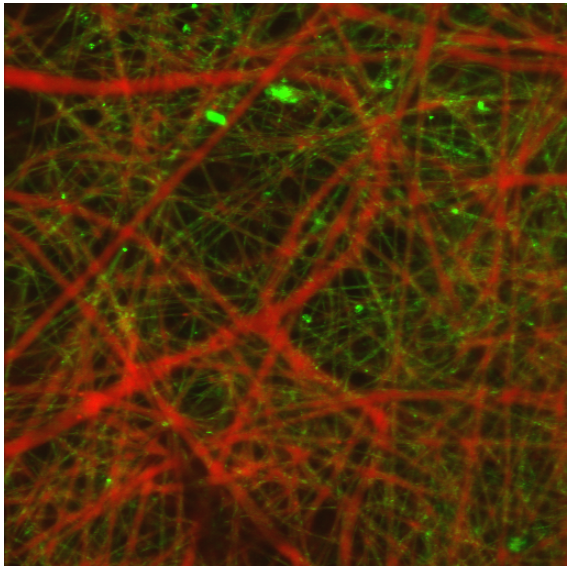


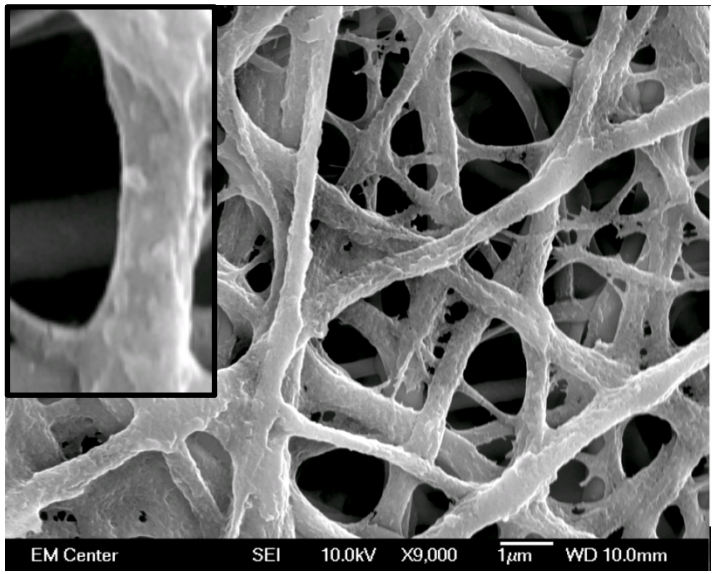
Nanofibers Modified with FGF-2/PCN Complexes



