DREXEL UNIVERSITY
School of
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Biomimetic Proteoglycans for Tissue Regeneration

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Biomimetic proteoglycans (PGs) mimic natural molecules

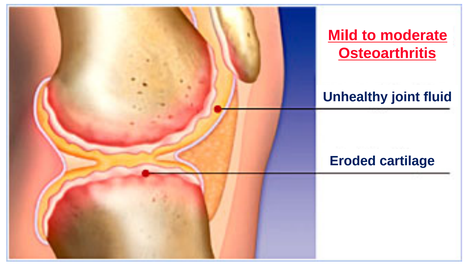
- Bottle brush architecture
- High osmotic pressure
- High water uptake

Natural Proteoglycans	Biomimetic Proteoglycans	Tissue
Aggrecan Chondroitin Sulfate (CS) Keratan sulfate (KS) MW ~ 2,000kDa ~ 100 CS bristles	Biomimetic Aggrecan Natural CS Enzymatically-resistant polymer core	Intervertebral disc (nucleus pulposus) Articular artilage
Versican Chondroitin Sulfate (CS) MW ~ 1,000kDa ~ 40 CS bristles	Biomimetic Versican	Skin Hair
Decorin Chondroitin Sulfate (CS) MW ~ 100kDa ~ 2-3 CS bristles	Biomimetic Decorin	Neural tissue Articular cartilage

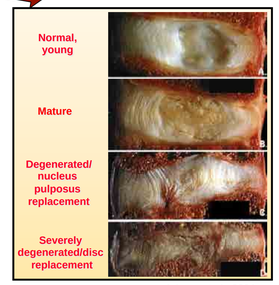
Biomimetic proteoglycan injection can be potentially used to restore functionality of degenerated and dehydrated tissue

http://dermalfillersmelbourne.tumblr.com/

Dermal fillers



Treatment of osteoarthritis

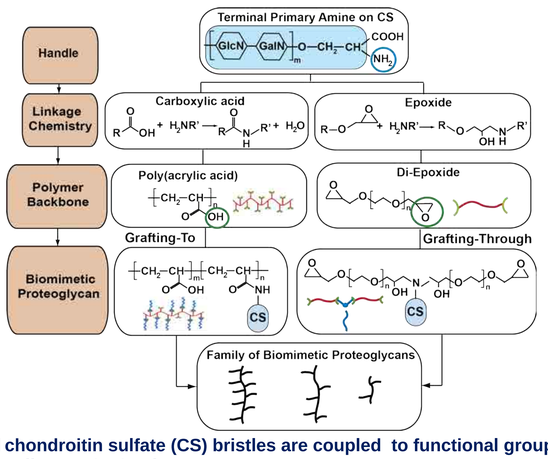


Adams et al., 2004[5].

Treatment of lower back pain

- Proteoglycans are natural nano-scale biomacromolecules that are essential for hydration and structural integrity of soft and connective tissues[1-3].
- With age and degeneration, PGs are enzymatically digested along the protein core and lost from the tissue matrix[4].
- Loss of proteoglycans results in a host of mechanical, hydration and nutritional deficits to tissue function[4-5].
- We propose to molecular engineer degenerated tissue and restore its function with an infusion of enzymatically-resistant biomimetic proteoglycans.

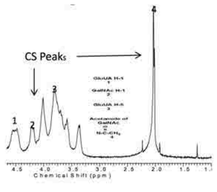
Synthesis of biomimetic PGs



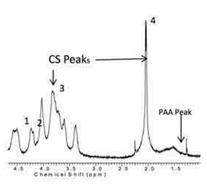
- Natural chondroitin sulfate (CS) bristles are coupled to functional groups on enzymatically-resistant polymer cores.

^{1}H-NMR Chemical structure

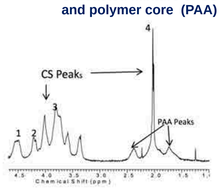
Natural Chondroitin Sulfate



Biomimetic aggrecan

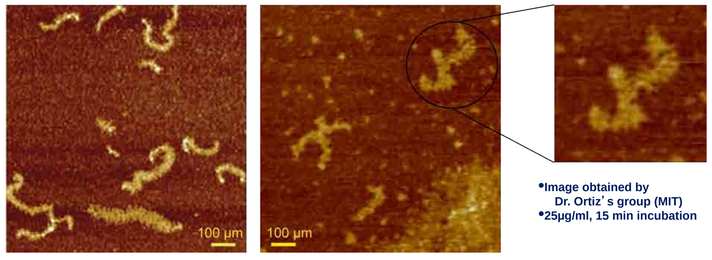


Unreacted mixture of CS and polymer core (PAA)



