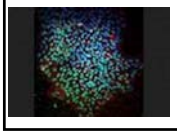
Regenerative Strategies for Replicating IVD Structure

2016 Castellvi Spine Symposium
May 19-22



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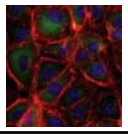
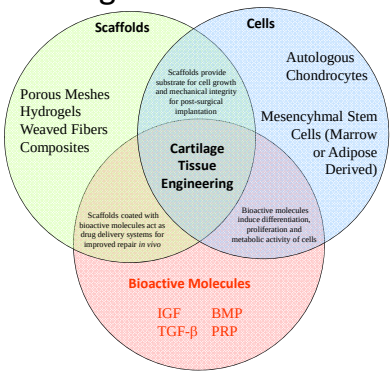


Disclosures:

- Aedicell, Inc.: Consultant, SAB member
- Histogen, Inc; Consultant
- Regen Labs; Consultant
- Suneris, Inc. Consultant/ Stock option holder

The Regenerative Triad

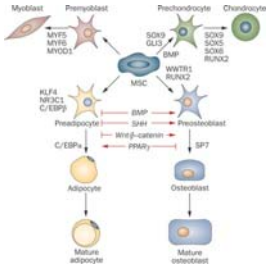


Characterization of MSC's

- Non hematopoietic multipotent cells
- High proliferative potential
- Ability to differentiate into:
  - Chondrocytes, osteoblasts, adipocytes, and stromal cells
- Immunomodulatory activity
  - Contribute to immune suppression
  - Tissue healing
- Potential use in tissue regeneration therapies
- Reserve to replace damaged and aged cells

multiple differentiation potential

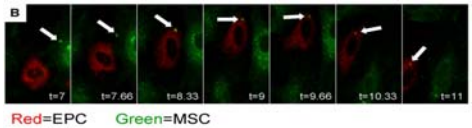
- Major source:
  - Bone marrow derived
  - Adipose derived
- Major applications in orthopaedics:
  - Spine fusion, Vertebral body repair (osteogenesis)
  - Cartilage, Intervertebral disc regeneration (chondrogenesis)



Takeda, I. *et al.* Wnt and PPARγ signaling in osteoblastogenesis and adipogenesis *Nat Rev Rheumatol.* 2009 Aug;5(8):442-7. Epub 2009 Jul 7.

Paracrine Stimulation by stem cells

- Coculture of endothelial cells and bone marrow MSC's



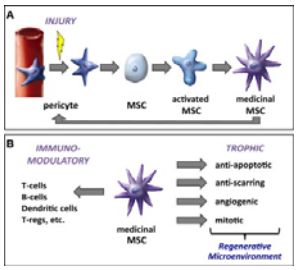
Red=EPC Green=MSC

A vesicle transported from MSC to endothelial cells. Time is indicated as hours

Aguirre A. *et al.* Dynamics of bone marrow-derived endothelial progenitor cell/mesenchymal stem cell interaction inco-culture and its implications in angiogenesis. *Biochem Biophys Res Commun.* 2010 Sep 17;400(2):284-91

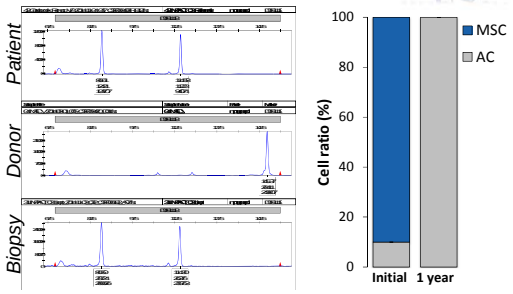
Mechanism of Action of MSC's as an Adjunct in Cartilage Repair

'We call MSCs medicinal signalling cells'



