Stem Cells for disk Regeneration Updates and Outcomes

Farhan Siddiqi, MD
Affiliate Assistant Professor
University South Florida
Trinity Spine Center

Disclosures

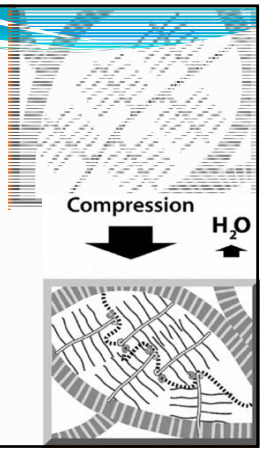
- Globus Royalties
- Foudation for Spinal Research, Education, Humanitarian Care – Board Member

Indications

- Axial Pain ?
  - What is the pain generator?
  - **Spine is a Black Box**
- Adjacent Segment Disease
  - Prevention
  - Treatment
- Augmentation of Arthrodesis
- Spinal Cord Injury
- Tumor Recurrence
- **Are Stem Cells a Hammer without a Nail?**
  - **Tool to head us in the right direction**

Anatomy

- Annulus
  - Fibroblast-like cells
  - Extracellular Matrix (ECM)
    - 60% Type 1 collagen
    - 25% Proteoglycan
- Nucleus
  - Chondrocyte-like nucleus cells
    - Thrive in low oxygen tension
    - Susceptible acidic environment
  - ECM
    - 25% Type 2 collagen
    - 70% Proteoglycan
    - Aggrecan most common (some Chondroitan sulfate and Keratan sulfate)
      - Osmotic pressure
      - Blocks Nerve in growth

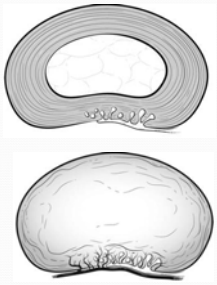


Pathology Review

- Cellular Senescence
  - Telomere destruction (aging)
- **Stress Induced** Premature Cellular Senescence (SIPS)
  - **Premature Aging**
  - Disrupts cell replication by DNA damage
  - Decreased number of active cells in disk
  - Decreased ECM production (Nerlich al, Spine 1997, Volvo Award Winner)
  - Phenotypic Changes
    - Increased Collagen Type 1 in NP
    - Inflammatory MMP / cytokines
    - **Decreased Aggrecan**
      - Inability to resist mechanical load (Spine 2002)

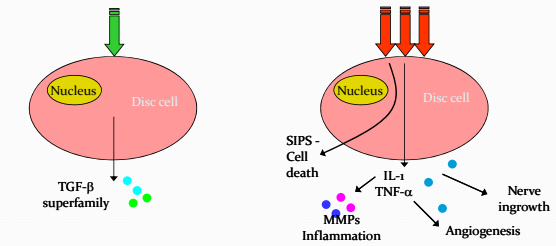
Pathology Review

- Sinuvertebral Nerve
  - In growth
    - Loss of inhibitory Aggrecan
  - Expression of Nerve Growth Factor
    - Exclusive to painful disks (Freedmont et al., J Path 2002)
  - Neurovascular In growth
    - Highly innervated vascular granulation tissue
    - Substance P, C fibers
    - Change in pH
  - **Death of NP cells**
    - **Loss of Collagen / Aggrecan**
  - **Cell Phenotype change**



