Regenerative and Biological Treatment of the Spine

Texas Pain Society
Annual Scientific Meeting
2016

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President ASIPP
Director of Clinical Research
Precision Spine Care | Texas Spine and Joint Hospital
Disclosure

Investigator:
- Mesoblast Phase II Trial
  - Mesenchymal Precursor Cells (MPC’s)
- Spinal Restoration Phase III Trial
  - Biostat Biologx (Fibrin Sealant)
- J and J
- rhGDF-5 Phase III Trial
What are we discussing?

- **Regenerative Medicine** involves delivering specific types of cells or cell products to diseased tissues or organs, where they will ultimately restore tissue and organ function.
- **Accomplished via** cell-based therapy or by using cell products, such as growth factors.
What are we discussing?

- Mesenchymal Stem Cells (MSC)
  - Adipose and Bone Marrow
- Platelet Rich Plasma (PRP)
- Amniotic derived products.
### Sources of Regenerative Agents

<table>
<thead>
<tr>
<th>Component</th>
<th>Bone Marrow Aspirate</th>
<th>Blood (PRP)</th>
<th>Adipose</th>
<th>Amniotic/Placental</th>
</tr>
</thead>
<tbody>
<tr>
<td>MSCs</td>
<td>++</td>
<td>–</td>
<td>+++</td>
<td>++</td>
</tr>
<tr>
<td>HSCs</td>
<td>+++</td>
<td>+</td>
<td>+</td>
<td>?</td>
</tr>
<tr>
<td>Endothelial PCs</td>
<td>+++</td>
<td>Low</td>
<td>+</td>
<td>?</td>
</tr>
<tr>
<td>Growth Factors</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>IL-1RAP</td>
<td>+++</td>
<td>+</td>
<td>?</td>
<td>?</td>
</tr>
<tr>
<td>Plasma Proteins (α-2 Macroglobulin)</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Structural Protein (Fibrinogen)</td>
<td>If activated</td>
<td>If activated</td>
<td>–</td>
<td>In some formulations</td>
</tr>
</tbody>
</table>
What is PRP

A concentrated solution of platelets in a small volume of plasma
What is in platelets?

- Platelets contain a variety of growth factors, coagulation factors, adhesion molecules, cytokines, chemokines and integrins.
- After activation, the platelets in PRP release these growth factors at concentrations significantly higher than the baseline blood levels.
Platelets

Thus, increased platelet count is a secondary measurement of growth factor concentration, which can be delivered to damaged tissues to promote healing.
Platelet Derived Growth factors

- Stimulates cell replication
- Promotes angiogenesis
- Promotes tissue granulation formation
- Promote growth of extra-cellular matrix
Cytokines for Healing

- Mitogenic (growth) factors
  - PDGF, FGF, HGF, IGF, etc.
  - Roughly 80% stored in platelets, 20% soluble in plasma

- Angiogenic factors (primarily VEGF)
  - Roughly 80% stored in platelets, 20% soluble in plasma

- Matrix-building proteins
  - Fibrinogen, fibronectin, vitronectin
  - Available in the plasma, not the platelets

- Anti-inflammatory proteins
  - Alpha-2-Macroglobulin (A2M), IL-1RAP (aka IRAP)
  - Available in the plasma, not the platelets
Mesenchymal Stem Cells

What is a Stem Cell?

A mesenchymal stem cell is a primitive cell with the ability to:

- Self-Replicate (undifferentiated state)
- Differentiate into multiple tissues
- Reduce Inflammation
- Fight Apoptosis (Cell Death)

Adult stem cells are the means by which our bodies naturally heal throughout our lifetime.
Mesenchymal Stem Cells: Environmentally Responsive Therapeutics for Regenerative Medicine

Murphy, Moncivais, Caplan
Experimental and Molecular Medicine
2013
Autologous Sources of MSC’s

- Bone marrow
- Adipose
- Synovial fluid
- other sources
Allogenic Sources of ASC’s

- Amniotic tissue
- Umbilical cord blood
- Commercial stem cell lines are currently under development
Where do we obtain these cells?

Human MSCs are generally isolated from bone marrow after separation by density gradient centrifugation or from processed lipoaspirate.

Bone Marrow or Adipose

Processing to Yield SVF

- Subcutaneous adipose is extracted through liposuction
- Connective tissue of the fat is disrupted by enzyme digestion (collagenase/neutral protease)
- Ultrasonic or mechanical disruption to free pericytes (MSCs) from vascular structures (not SVF)
Processed lipoaspirate-derived Adult Stem Cells

Adipose tissue is an abundant source of mesenchymal stem cells
Bone Marrow Aspirate
Bone marrow msc’s exist in suspension - simple centrifugation can isolate the cells in the buffy coat layer.