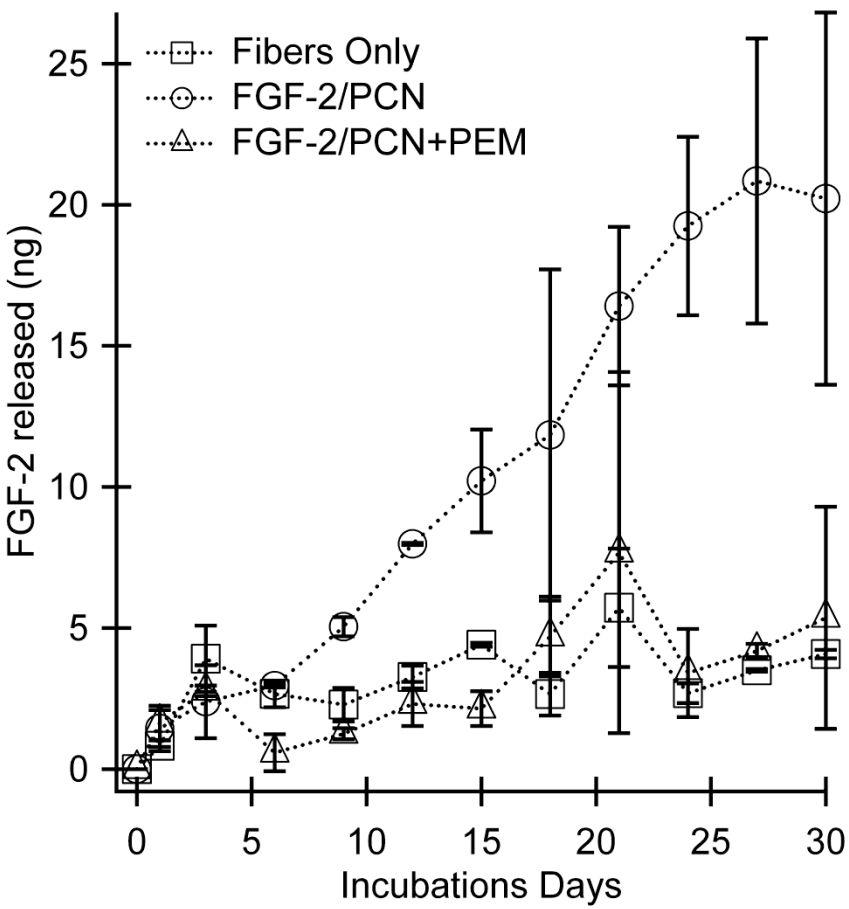


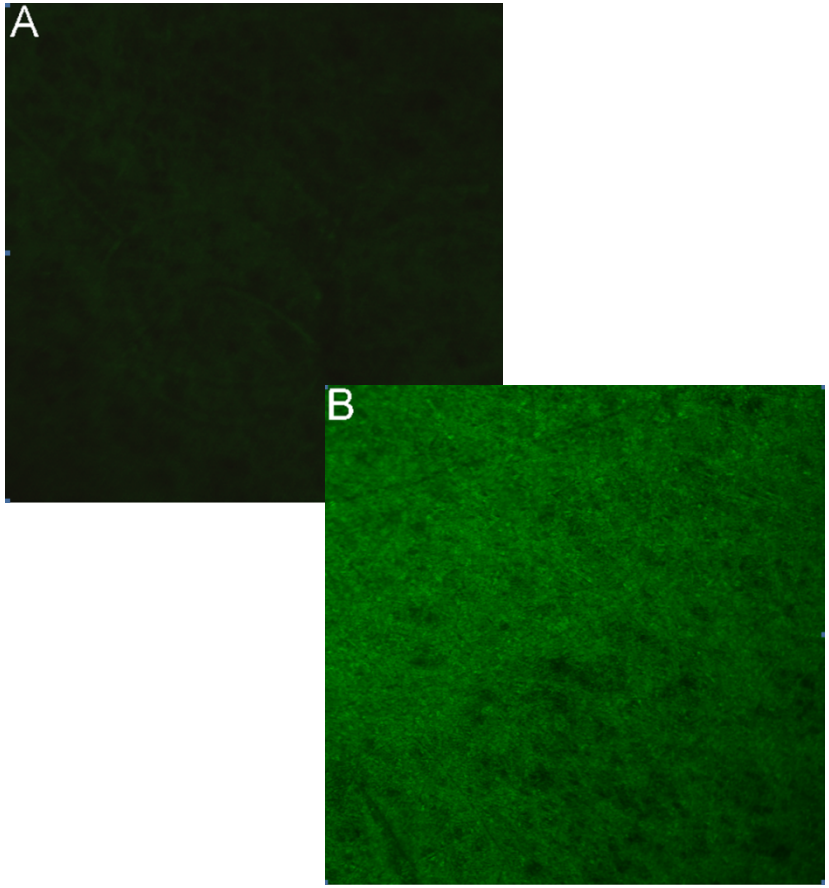




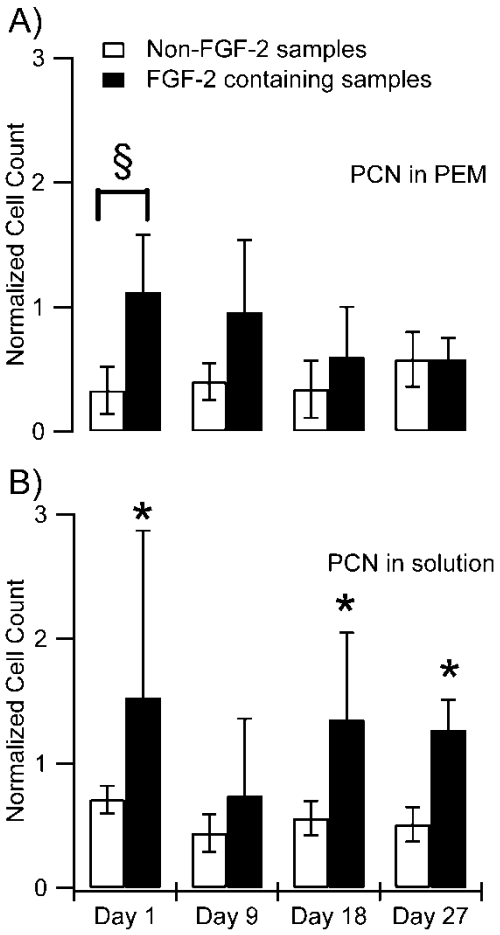


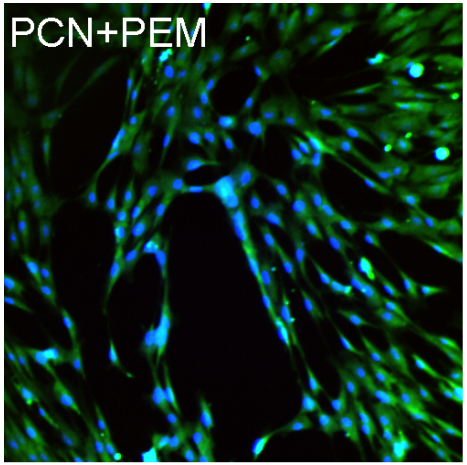
FGF-2 Release from Nanofibers

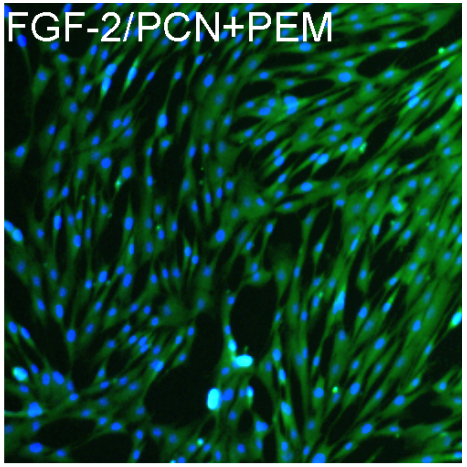


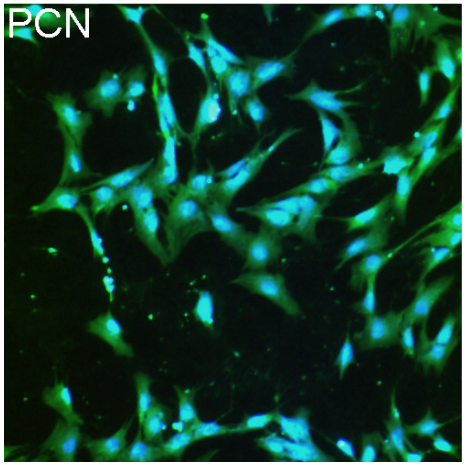


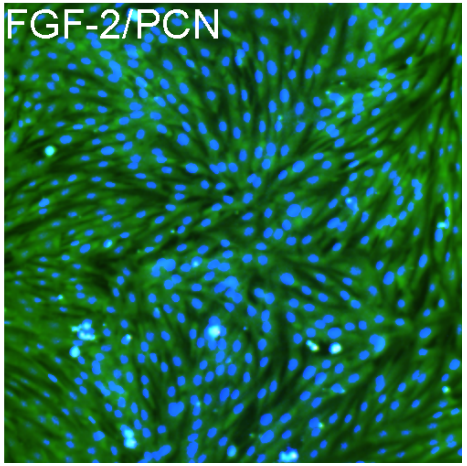
FGF-2 Activity After Release from Nanofibers