Physical changes are not the whole story in IVD degeneration

Interactions Between Load and Cytokines in IVD



Cartilage Anabolism

Synthesis of Collagen II and Aggrecan

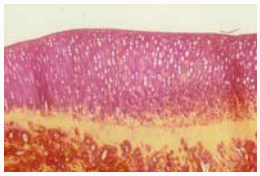
Cartilage Catabolism

Breakdown of matrix, synthesis of abnormal PGs and collagen

Stem Cell Treatment of IVD Degeneration

The Feinstein Institute for Medical Research

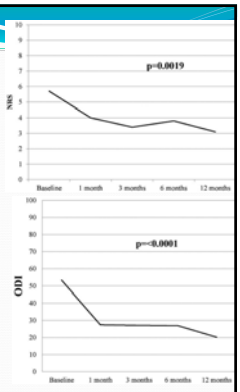
- 1987 D. Grande publishes on the regenerative potential of transplanted chondrocytes
  - 1. Where do we get the cells?
  - 2. Donor Site Morbidity
- 1988 Maureen Owen publishes landmark research; marrow derived stromal cells differentiate into multiple lineages including chondrocytes (Owen et al, J Cell Sci, 1988)
- Injection of MSCs into IVD:
  - **MSCs survive >1 yr and differentiate into NP like cells** (e.g. Sakai+ 2003, Crevensten+ 2004, Sakai+ 2005, LeMaitre+ 2009, Ganey et al 2003)
  - **Recovery of disc height** (e.g. Sakai+ 2003, Crevensten+ 2004)
  - **Increased ECM Expression / Synthesis** (e.g. Zhang+ 2005, Sakai+ 2005, Ho+ 2008)
  - **Recovered MRI signal** (Sakai+ 2003, 2005, Ho+ 2008)
  - **Loss of Vacuum Phenomenon** (Yoshikawa, 2010)



Grande et al, Anat Rec, 1987

Differentiated Cells

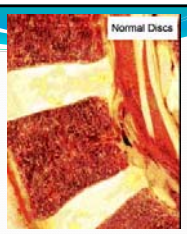
- Euro Disc RCT
  - Chondrocyte harvested during MLD
  - Re-implanted after culture and expansion vs. placebo
  - Interim Results
    - 28 pts 2 yr F/U
    - Statistically Improved ODI, SF-36 vs. control
    - 2 yr F/U
      - 25% disks collapsed
      - Versus 48% control
- Juvenile Chondrocytes
  - Isto NuQu Phase 1 and Phase 2
  - 15 patients
  - ODI, NRS, SF-36 all improved
  - 77% improved on imaging 6 months
  - 80% avoided operative intervention at 12 months
  - 20% required TDR

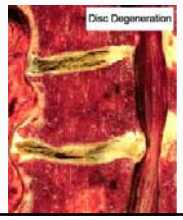


Motivation

- Cartilage Cells have minimal reparative ability
  - Progressive degeneration
- Can we MOTIVATE chondrocytes to repair the disk?
- Are there healthy chondrocytes available?
  - If not, can we implant them?
  - Will the survive?
  - Where do we obtain them?
- If none available? How do we make them?
- How do we get them to repair disk?

- THE STEM CELL
  - Anabolic
  - Anti-inflammatory
  - Growth Factor Production
  - Prevention of nocioceptive nerve ingrowth
  - Stimulate native NP cells
  - Attract additional cell to migrate to disk





Allogenic Mesenchymal Stem Cells

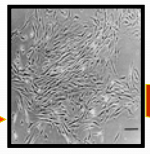
- Mesoblast Phase 2 trial
  - 100 patients
  - Saline vs. HA vs. 6 million MSC vs. 18 million MSC
  - Results
    - VAS 7 to 3 in MSC
    - 70% reached 50% pain relief
    - 50% reached VAS<2
    - 43% reduction in ODI (39% reached ODI of <20)
    - 42% reduction in opioids
    - 3-6% of MSC needed additional procedure
    - 25% of control needed procedure