Macromolecular architecture (AFM)

Natural Aggrecan Biomimetic Aggrecan

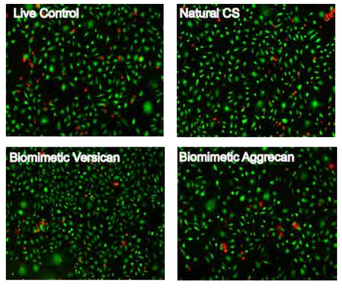


- Image obtained by Dr. Ortiz's group (MIT)
- 25µg/ml, 15 min incubation

- Biomimetic aggrecan has a bottle brush architecture similar to a natural molecule.

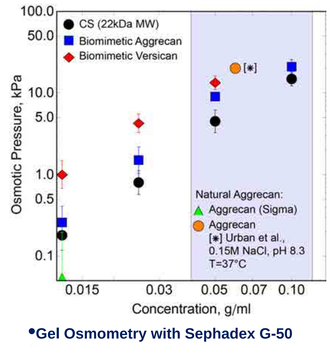
Cytotoxicity

Live/Dead Assay @ 2 mg/ml



- L-929 Fibroblasts (12500 cells/cm^2).
- 48h dosing with biomimetic PG. solutions at 2 mg/ml and 0.2 mg/ml.
- Biomimetic PGs do hot exhibit cytotoxic effects.

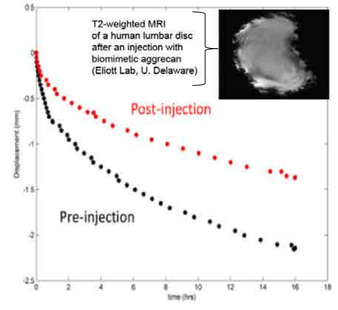
Osmotic pressure



- Gel Osmometry with Sephadex G-50
- 1X PBS, pH 7.4, T = 25°C
- Shaded region – physiologically relevant range of concentrations.

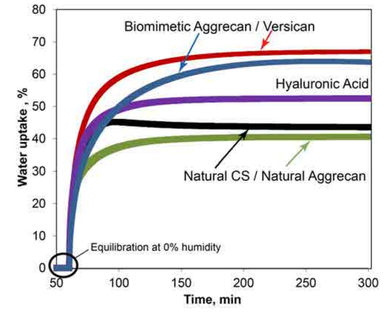
Pilot experimental studies

Intervertebral disc injection



- Biomimetic aggrecan was injected into a human lumbar cadaveric disc (T12-L1, 76 yo male).
- The disc was pre-conditioned and tested in diurnal compressive stress and recovery (16hr 400N and 8hr 50N).
- Reduced creep displacement correlates with an increased osmotic pressure in the disc.

Water uptake



- Water uptake measurements were performed at 90% relative humidity, 37°C.
- Biomimetic PGs show considerable improvement in water uptake (~60-63%) compared with CS (45%), natural aggrecan (43%) and even hyaluronic acid (52%), which is widely used as an excellent moisturizer and a dermal filler.

Conclusions

- Biomimetic proteoglycans have been successfully fabricated by coupling natural CS bristles to an enzymatically-resistant polymer core.
- Biomimetic PGs mimic a bottle brush architecture of natural molecules
- Biomimetic PGs do not exhibit cytotoxic effects at moderate concentrations.
- Organization of CS bristles in a bottle brush configuration leads to an increase in osmotic potential.
- Biomimetic PGs show considerable improvement in water uptake as compared to CS, natural aggrecan and hyaluronic acid.
- Biomimetic aggrecan can be delivered to a lumbar disc without over-pressurization or damage to a disc.
- Introduction of biomimetic aggrecan into a lumbar disc leads to an increase in osmotic pressure and water retention within the disc what helps to stabilize its compressive biomechanics.

We would like to acknowledge the Coulter Foundation for funding.

References:

1. Roughley, P.(2004). Spine, 29(23).
2. Seog J, Dean D, Plaas AHK, Wong-Palms S, Grodzinsky AJ, Ortiz C. Macromolecules. 2002;35(14):5601-15.
3. Chris Kiani et al. Cell Research 2002; 12 (1): 19-32
4. Kern Singh MD et al. Spine Vol. 34 No. 1 (2008) 10-16
5. Adams, M. A., Dolan, P. & McNally, D.S. (2009). Matrix Biol, 28, 384-9.

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