The Glycome: Sweet Opportunities to Engineer Biomaterials

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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
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
Act 1: Polysaccharide Nanostructure


Polysaccharide Nanostructure Controls Biochemistry and Biophysics


Compression of articular cartilage
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

Abnormal function
Injury!

MMPs (catabolic)
ADAMTS-4
ADAMTS-5

Catabolism and anabolism are not balanced

Osteoarthritis
Rheumatoid Arthritis
Polysaccharides have Useful Biological Functionality

- Antimicrobial
- Support mammalian cell growth
- Promotes wound healing, fibroblast migration, growth factor (PDGF, TGFβ1) and interleukin (leukotriene B₄, IL-1, IL-8)
- Used for blood vessel, skin, bone, cartilage, and liver tissue engineering
Polysaccharides have Useful Biological Functionality

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

\[ pK_a = 2.9 \]
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).

![Chemical Structures]

Chondroitin-6-Sulfate

Heparin

\[ X = \text{OH, } \text{OSO}_3^- \]

\[ Y = \text{NHAc, } \text{NH}_2, \text{NHSO}_3^- \]
Act 2: Polysaccharide Nanostructures we Can Engineer

- Polyelectrolyte Multilayers
- Polyelectrolyte Complex Nanoparticles
- Electrospun Nanofibers

Polyelectrolyte Complexation

GAG-rich Nanogel

Electrospun Nanofibers

300 - 600 nm
A Combination of Techniques Provides a Detailed Understanding of PEM Formation

Increasing polyelectrolyte strength

Chitosan (CHI)  Trimethyl chitosan (TMC)

Hyaluronan (HA)  Chondroitin Sulfate (CS)  Heparin (HEP)

X = OH, OSO₃⁻
Y = NHAc, NH₂, NHSO₃⁻
Controlling Polyelectrolyte Multilayer Assembly

- Chitosan-Hyaluronan
- Chitosan-Heparin
- Weak Polycation-Weak Polyanion (CHI-HA)
- Weak Polycation-Strong Polyanion (CHI-CS and CHI-HEP)

- FT-SPR (wet) Ellipsometry (dry)
- Polyanion strength
- Polyanion molecular weight

- Binding Energy (eV)
- Wavenumber (cm⁻¹)
- PEM Thickness (nm)
- % Swelling
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

![Graphs showing distribution of PCN sizes with charge mixing ratio](image1.png)

![Microscopy images of PCN samples](image2.png)
Act 2: Polysaccharide Nanostructures we can Engineer

Polyelectrolyte Multilayers

Polyelectrolyte Complex Nanoparticles

Electrospun Nanofibers

- Electrostatic complexation
- GAG-rich Nanogel
- GAG
- Chitosan

300 – 600 nm
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

A

Binding energy (eV)

F1s  O1s  N1s  C1s  

As spun  

NH2OH treated

800  700  600  500  400  300  200  100

B

Binding energy (eV)

N1s

S2p

As spun  

NH2OH treated  

Heparin coated

PEM-3

PEM-5

PEM-11

405  401  397  393  169  165  161

NH2OH-treated nanofibers  

Heparin-coated nanofibers
Electrospinning Nanofibers and Modifying with Multilayers
Act 3: MSC Responses to FGF-2 Delivered from Polysaccharide Nanoassemblies

Polysaccharides

FGF-2

Cell Response?

Nanostructures

\[
X = \text{OH, OSO}_3^-
\]
\[
Y = \text{NHAc, NH}_2, \text{NHSO}_3^-
\]
PM-IRRAS Confirms Protein Stability

Absorbance

PEM$_{\text{Hep}}$ + FGF$_{\text{ad}}$

PEM$_{\text{Hep}}$

FGF

Absorbance

Wavenumber (cm$^{-1}$)

Absorption (A.U.)

2$^{\text{nd}}$ derivative

Residual

Wavenumber (cm$^{-1}$)
FGF-2 Adsorption and Release from PEMs
Can We Design Polysaccharide Nanoassemblies with Biological Function?
ECM and Proteoglycan Mimetic Structures

Chitosan or TMC + Electrostatic complexation + Heparin or CS → GAG-rich PCNs

\[ D_H = 200 – 600 \text{ nm} \]

Aggrecan

\[ D_H = 404 \text{ nm} \]

\[ X = \text{OH, OSO}_3^- \]
\[ Y = \text{NHAc, NH}_2, \text{NHSO}_3^- \]

Electrostatic complexation
ECM and Proteoglycan Mimetic Structures

Aggrecan-mimetic nanoparticles

glycosaminoglycans

growth factors

Aggrecan

Preconditioning time (days)

FGF activity

Control

Hep-Chi

Hep-TMC

Aggrecan

FGF-2 sol.

Day 0

Day 7

Day 14

B

C

D

E
Nanofibers Modified with FGF-2/PCN Complexes
FGF-2 Release from Nanofibers

![Graph showing FGF-2 release over incubation days for different conditions: Fibers Only, FGF-2/PCN, FGF-2/PCN+PEM.](image)

![Image A](image)

![Image B](image)
FGF-2 Activity After Release from Nanofibers

A) Non-FGF-2 samples
- PCN in PEM

B) FGF-2 containing samples
- PCN in solution
- Day 1
- Day 9
- Day 18
- Day 27

PCN+PEM

FGF-2/PCN+PEM

PCN

FGF-2/PCN
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.

Three Methods for Coating Bone

Method 1: PEMs

Method 2: Foam+PEMs

Method 3: Nanofibers+PEMs

Diaphyseal surface
PEM Coating on Bone

Polycation adsorption
Charged surface
Polyanion adsorption
Repeat

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<th>O1s</th>
<th>N1s</th>
<th>Ca2p</th>
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PEM Coating on Bone

Binding energy [eV]

O1s

N1s

C1s

S2p

PEM$_{30}$
on Au

alcohol, ether 532.9
carboxylic acid 533.4

PEM$_{10}$
on Bone

sulfate 531.8
ammonium 402.1

amine 399.6

aliphatic 285.0

Untreated

Bone

ester 533.7
phosphate; carbonate 531.4

amine 400.1

alcohol; amine; ether 286.3

ester 289.3

amine 288.2

aliphatic 285.0

alcohol, ether 532.3

alcohol; amine; ether 286.3

amid ester 289.3

amine 288.2

aliphatic 285.0
Promote MSC attachment and growth without adhesion ligands or growth factor addition and inhibit bacterial growth.

S. aureus

E. coli

- Sonicated bone
- Bone + PUA
- Bone + PEM₆
- Bone + PEM₆ + Fibronectin

![Graph showing bacterial growth inhibition](image-url)
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

**Polypelectrolyte Multilayers**

- Hyaluronan molecule
- Keratan sulfate
- Chondroitin sulfate
- Core protein
- Link proteins
- Aggrecan aggregate

**Electrospun Nanofibers**