Caplan and Correa. Cell Stem Cell 2011;9:11

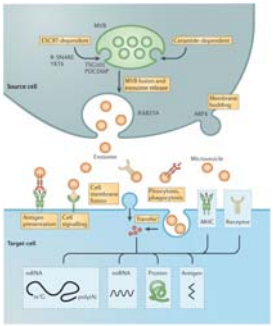
MSCs stimulate cartilage repair



90 % allogeneic MSCs facilitate 100% autologous repair tissue

Vonk, Saris. UMC Utrecht, IMPACT trial

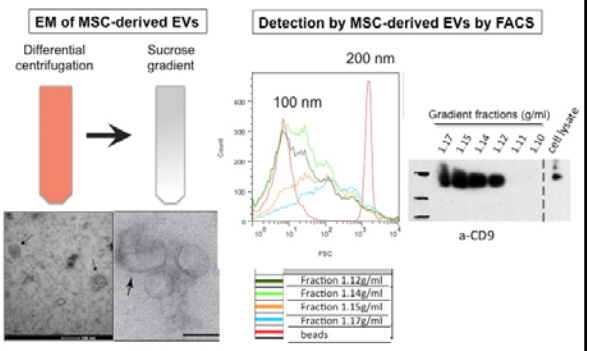
Extracellular vesicles - biogenesis



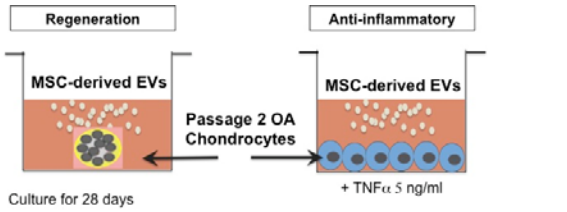
Exosomes: membrane enclosed particles

Andaloussi et al. Nat Rev Drug Discov. 2013;12:347-57

Characterization MSC-EVs



Methods



Culture for 28 days

GMP-grade MSCs passage 5

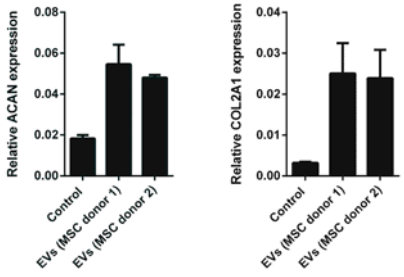
MSC-derived EVs added every 5 days

Readout: Histology
PGs - DMMB
Gene expression

Culture for 48 hours

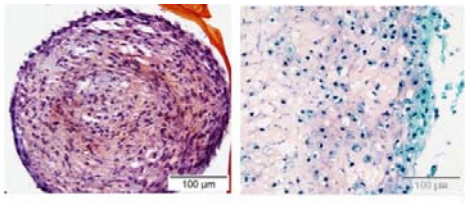
Readout: COX2 gene expression
PGE2 protein expression

Induction of ACAN and COL2A1 gene expression



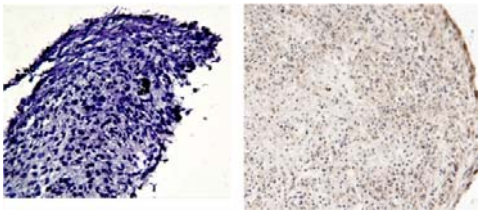
Proteoglycan staining

Control MSC-derived EVs

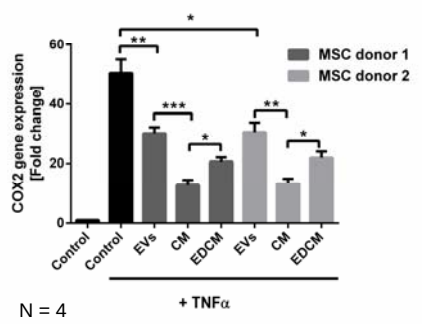


Type II collagen staining

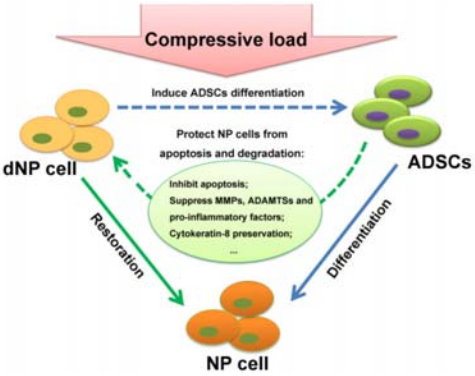
Control MSC-derived EVs



Inhibition of COX2 gene expression



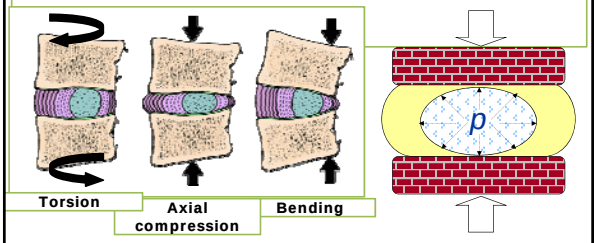
N = 4



Sun, Z. *et al*. Adipose-derived stromal cells protect intervertebral disc cells in compression: Implications for stem cell regenerative disc therapy. Int J Biol Sci. 11(2): 133-143. 2015

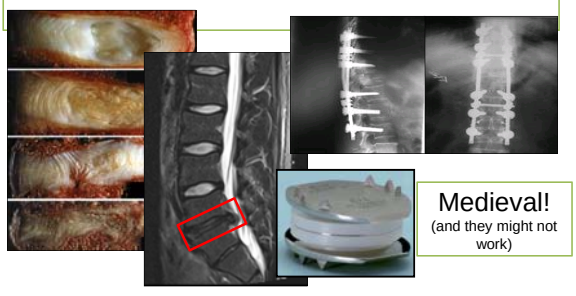
Mechanical Loading of the Disc

- **Multi-directional loading**: Compression, torsion, bending
- Operates as a **thick walled pressure vessel**
  - NP swelling pressure: disc height & load support
  - AF stiffness: minimize bulging and strain; load support



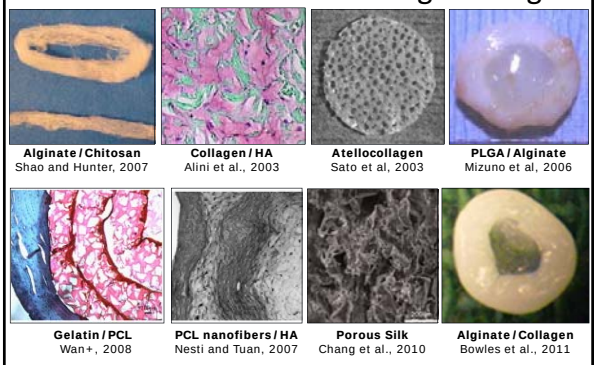
Low Back Pain and Disc Degeneration

- Annual societal burden over $100 billion
- #1 cause of activity limitation in adults < 45 y.o.