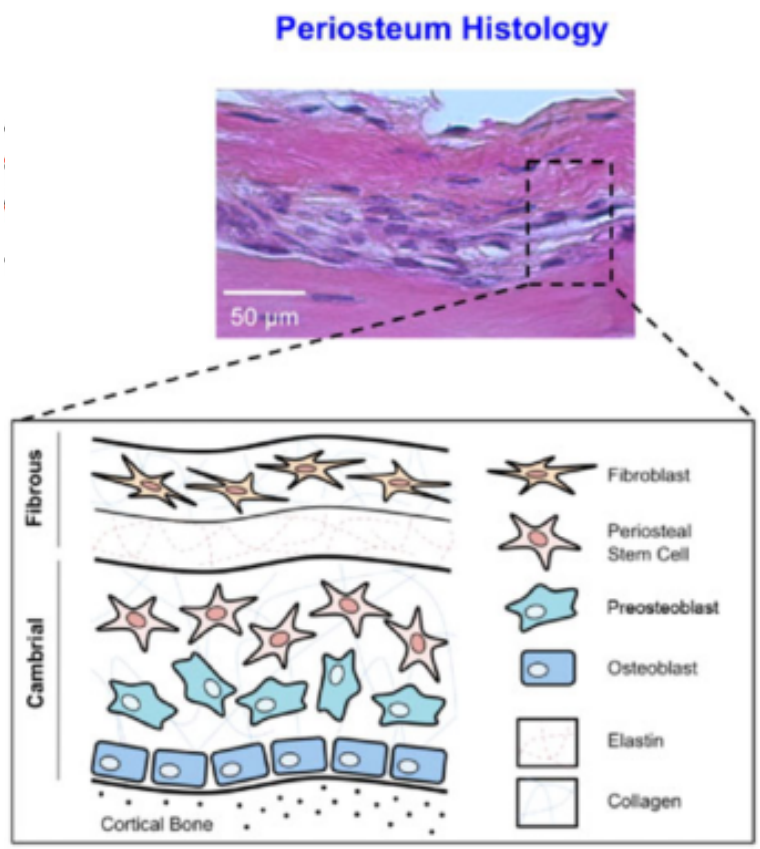






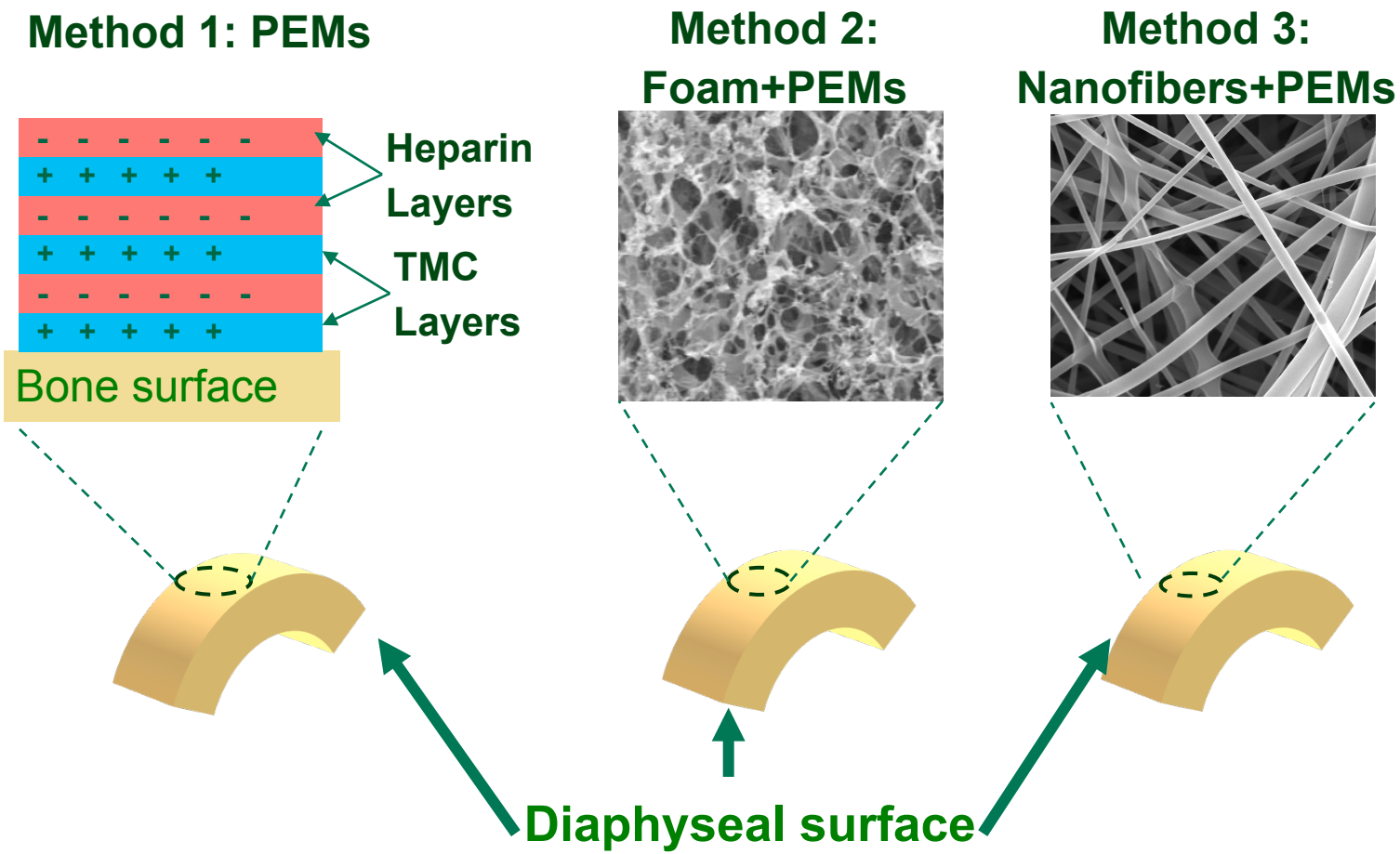
Act 4: A Tissue Engineering Application

- Small bone defects heal
- Large (critical sized) defects may require bone *autograft*
- Disease and/or extensive injury can preclude *autograft* → use *allograft.*
- *Allografts* are devitalized to prevent disease transmission and immune rejection.
- Devitalization includes removal of periosteum – essential for healing.
- Up to 60 % failure in 10 years.