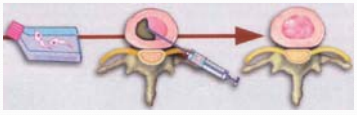
Clinical Paradigm in Spine - Autologous

Degen. Disc disease

BMA

Laboratory Processing

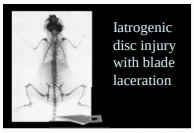




Implantation Into Disk

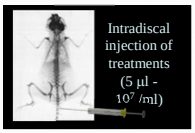
Study Design

20 adult ♂ Sprague Dawley Rats



Iatrogenic disc injury with blade laceration

3 weeks post-operative recovery



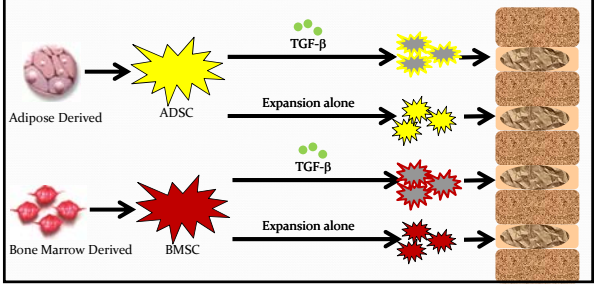
Intradiscal injection of treatments (5 µl - 10^7 /ml)

Intradiscal Injections (1 million cells / ml)

- Uninjured Control
- Injured No Treatment
- MSC's
- TGF-B differentiated MSC's

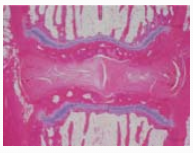
Purpose:

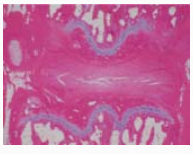
- Evaluation of the regenerative potential of autologous mesenchymal stem cells from two source tissues (adipose or bone marrow) cultured with or without the morphogen TGFb in a rat degenerative disc model.



Histological Results

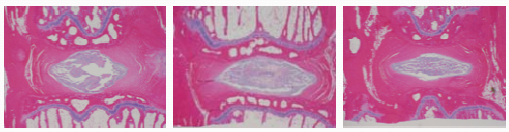
- Controls:
  - All with significant disc injury
  - Loss of disc volume and scar formation (100%)





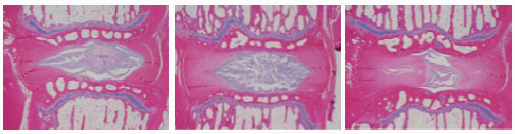
Histological Results

- BMSC alone
  - 100% injured discs were repaired with native histological morphology
  - 30.9% increase in disk volume



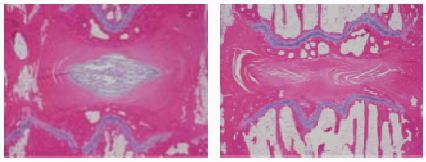
Histological Results

- BMSC with TGF-β
  - Native histological repair in 67% Samples
  - Non-native nucleus tissue in 33% Samples
  - 29.8% increase in disk volume



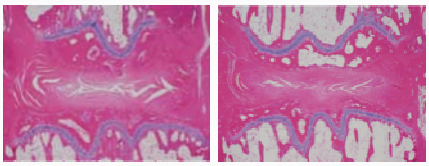
Histological Results

- ADSC without TGF-β
  - Native repair in 50% samples
  - No repair in 50% samples
  - 50% of treated disks showed regeneration and volume increase of 43.6%



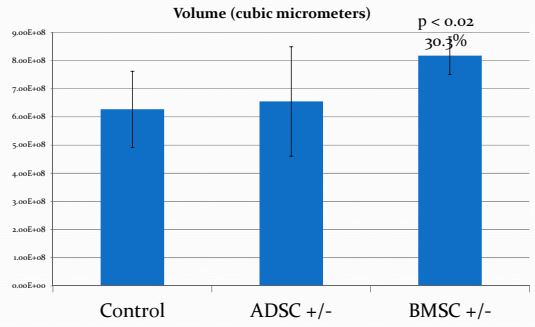
Histological Results

- ADSC with TGF-β
  - No reparative process seen in any sample



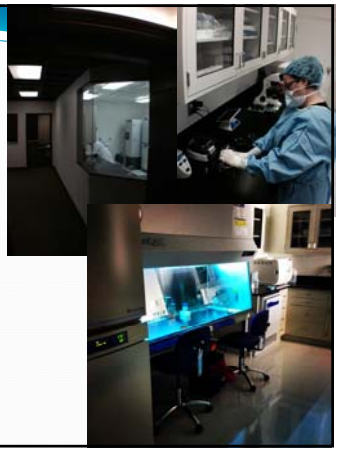
Results: Tissue Origin Influence on Disc Volume