Medieval!
(and they might not work)

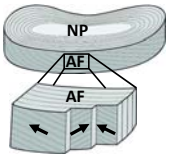
Materials for Disc Tissue Engineering

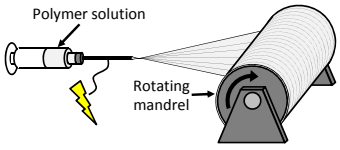


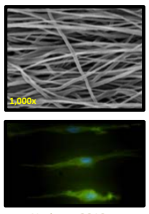
Alginate / Chitosan Shao and Hunter, 2007
Collagen / HA Alini et al., 2003
Atellocollagen Sato et al, 2003
PLGA / Alginate Mizuno et al, 2006
Gelatin / PCL Wan+, 2008
PCL nanofibers / HA Nesti and Tuan, 2007
Porous Silk Chang et al., 2010
Alginate / Collagen Bowles et al., 2011

Engineered Annulus Fibrosus

- Hierarchical structure:
  - Lamellae: discrete fibrous sheets
  - Fiber alignment: 20° to 50° to the transverse plane (Holzapfel+ 2005)
- Electrospun polymers recreate AF fiber architecture (Nerurkar+ 2007, 2008, 2009, 2010; Driscoll+ 2011)

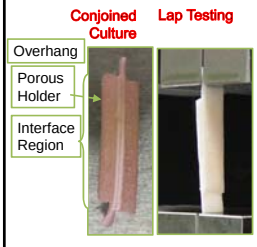


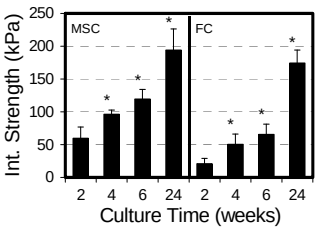




Nathan+ 2010

Multi-Lamellar Nanofibrous Assemblies



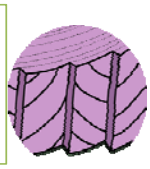


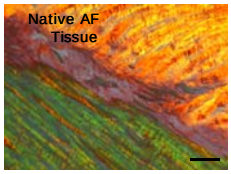
Mechanically viable interface forms between apposed MSC-laden nanofiber layers

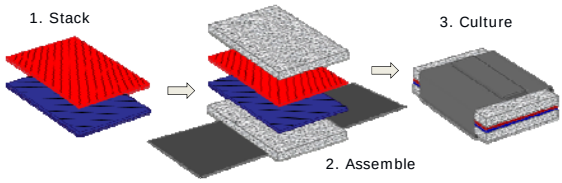
Baker et al., 2007

Fabrication of Ordered Bi-Layers

Goal: Fabrication of biologic angle-ply laminates to recreate the unique structural hierarchy of the native AF



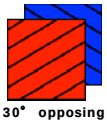


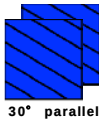


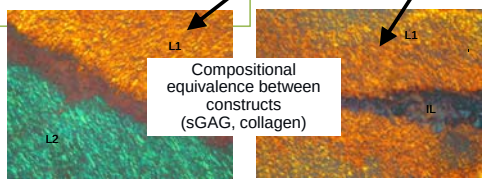
Nerurkar et al., 2009

Evaluation of Ordered Bi-Layers

- Fabrication of MSC seeded angle-ply biologic laminates
  - Opposing: +/- 30º
  - Parallel: +/+ 30º
- Culture in vitro for up to 10 weeks
- Mechanical, biochemical, and histological evaluation





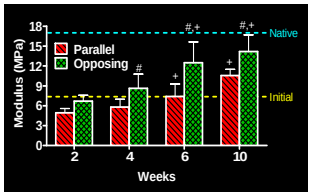


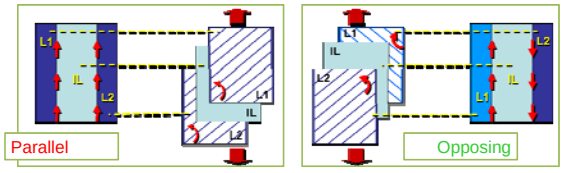
Compositional equivalence between constructs (sGAG, collagen)

Nerurkar et al., 2009

Mechanics of Ordered Bi-Layers

- Uniaxial and Biaxial Testing: opposing bi-layers are significantly stiffer than parallel bi-layers (#p<0.05)





Nerurkar et al., 2009; Driscoll et al., 2012

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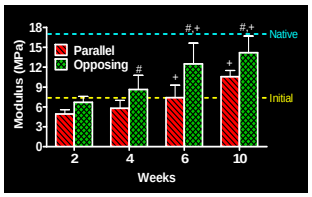
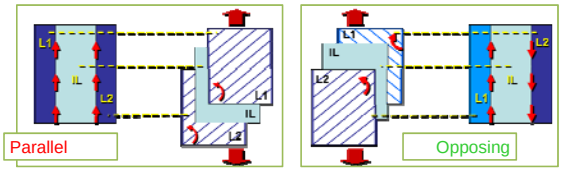
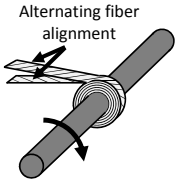
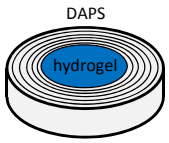