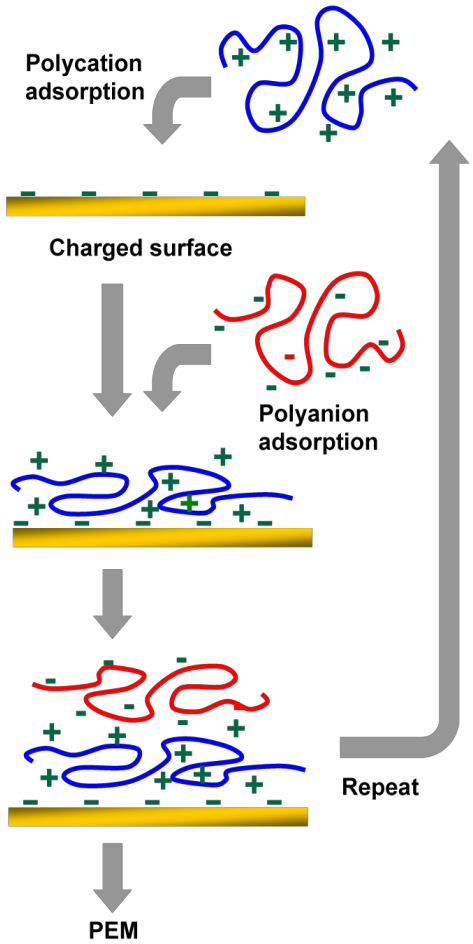


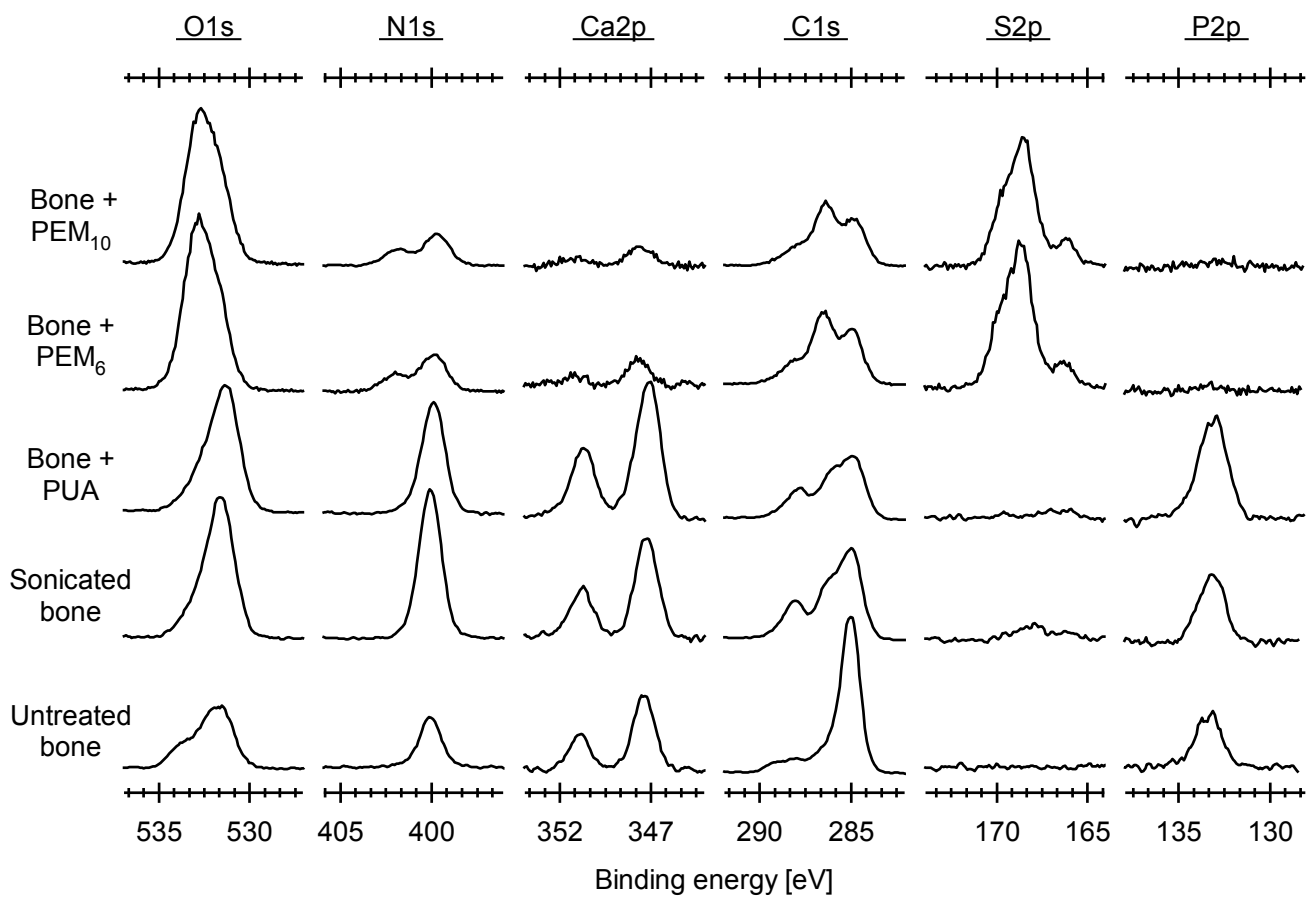
Hoffman, Michael D. Clin Orthop Relat Res. 2012