The Feinstein Institute for Medical Research

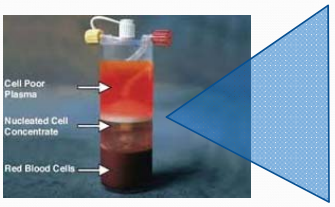


Human Trials

- ISO level 3 Cleanroom Cell Biology Lab
- Inclusion
  - Axial Pain (1 to 2 level disease)
    - Pfirman 2-4 disease
  - Prevention of Adjacent Segment Disease
  - Post Diskectomy



Bone Marrow Concentrate



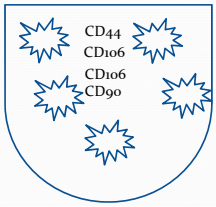
- Non-Stem Cells
  - 99% - 99.9%
    - WBC, Macrophages, Precursors
- 0.1% - 1% Stem cells
  - Hematopoetic Stem cells
    - 0.08% - 0.8%
  - Mesenchymal stem cells
    - 0.02 – 0.2%

Results – avg. 12 months - 52 patients

- Pain reduction – Single Level
  - 59.1%
  - P<0.001
- ODI Reduction- Single Level
  - 67.6%
  - P<0.001
- Multilevel
  - Pain 72.3% (p<0.01)
  - ODI 39.3% (p<0.01)
- Buffy Coat Only hist. cohort
  - Pain – 55.7% (p<0.0001)
  - ODI – 58.1% (p<0.0001)

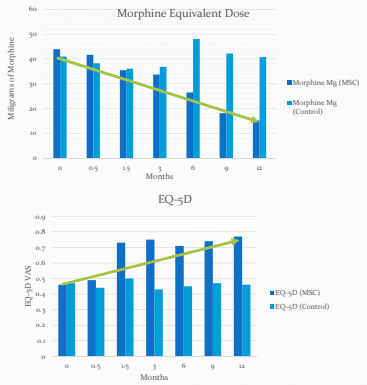
What is being injected?
Hybrid transplant

Avoid donor site morbidity
Increase cells available for treatment



Results – 12 months – 1 and 2 Levels

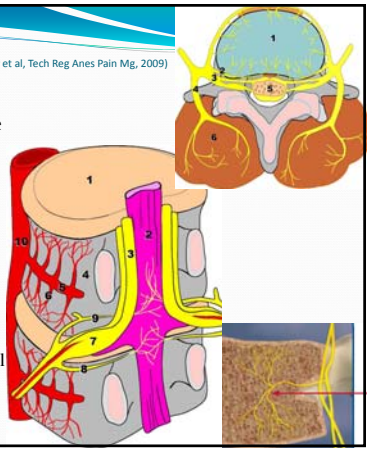
- Variable Cost
  - $2793
    - Includes $750 for surgeon
- Fixed Cost
  - $3200
    - Including Lab Construction Amortized over 200 patients
- ICER
  - $5993 = $19,332 0.31
  - By Contrast SPORT for spondylolisthesis
    - $115,600
    - DDD fusion likely worse ICER



Thank you

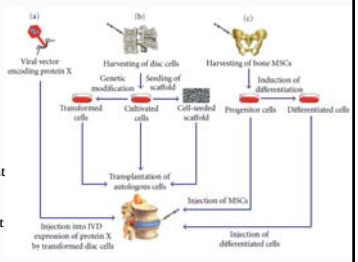
Anatomy (Shankar et al, Tech Reg Anes Pain Mg, 2009)

- Anterior / Central
  - Basivertebral Nerve
  - Rami Communicantes
- Posterior
  - Sinuvertebral Nerve
    - DRG
    - 3 mm of annulus/PLL
  - Branches
    - Cephlad and Caudal
    - Multiple segments
    - Discogram ineffective