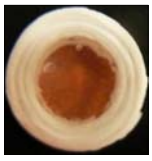


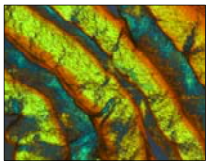
Nerurkar et al., 2009; Driscoll et al., 2012

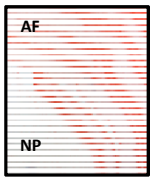
Disc-like Angle Ply Structures (DAPS)

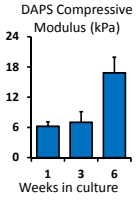








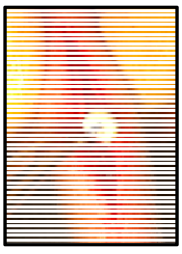


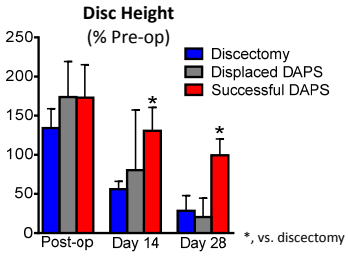


Nerurkar+ 2009, 2010

Rat Caudal Spine Model

- Implantation of Acellular DAPS
  - In 50% of DAPS surgeries DAPS are displaced from the disc space





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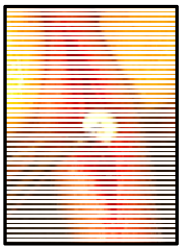
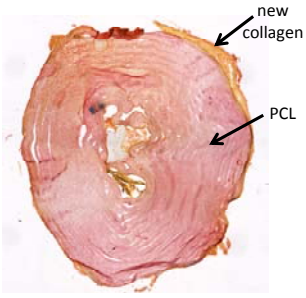
Rat Caudal Spine Model

- Implantation of Acellular DAPS
  - Little matrix deposition within dense PCL scaffold





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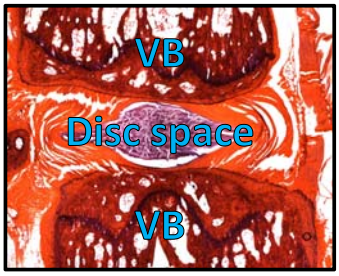
I. DAPS Implantations with External Fixation

- DAPS are retained in 100% of surgeries

Ventral



Dorsal (Incision)

I. DAPS Implantations with External Fixation

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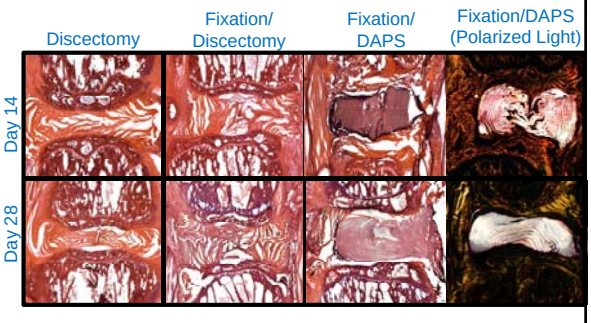

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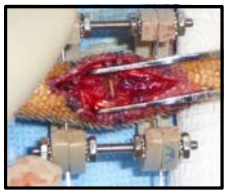
Objectives

- I. Improve retention of DAPS implanted into the rat caudal spine.
- II. Improve matrix formation in implanted DAPS.

I. DAPS Implantations with External Fixation

- Radiolucent ring-type external fixator (Iatridis+ 1999)
- Implant: AF-only DAPS, electrospun PCL
- Procedure: Insert wires, apply fixator, discectomy, implant
- Groups: (n=2-5/group)
  - Fixation+DAPS: 14 days, 28 days
  - Fixation+Discectomy: 14 days, 28 days

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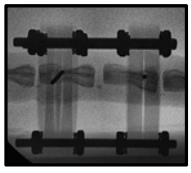
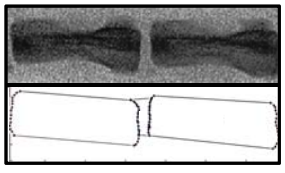
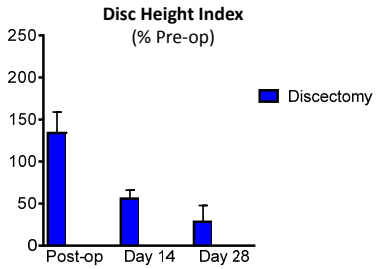
- Procedure: Insert wires, apply fixator, discectomy, implant
- Groups:
  - Fixation+DAPS: 14 days, 28 days
  - Fixation+Discectomy: 14 days, 28 days
- Analysis: Fluoroscopy – DHI (Masuda+ 2005) , µCT, Histology

I. DAPS Implantations with External Fixation

- Fixation preserves disc height for DAPS and discectomy groups



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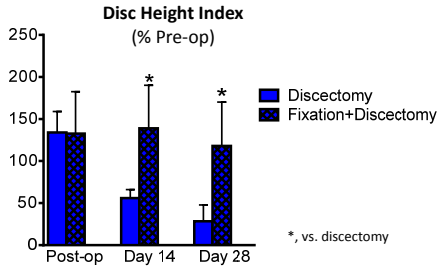
I. DAPS Implantations with External Fixation