Three Methods for Coating Bone

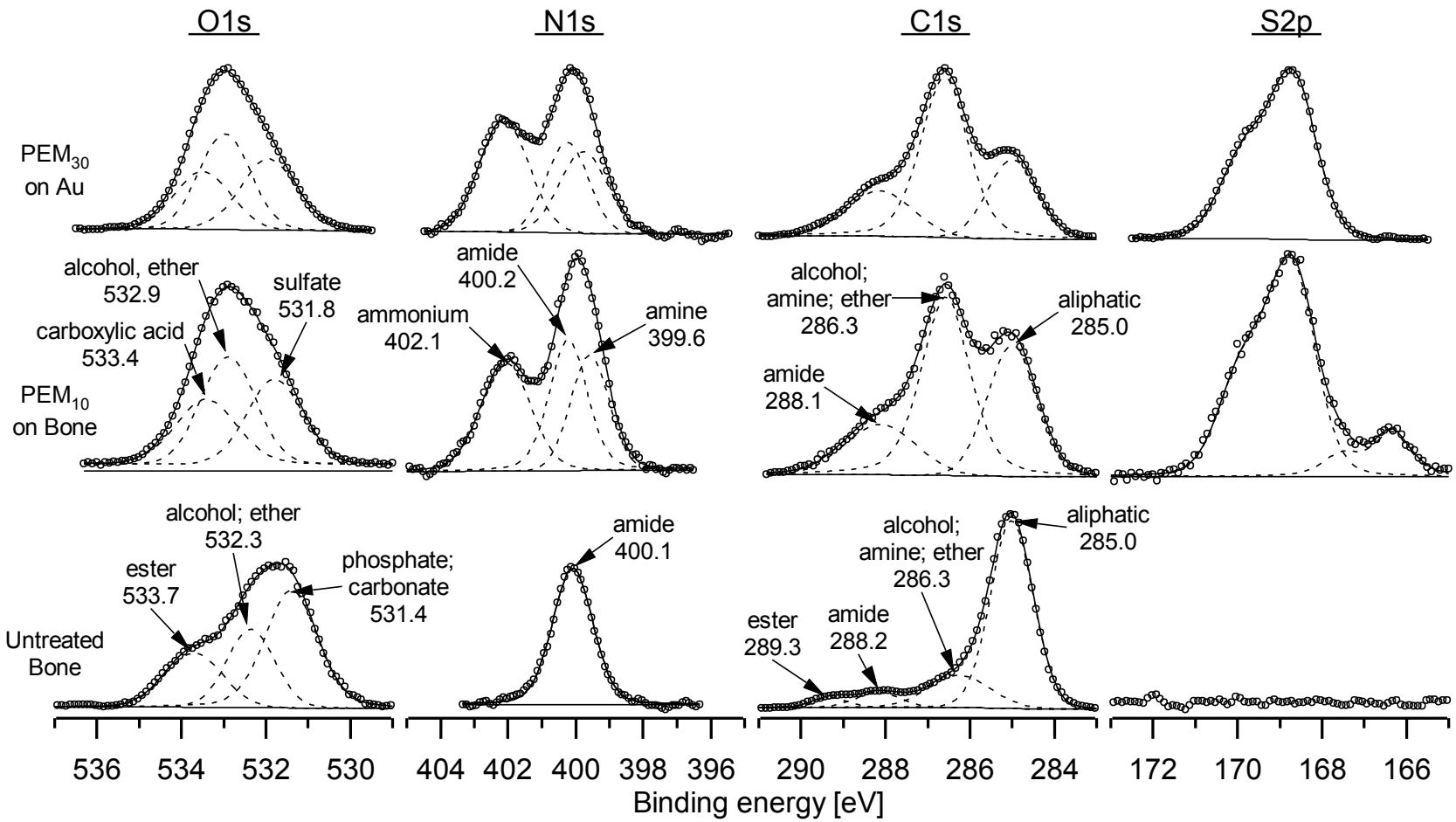


PEM Coating on Bone



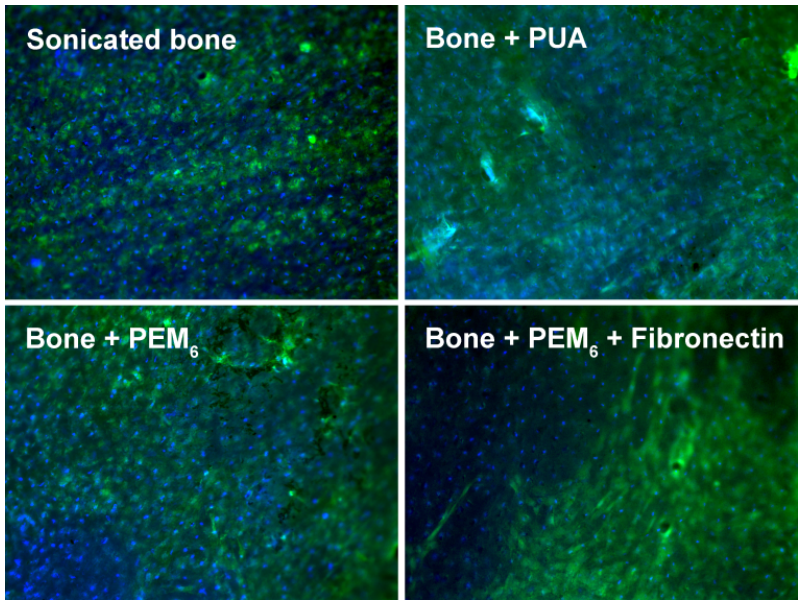


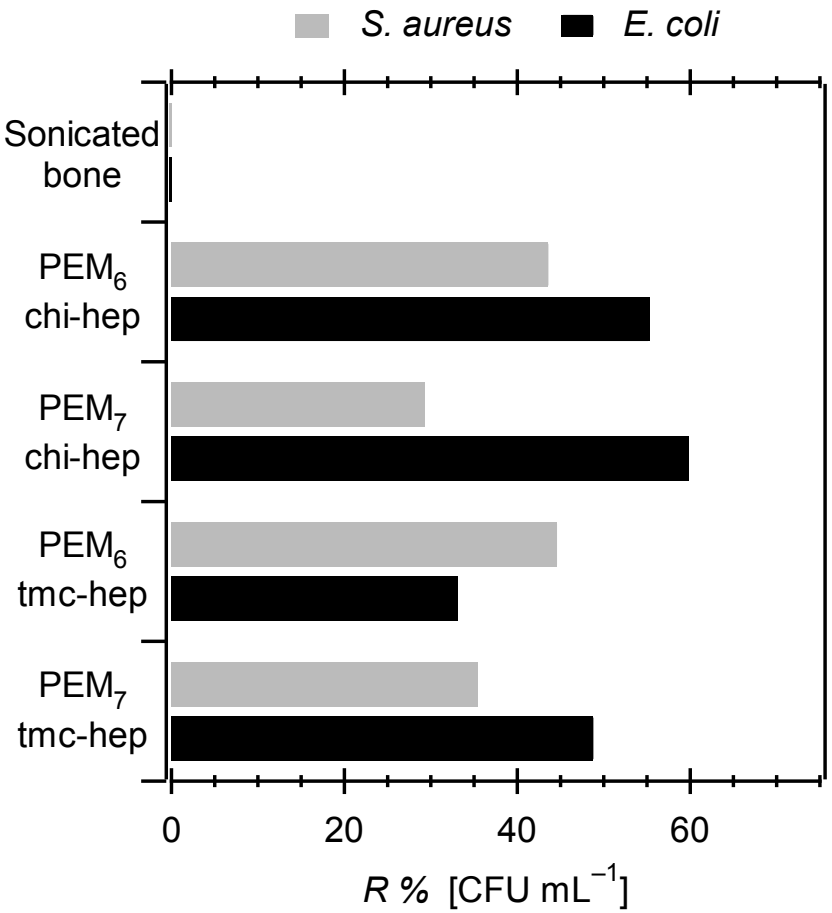
PEM Coating on Bone



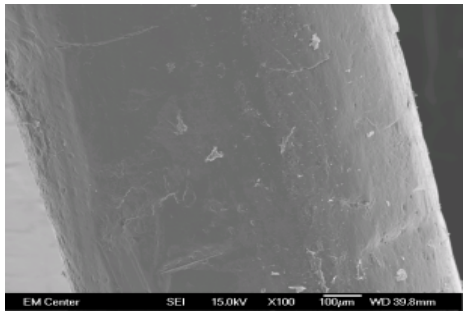
PEM Coating on Bone

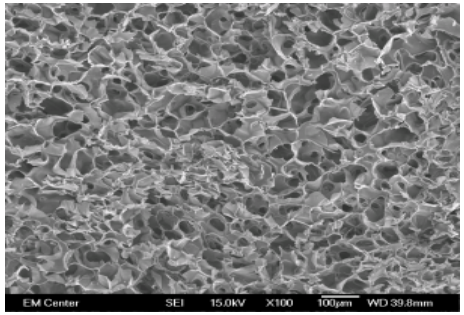
Promote MSC attachment and growth without adhesion ligands or growth factor addition and inhibit bacterial growth