Concentrates cell numbers 2-4x (from a baseline of 0.02% of cells up to 0.06%)

Dragoo Tobi 2015
Dr. Philippe Hernigou, MD

- Chief of Orthopaedic Surgery, Henri Mondor Hospital, University of Paris France
- Treated more than 5,000 patients with autologous bone marrow concentrate (BMC) since 1990
- Found correlations between stem cell content of BMC and clinical outcomes in non-unions, AVN, rotator cuff, and articular cartilage repair
- Established no increased risk of cancer when using the patient’s own cells with 15+ years follow-up compared to the general French population
PRP and MSC
Mechanisms of Therapeutic Benefit
Framework for Regenerative Therapies

Types of Therapeutic Benefits
- Pain Relief
- Extracellular matrix repair/replacement

Therapeutic Strategies
- **Indirect** (e.g., PRP): Influence Host Cells
  - Proteins, Enzyme Inhibitors, Growth Factors

- **Direct** (e.g., BMC): Contains Viable Progenitor Cells
  - Paracrine influence (releasing biochemical agents)
  - Differentiation into adult tissue cells (less likely)
Inflammation

- Inflammation = Pain
- Pro-inflammatory cytokines stimulate anti-microbial agents (macrophages, granulocytes) to clean up a potentially contaminated wound and digest damaged parts of tissue/extracellular matrix (ECM)
- Inflammatory (e.g. TNF-a, interleukins) and mitogenic (e.g. PDGF, FGF) factors also recruit MSCs into the damaged site from nearby blood vessels
- Prolonged inflammation in a non-healing injury leads to cell death and further deterioration of the tissue
Why We Fail to Heal

- Injuries in or adjacent to vascular tissue release cytokines to recruit MSCs and other progenitors
- “Critical size defects” are non-healing injuries that do not have proximity to blood vessels or lack sufficient “scaffold” to build and remodel matrix proteins
- Most cartilaginous injuries cannot overcome the inflammation stage of healing
Non-Surgical Strategies

- Rest, icing, physical therapy
- Steroids – reduces inflammation (pain), long term is cytotoxic to chondrocytes
- Platelet-rich plasma (PRP) – INDIRECT therapy, objective is to release cytokines from platelets to stimulate local cells to move in, overcome inflammation generators, and remodel tissue
- Stem Cells (Bone Marrow Concentrate) – DIRECT therapy, delivers MSCs, HSCs, and other progenitor cells along with platelets to accelerate the healing process or provide cells where they are otherwise unavailable
How does this pertain to the painful disc?
Disc Nucleus

- Nucleus Pulposus is a gel-like matrix containing proteoglycans and type II collagen. The negative charge of the glycosaminoglycans attracts and holds onto water.
- Hydration helps with maintenance of disc height and load-bearing capacity of the disc.
- Chondrocytes within the NP synthesize and maintain matrix.
The Lumbar Discovertebral Complex

- Homeostasis: Chondrocytes control synthesis and degradation of the nuclear metrix:
  - Proteoglycans, collagen, H₂O

- Hostile biochemical environment
  - No direct blood supply
  - Low O₂ tension
  - Anabolic metabolism pH (6.9-7.1)

- A variety of insults may upset this homeostatic balance
  - Metabolic disease (DM, Ochronosis)
  - Genetic factors
  - Traumatic endplate injury
  - Nutritional (smoking, vascular disease)
  - Infectious
Disc Age Related Change or “Degeneration”

Consequence of Imbalance of Synthesis / Degradation

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<thead>
<tr>
<th>Molecular</th>
<th>Nuclear Matrix</th>
<th>Annulus</th>
</tr>
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<tbody>
<tr>
<td>Altered PGs</td>
<td>Cross-linking</td>
<td>Cross-linking</td>
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<tr>
<td>Dehydration</td>
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<table>
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<tr>
<td>Cracks</td>
<td>Fibrosis</td>
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<td>Tears</td>
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<tr>
<td>Thinning</td>
<td>Fragmentation</td>
<td>Stiffening</td>
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<tr>
<th>Biomechanical</th>
<th>Nuclear Matrix</th>
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<tbody>
<tr>
<td>Depressurized</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stiffening</td>
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<tr>
<th>Imaging</th>
<th>Nuclear Matrix</th>
<th>Annulus</th>
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<tbody>
<tr>
<td>Loss of T2 signal</td>
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Internal Disc Disruption: Etiology