- Fixation preserves disc height for DAPS and discectomy groups



Martin+ Acta Biomat 2014

I. DAPS Implantations with External Fixation

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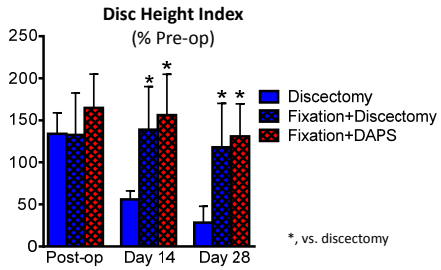

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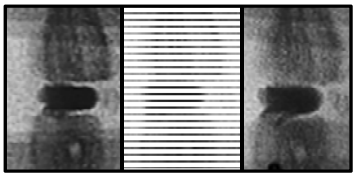
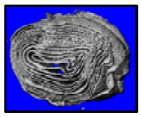
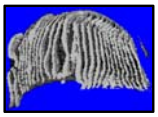
I. DAPS Implantations with External Fixation

- rDAPS maintain position with fixation



Post-op Day 14 Day 28

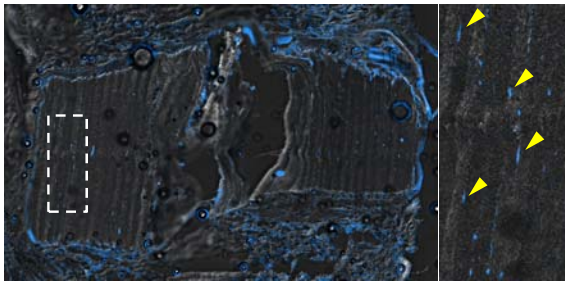




Martin+ Trans ORS 2014

I. Development and Validation of Model

- Surgery 2: Implantation of DAPS with External Fixation
  - Sparse cellularity, cells with elongated nuclei

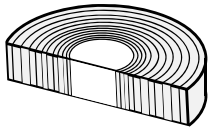


Martin+ Acta Biomat 2014

II. Sacrificial Layer DAPS (sDAPS)

- Fabrication of sDAPS
  - Water soluble PEO or PLGA layers between PCL layers

PCL



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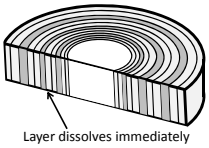
PCL PEO



II. Sacrificial Layer DAPS (sDAPS)

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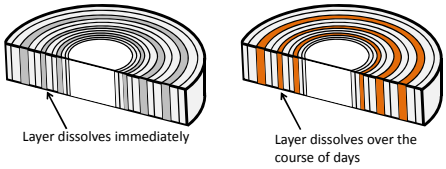
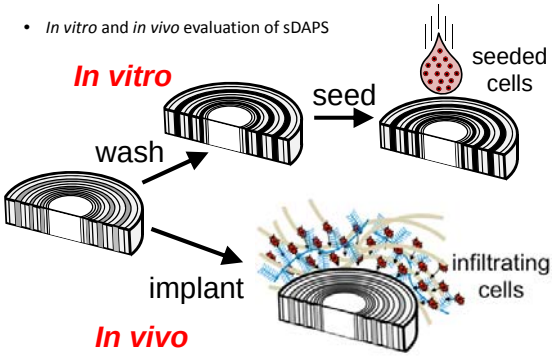
  - Water soluble PEO or PLGA layers between PCL layers

PCL PEO PLGA



II. Sacrificial Layer DAPS (sDAPS)

- *In vitro* and *in vivo* evaluation of sDAPS



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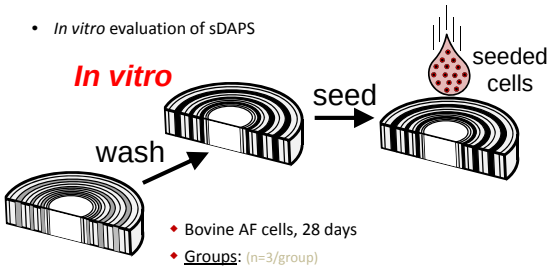
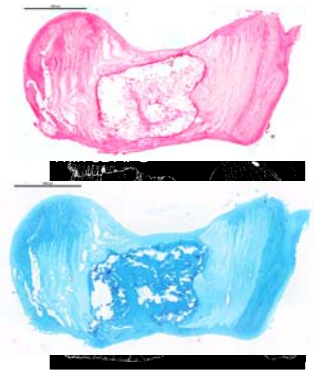
- *In vitro* evaluation of sDAPS



- Bovine AF cells, 28 days
- Groups: (n=3/group)
  - DAPS
  - **Thin** sDAPS – 125 µm PEO
  - **Thick** sDAPS – 250 µm PEO
- Analysis: Histology – DAPI

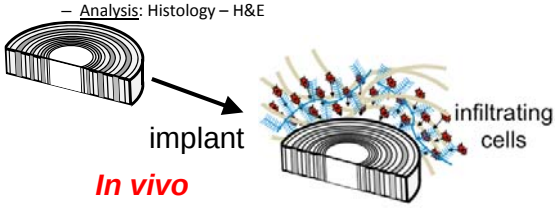
II. Sacrificial Layer DAPS (sDAPS)

- *In vitro* evaluation of sDAPS
  - PCL-only DAPS were poorly infiltrated
  - AF cells penetrated full height of both thick and thin sDAPS
- Long term culture
  - NP cell seeded Hyaluronic Acid NP region
  - AF cell-seeded AF region
  - 5 and 10 weeks
- Increasing matrix content and mechanics