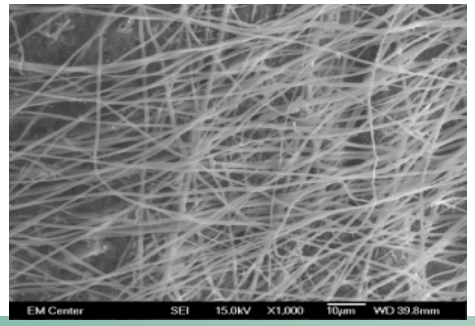




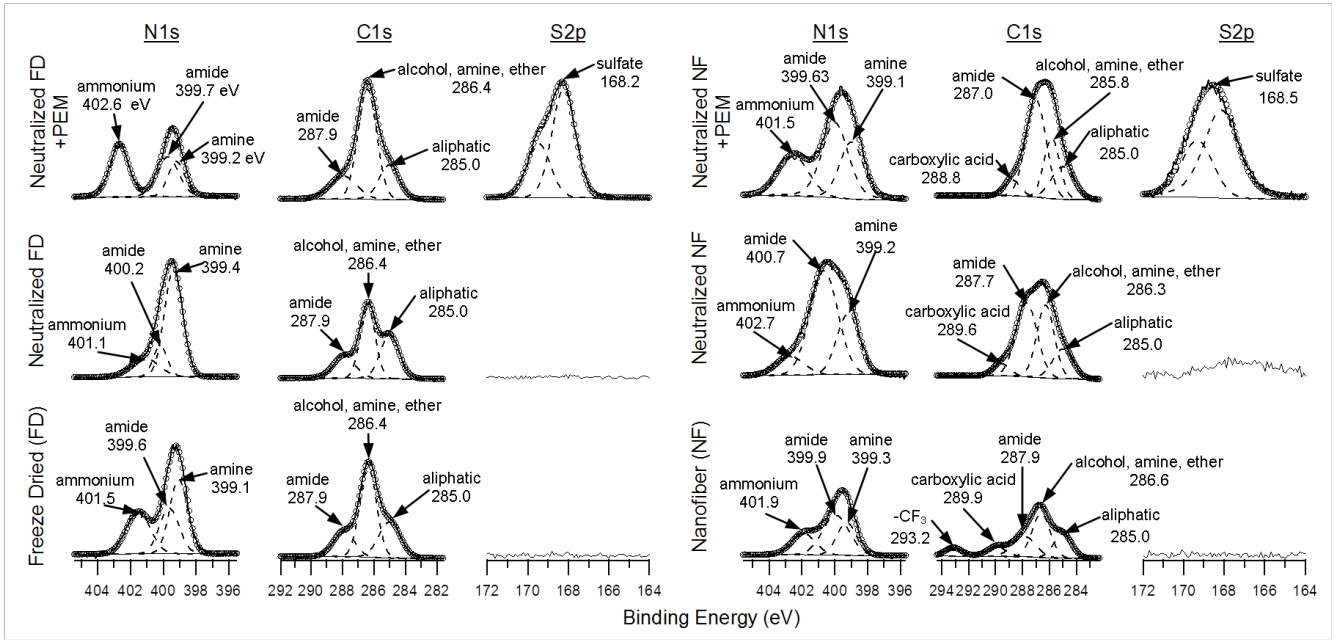
PEM, FD, and NF Coatings





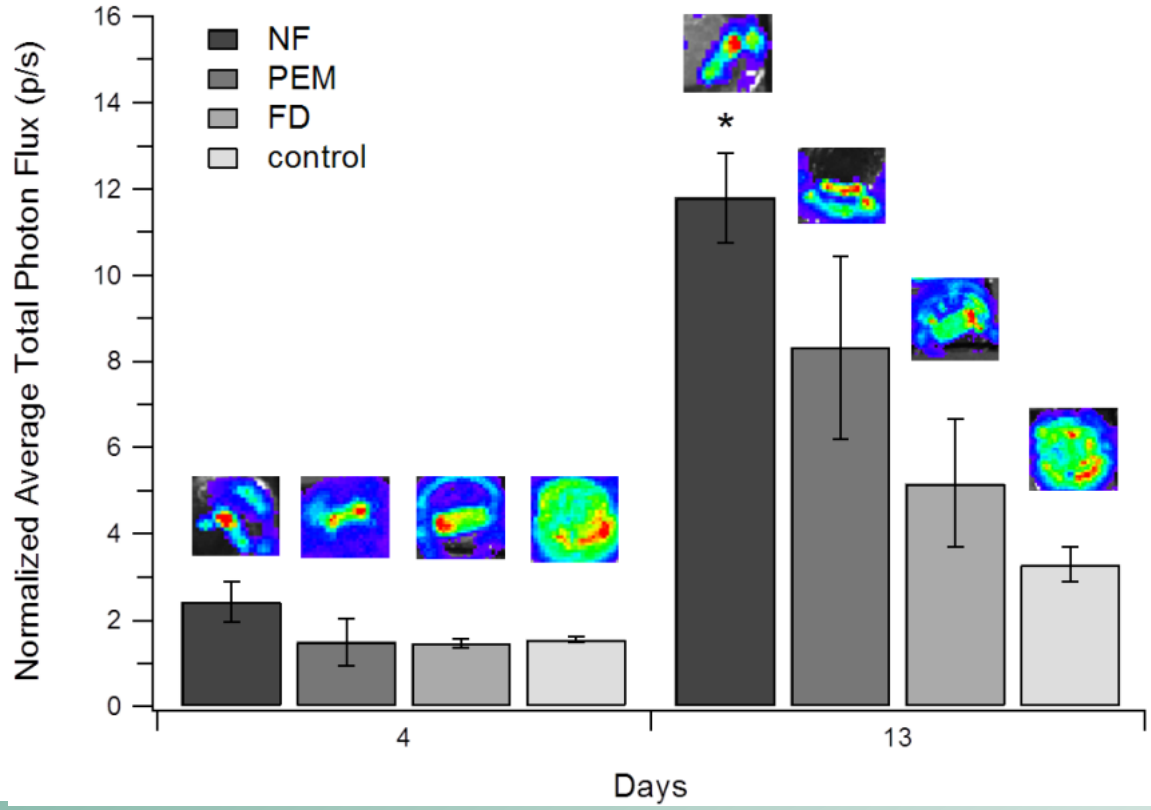


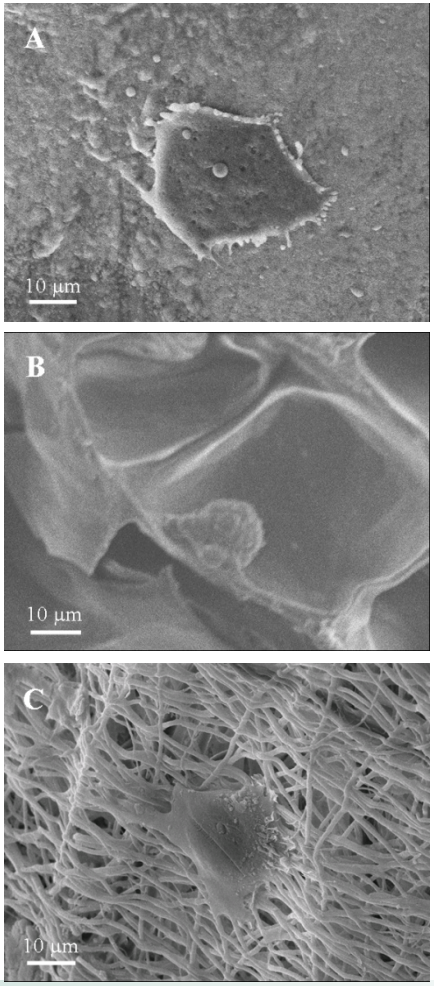
FD and NF scaffolds are durable to multiple aqueous PEM processing steps and MSC culture.