Treatments

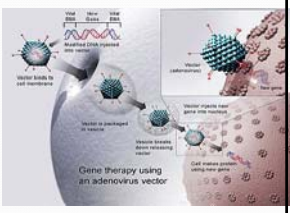
- Interventional Treatment
  - Cortocosteroid Injections
  - IDET
  - Radiofrequency
  - PELD
  - Oxygen Treatment
  - Decompression Surgery
  - Fusion
  - Joint/Disk Replacement
- Cell Based Therapy
  - Differentiated Cell Transplant
  - Undifferentiated Cell Transplant
  - Autograft / Allograft
- Growth Factor Therapy
  - Direct Growth Factor Treatment
  - Gene Therapy
- Tissue Engineering
  - Grow a better disk and implant it
  - Biologically active implants



Drazin et al, Adv. Ortho, 2012

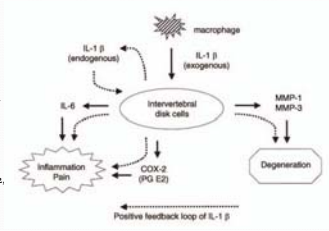
Gene Therapy

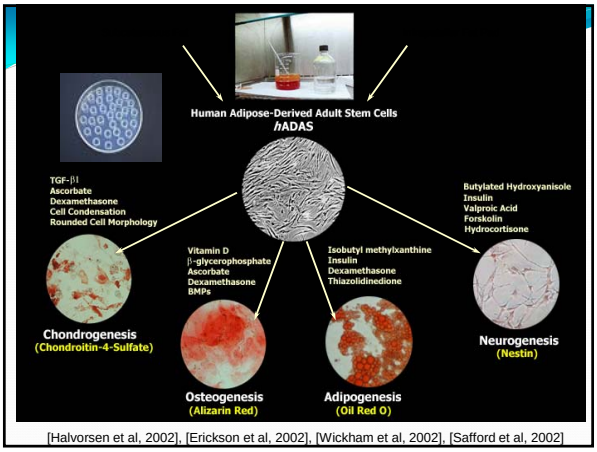
- Gene Therapy
  - Adenovirus
    - TGF-B1 to rabbit (Nisheda et al. Spine 1999)
      - 100% increase Proteogycan synthesis
    - IL-1 antagonist to human cells in vitro (Me Maitre et al, Arthritis res 2007)
    - TGF-B1, IGF, BMP-2 (Moon et al. Spine 2008)
      - 470% increase proteoglycan
      - Human cells
    - Telomerase transfection
      - Halts cellular senescence
  - Variable transfection
  - Mutation
  - Immune response senses virus
  - Patients don't like being given infections



Growth Factor Therapy

- Protein Based Therapy
  - IL-1 prototypic inflammatory molecule
    - Decrease it's production
  - BMP-7/OP-1 (An et al, Spine 2005)
    - ECM increase
    - Biomechanical Properties (Masuda et. Al, Spine 2006)
    - Decreased IL-1 cascade
    - Clinical Trial – Strategically Delayed
  - GDF-5 (J&J)
    - Increased aggrecan and collagen type2, decreased MMP (Wash et al, Spine 2004)
    - Single Injection only
    - Clinical Trial – Single Injection
  - **MAJOR PROBLEM**
    - **FOR THIS TO WORK – THERE HAVE TO BE VIABLE CELLS FOR THE GF TO INTERACT WITH**





[Halvorsen et al, 2002], [Erickson et al, 2002], [Wickham et al, 2002], [Safford et al, 2002]

Challenging Biochemical Characteristics of the Degenerative Intervertebral Disk

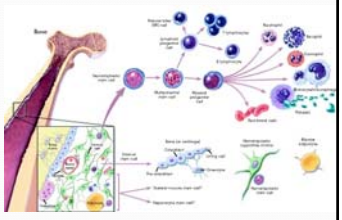
IVD Characteristic	IVD Components Affected	Therapeutic Challenge
Avascular	Disk cells	Tenuous nutritional support for therapies that rely on implantation of cells. Implantation of cells would increase lactic acid, which is cleared slowly, and it would decrease pH.
	ECM	Cannot use intravascular delivery of agents to modulate cytokine or growth factor signaling
Relatively acellular	ECM	Synthetic burden of ECM maintenance is susceptible to increases in apoptosis rate
Acidic	Disk cells	Acidity decreases production of ECM and would decrease the effectiveness of implanted cells
	ECM	Acidity does not affect MMP activity but does enhance the activity of cathepsins