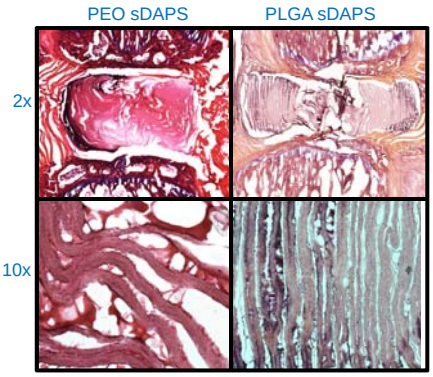
II. Sacrificial Layer DAPS (sDAPS)

- *In vivo* evaluation of acellular sDAPS
  - Rat caudal disc model
  - Groups:
    - PEO sDAPS: thin, 14 days (n=3/group)
    - PLGA sDAPS: thin, 14 days (n=3/group)
  - Analysis: Histology – H&E



In vivo

II. Sacrificial Layer DAPS (sDAPS)

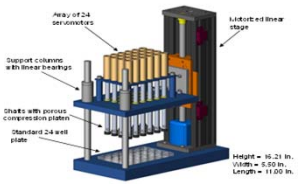


Discussion

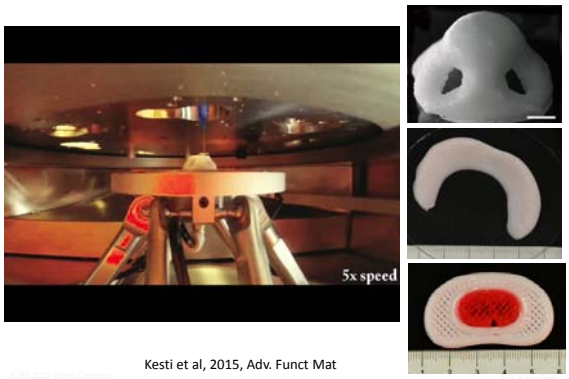
- Developed an engineered disc that mimics the hierarchical structure of the native AF in a rat tail model.
- External fixation improves implant retention.
  - Improvement from ~50% to 100% successful
  - Useful for evaluation of mechanical stimulation and remobilization
- Sacrificial layers improve cellular ingress and matrix deposition.
  - Useful in vitro paradigm for construction of cell-seeded DAPS
  - Polymer dissolution time should be tuned for in vivo application

Future Directions

- An *in vivo* model to screen:
  - Acellular sDAPS with hydrogel NP
  - Cell-seeded DAPS (comprised of both native NP and AF cells as well as MSCs)
  - Effect of pre-maturation and mechanical stimulation of cell-seeded DAPS and functional outcomes in vivo
  - Methods to improve integration with vertebral endplate
  - Up-scaling to larger species

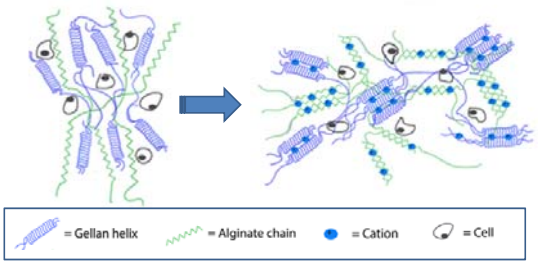


BioPrinting Cartilage Structures



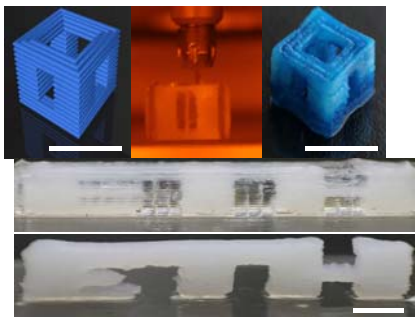
Kesti et al, 2015, Adv. Funct Mat

Bio-Ink Composed of Gellan and Alginate (Gelation upon addition of cations)



50

Bioprinting de novo Cartilage with ECM-based Bioink

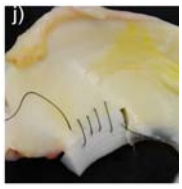


3D printed structure before and after elution of the support material. Scale bar 2.5 mm

3D Bio printed Components

Multi material samples with varying geometry and structure.

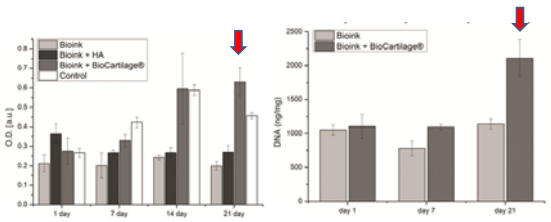
How Strong, Flexible, Tough is the Printed Material?