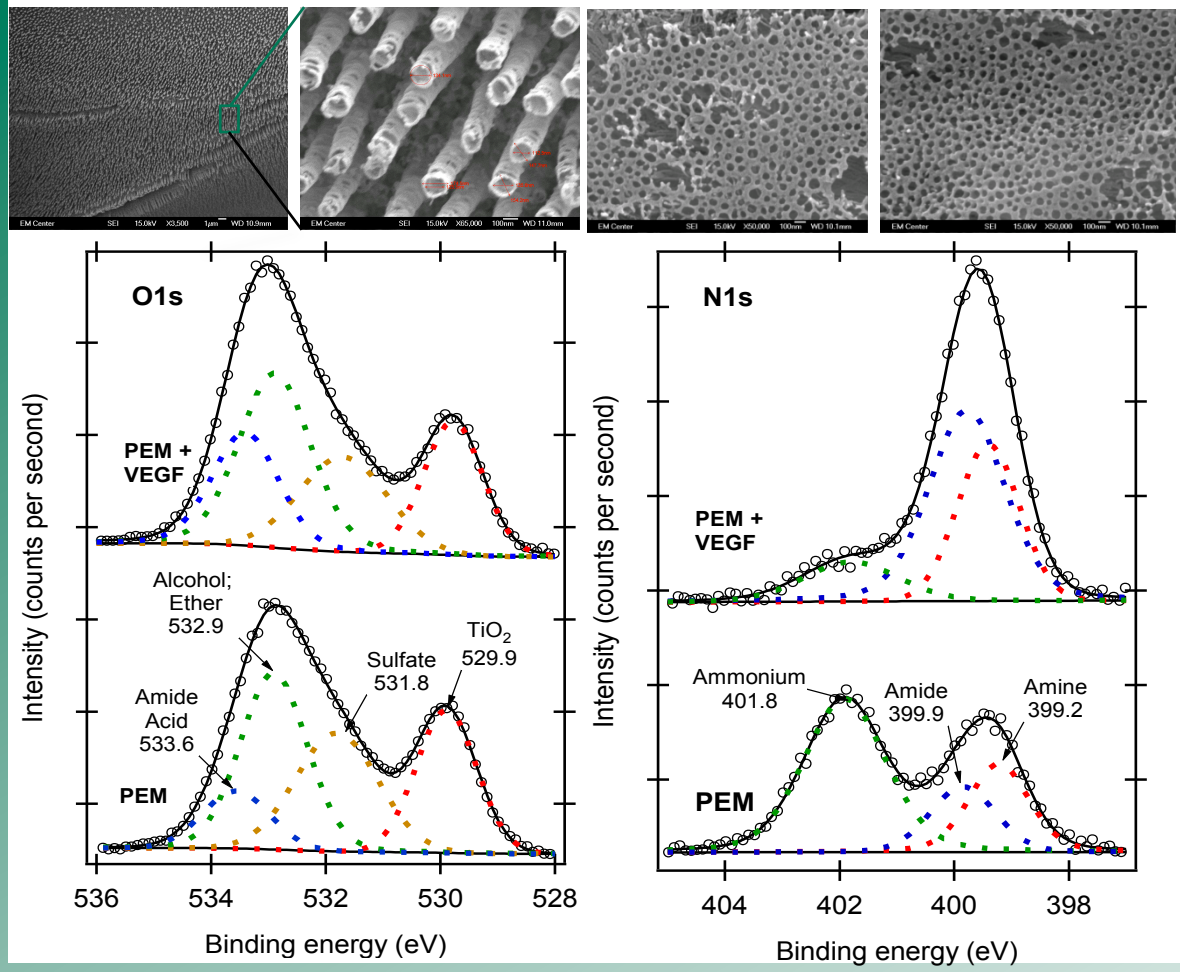
PEM, FD, and NF Coatings

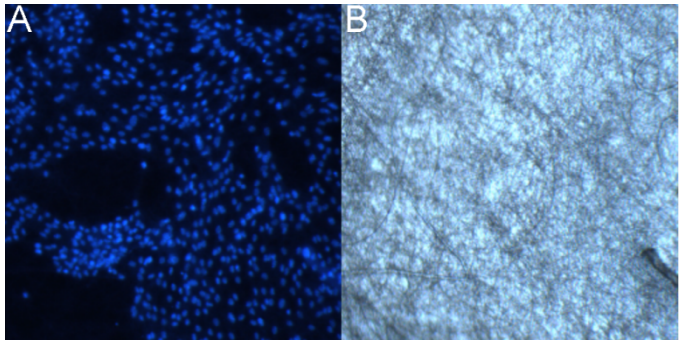
Luciferase expressing MSCs do best on NF coatings.



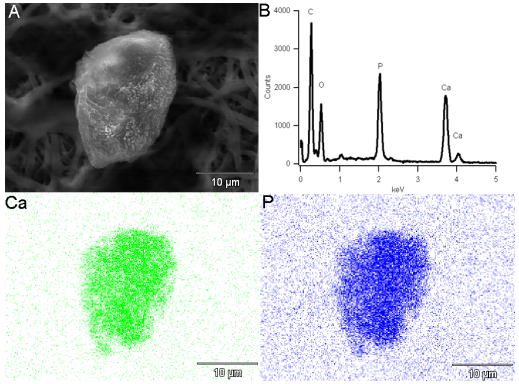


Epilogue: Trailer for Upcoming Episodes





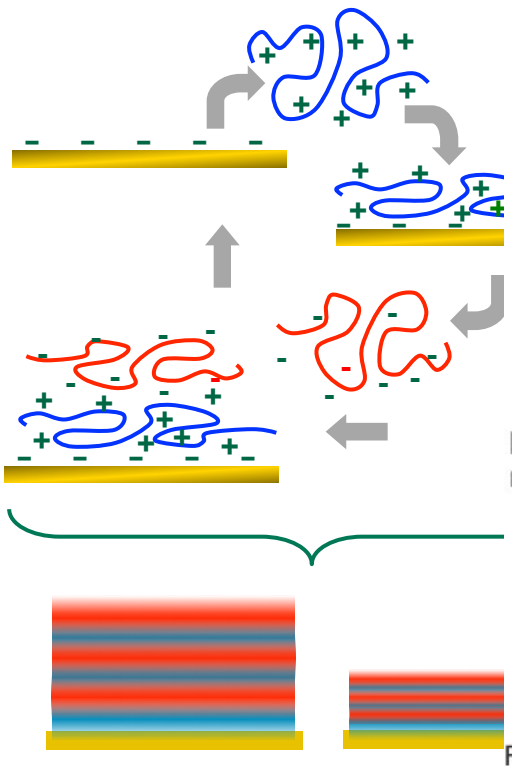