ECM = extracellular matrix, IVD = intervertebral disk, MMP = matrix metalloproteinase

Keppler et al. JAAOS 2011

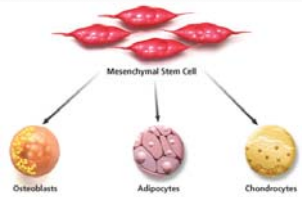
What is a Stem Cell?

- Undifferentiated cell capable of differentiating into multiple phenotypes
- Types
  - Embryonic (Pluripotent)
    - Differentiate into anything
  - Adult
    - Mesenchymal Stem Cell
    - Marrow Stromal Cell
    - Differentiate into cell types found in their lineage only
    - Capable of differentiating into cells of only one germ cell layer type
  - Induced Embryonic Stem Cells
    - Adult cells reprogrammed to become pluripotent



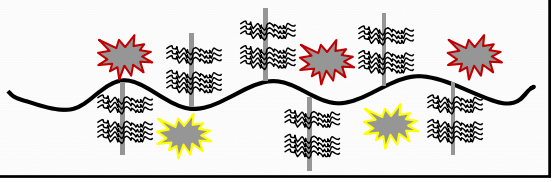
What is the Evidence?

- Stem Cell implantation
  - Cell Survival > 1 yr (Ganey et al. Spine, 2003)
  - Continued production of ECM (Zhang, Clin Orth RR, 2005)
  - MSC exposed to hypoxia – Disk-like chondrocytes Spine 2004, 2011, 2012)
  - MSC transplantation into IVD (Yoshikawa et al., Spine 2010)
    - Decreased vacuum phenomenon



Hypothesis

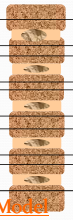
- **Therapeutic injection of mesenchymal stem cells can repair the intervertebral disk.**
- **Such repair is initiated by extracellular matrix signaling alone without the aid of exogenous TGF-β to induce differentiation.**



Methods: Study Design

The Feinstein Institute for Medical Research

- 20 rats



Injury		Treatment		Group
Puncture	+	PBS	=	SHAM
Puncture	+	ADSC alone	=	ADSC-
Puncture	+	BMSC alone	=	BMSC-
Puncture	+	ADSC + TGF-β	=	ADSC+
Puncture	+	BMSC + TGF-β	=	BMSC+

Puncture Injury Model

- Disc space (Co3-4, Co4-5, Co5-6, Co6-7).
- 27G Needle
- 4mm Deep Insertion
- Rotated 180° then held in place for 30 sec

Masuda +, 2005, Sobajima+ 2005, Zhang+, 2009

RX 4 weeks PO

- MSCs were isolated from bone marrow and adipose tissue on initial surgery
- MSCs expanded to passage 2 +/- TGFb
- 5 µl injection at 4 weeks post injury

Methods

- 20 Rats x 4 disc segments / rat = 100 segments
- 4 groups of following

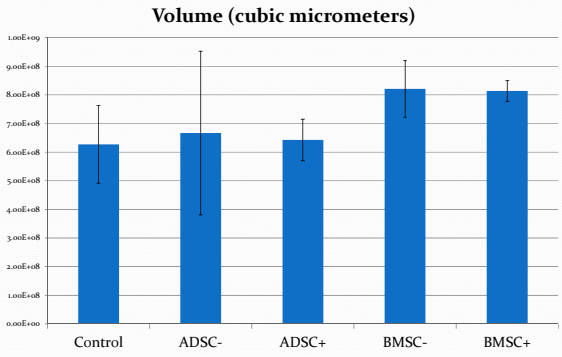
	Co5-6	Co6-7	Co7-8	Co8-9
Rat 1	ADSC +	ADSC -	BMSC +	Injury only
Rat 2	BMSC -	ADSC +	ADSC -	Injury only
Rat 3	BMSC +	BMSC -	ADSC +	Injury only
Rat 4	ADSC -	BMSC +	BMSC -	Injury only
Rat 5	ADSC +	ADSC -	BMSC +	Injury only