Kesti et al, 2015, Adv. Funct Mat, in press

53

Metabolic activity and **proliferation** increased in the presence of BioCartilage



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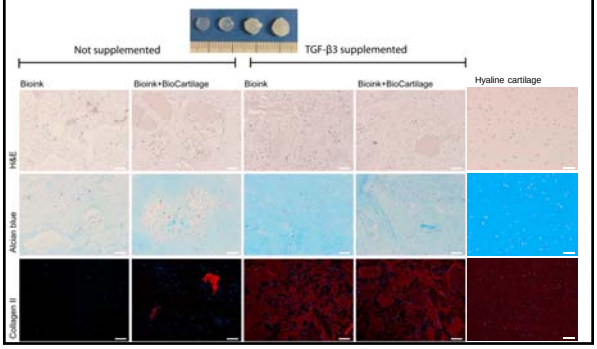
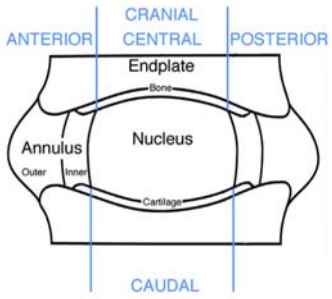
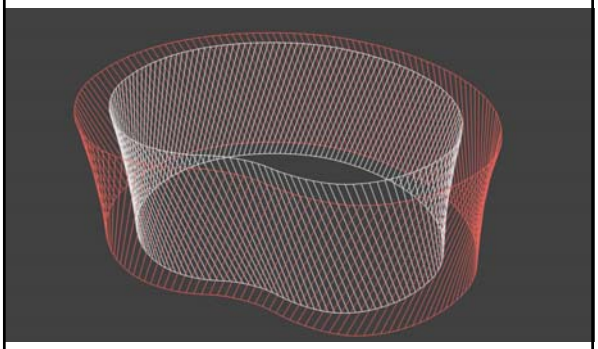
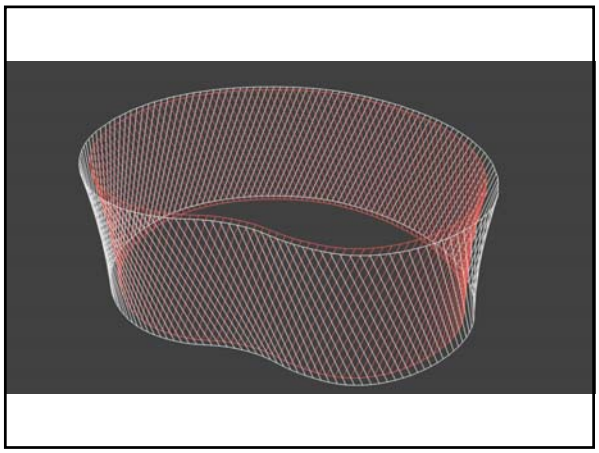
In vitro (8 weeks)

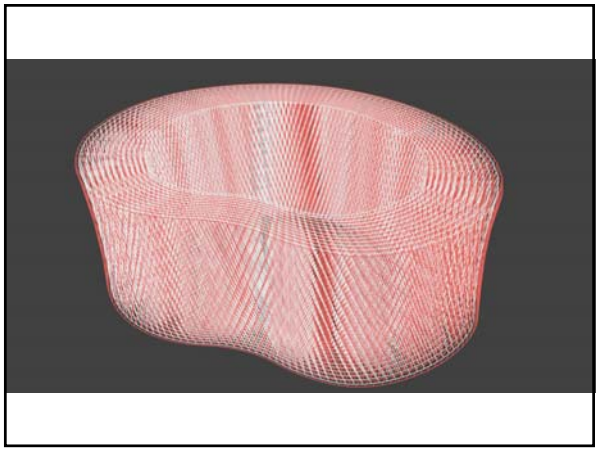


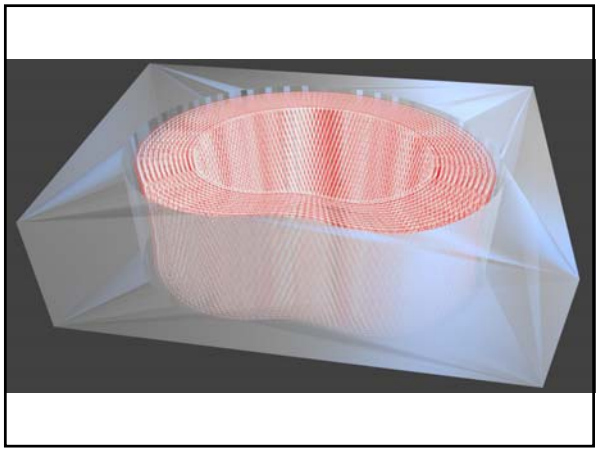


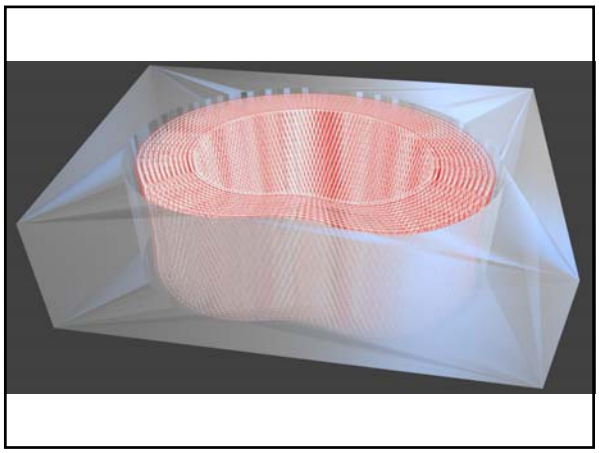
Fields, A. J. *et al*. Innervation of pathologies in the lumbar vertebral end plate and intervertebral disc. Spine J. 14: 513-521. 2013