MSC-nanofiber constructs



Octa-calcium phosphate

Polysaccharides: Sweet Opportunities to Engineer Biomaterials

Polyelectrolyte Multilayers



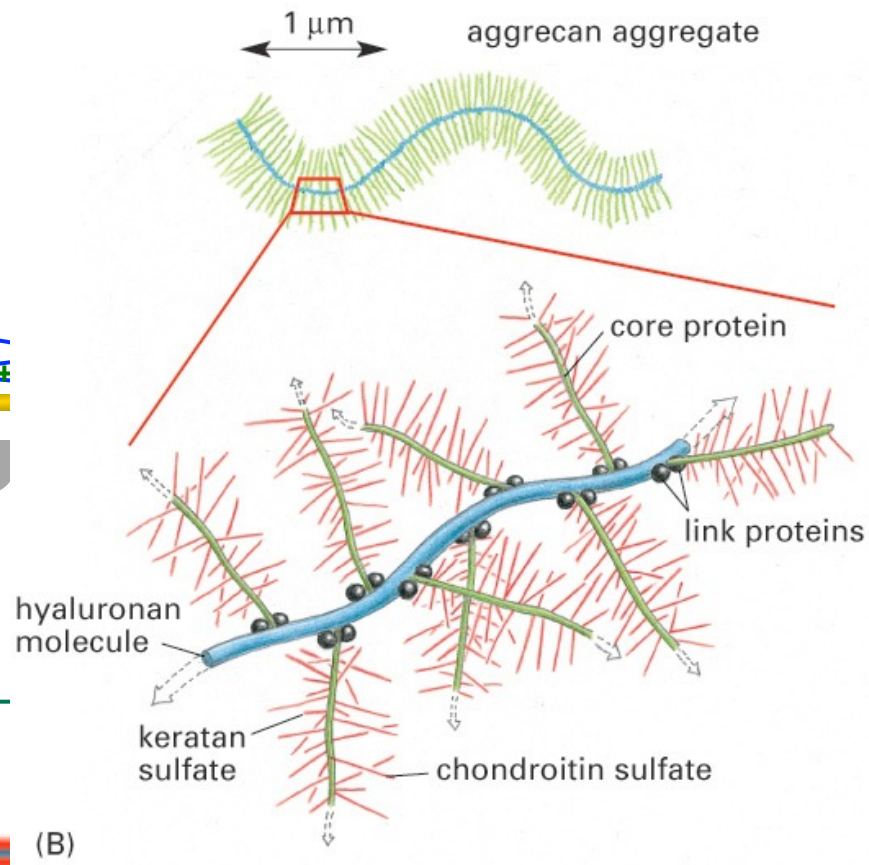


Figure 19–41 part 2 of 2. Molecular Biology of the Cell, 4th Edition.

Electrospun Nanofibers