Timeline

- **Index Surgery**
  - Disc injuries made with 27g needle
  - Unrestricted activity x 4 weeks
  - Harvest adipose or marrow cells
- **Repair Surgery**
  - Injections of cells made with 27g needle
  - 5,000 cells in 5 µL per injection ($1x10^6$ per ml)
  - Unrestricted activity x 6 weeks
- Sacrifice / Histological analysis

Results: Disc Volume

The Feinstein Institute for Medical Research

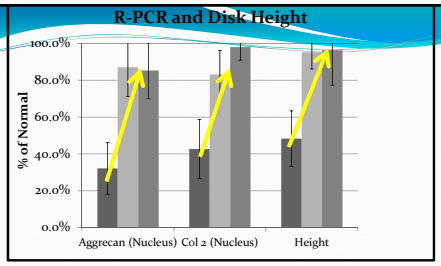


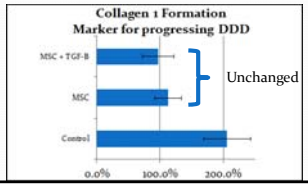
Discussion:

- Our study explored the combination of TGF-b with MSC's from different tissue origins in the treatment of degenerated IVD's.
- It was our hypothesis that MSC's would regenerate the degenerative process in the IVD.
- Additionally, MSC's can initiate the regenerative process based on extracellular matrix signaling alone without the addition of TGF-B

Conclusions:

- Undifferentiated Bone Marrow derived MSCs provide a therapeutic benefit to degenerated IVDs.
- Bone Marrow derived MSCs histologically regenerated disk without the need of TGF-B
- Only 50% of Adipose Derived MSCs exhibited a therapeutic response
- Adipose Derived MSCs pre-treated with TGF-b exhibited no therapeutic response.
- Bone marrow derived MSC's, regenerated the disk volume by 30.3% and are highly therapeutic histologically when compared to adipose derived MSC's ($p<0.02$)
- In humans, we would recommend autologous bone marrow derived MSC's for further study to confirm clinical efficacy as reduction of pain may or may not be linked to histological regeneration or nuclear volume restoration.





Bio-Filter Concept

- Negative Cell Selection
  - Flow Cytometry confirmation
  - **Typical centrifugation system used in OR (Bufffy Coat)**
    - Only about 15 million cells per ml
      - 150,000 total stem cells
        - **30,000 MSC's /ml**
        - Mixed with 14.7 million other cells (**0.2% purity**)
  - **Lab grade equipment (multiple spin and wash): 600 million cells/ml**
    - 40 times more concentrated
    - 1% are stem cells (mixed MSC and HSC)
    - 6 million stem cells/ml
      - **1.2 million MSC's/ml**
      - Mixed with 599 million other cells (**0.2% purity**)
  - **Biofiltration: 87% cells are stem cells and chondrogenic precursors**
  - Average
    - Hematopoetic Stem cells: 4.4 million /ml
    - Mesenchyal Stem cells: 800,000 / ml – 1.6 million / ml
      - Based upon how much marrow is aspirated
        - 120 cc
        - 240 cc
  - **Biofiltration yields an 87% pure chondrogenic or osteogenic population**
    - **Compared to 0.2% purity of useful cells in typical OR system**
- Specific for Cells needed for end goal
  - Fusion / Fracture Repair (osteoblast and osteoclast)
  - Disk regeneration (MSC, chondrocyte lineage)
  - Oncology (Natural Killer Cells)
    - Cytotoxic to tumor cells / Reduction in Recurrence