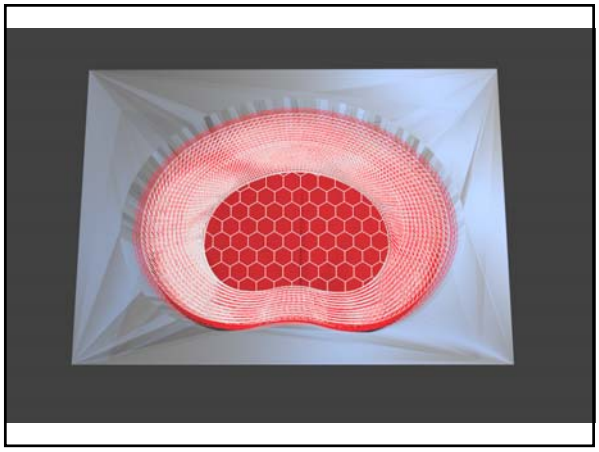


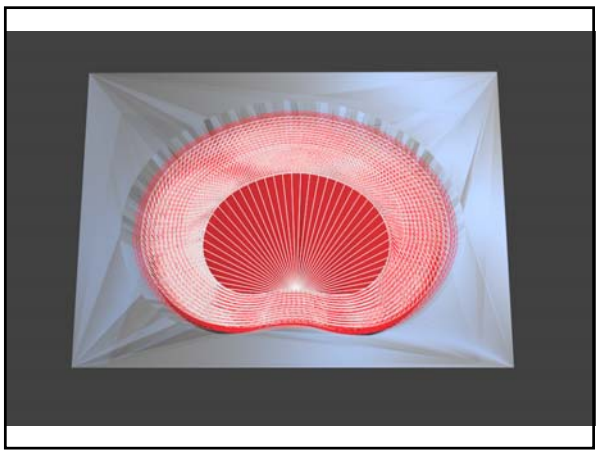


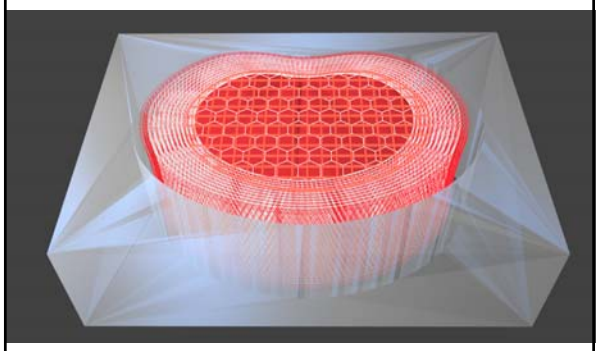


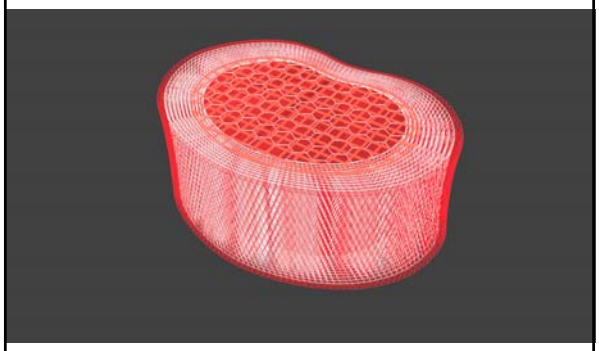




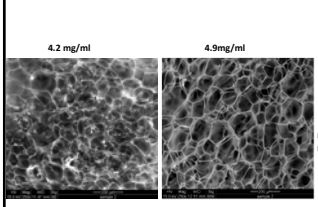


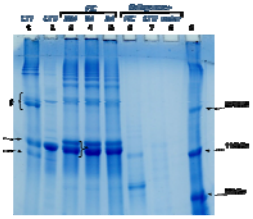






Novel Collagen II Scaffold and Gels





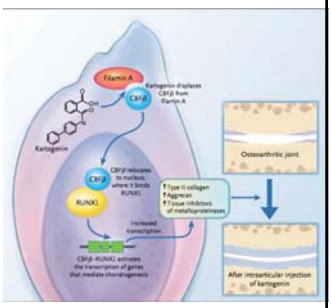
Collagens was extracted from jellyfish umbrella. Pepsinized jellyfish collagens (PIC) were loaded on SDS-PAGE gel (Lane 3-5) . Type I (Lane 1) and type II (Lane 2) collagens was used as collagen control. PIC and collagen type II std were treated with collagenase. Molecular mass markers (kDa) are indicated in Lane

Thank You!

- Dept. of Orthopaedic Surgery
- Dept. of Thoracic Surgery
- Dept. of Otolaryngology
- North Shore-LIJ Health System
- Hofstra North Shore-LIJ School of Medicine

Kartogenin Pathway

- Kartogenin binds with filamin A, which normally sits bound with core-binding factor β (CBFβ)
- Kartogenin displaces CBFβ, where it migrates to the nucleus and binds with RUNX-1 (transcription factor)
- CBFβ-RUNX-1 complex increases the transcription of type II collagen, aggrecan, and metalloproteinase inhibitors
- Encourage differentiation into chondrocytes



MSC-Derived EVs:

- Stimulate cartilage regeneration
- Enhance proliferation
- Possess anti-inflammatory properties

- Clinically safer - no DNA carried
- Cheaper in production and storage
- Easier to biologically manipulate

- Regenerative and anti-inflammatory properties →
→ Potential therapy for OA