- Of the several insults previously described, IDD appears to be most closely associated with endplate fracture

- Experimental endplate fracture causes, over time:
  - reduction in water, proteoglycans
  - Delamination
  - reduction in pressure within the nucleus

Internal Disc Disruption: Etiology

- Endplate Fatigue Fracture
- Precipitates degradation of nuclear matrix
  - Inflammatory response
  - Nutritional / biochemical (pH) insults
- Nuclear dehydration
  - Unable to accept and disburse load
  - Load to transferred to posterior annulus
  - Radial Fissuring
Homeostasis:
The Balance of Synthesis and Degradation
Chondrocyte

Degradation:
- TNFα
- IL-1

Synthesis:
- TGF
- bFGF
- IFG

Proteoglycans

Collagen

Nuclear Matrix

Metalloproteases
- O₂
- NO

Annulus

Water

© Nik Bogduk, 2012
Disc Degeneration

- Can biologics slow or even reverse the cascade of DDD?
- Will transplantation of cells into the disc improve the production of proteoglycan rich extracellular matrix and lead to better hydration and biomechanical properties?
Therapeutic effect of MSC’s intradiscal

- MSC’s have been shown to differentiate towards chondrocyte-like cells - phenotypically similar to NP cells.
- MSC’s promote regeneration by stimulating native NP cells.
- Upregulation of extracellular matrix proteoglycan and Type II collagen synthesis by native NP cells has been demonstrated during coculture of MSC’s with degenerative NP cells.
  - Svanvik et al. 2010
IT’S BEEN SAID....

The Disc is where good therapies go to DIE
Animal Data
Meta-analysis of Animal Data
Meta-analysis of Animal Data

- 6 rct’s in animals met criteria
- Looked at the association between disc stem cell transplant and subsequent change of disc height

Research paper:

Efficacy of intervertebral disc regeneration with stem cells — A systematic review and meta-analysis of animal controlled trials

Zhen Wang, Carmen M. Perez-Terric, Jay Smith, William D. Mauck, Randy A. Shuler, Timothy P. Maus, Tai-Hua Yang, Mohammad Hassan Mirad, Shanhua Gao, Marisa J. Terry, Jason P. Dauffenbach, Mathew J. Bingee, Jason S. Eldridge, Khaled Mohammed, Khalid Benkhadra, Andre J. van Wijnen, Wenchun Qu.
Meta-analysis of animal data

- Overall, IVD stem cell transplant was associated with 23.6% increase in disc height index (95% CI, 19.7-23.5; p<0.001)
- Of all the six studies, none showed decrease of disc height index in the transplant group compared with the controlled group.
- The increase in disc height index was statistically significant.
Meta-analysis of animal data

- The findings of this meta-analysis indicate that cell therapy may arrest or reverse the IVD degenerative process.
Stem Cells and the Intervertebral Disc
Human Trials
Allogeneic MSC’s
Mesoblast
Safety and Preliminary Efficacy Study for Disc Repair (Mesoblast)

• MPC’s for Lumbar Disc Disease in Adults
• Primary Objective: Safety @ 6 months
• Secondary Objective: Efficacy
• 100 Patients Worldwide
• Randomized to:
  • Normal Saline
  • Hyaluronic Acid (HA)
  • Low Dose (6 Million) MPC’s in HA
  • High Dose (18 Million) MPC’s in HA

1. Safety and Preliminary Efficacy Study of Mesenchymal Precursor Cells (MPCs) in Subjects With Lumbar Back Pain
**Study Design**

- Prospective, multi-center, randomized, double-blind, controlled study
- Patients and radiographic evaluators blinded to treatment
- Follow-up: 1, 3, 6, 12, 24 & 36 months
MPC groups have a greater proportion of patients with at least a 50% improvement in back pain or minimal/no residual back pain at 12 months relative to controls.

Proportion of patients with 50% back pain reduction @ 12 months:
- Pooled Controls: $33.0\%$ (95% CI: $21.5\% - 44.8\%$)
- 6M MPCs: $18.2\%$ (95% CI: $12.1\% - 24.2\%$)

Proportion of patients with minimal to no back pain @ 12 months:
- Pooled Controls: $120.0\%$ (95% CI: $100.0\% - 140.0\%$)
- 6M MPCs: $100.0\%$ (95% CI: $80.0\% - 120.0\%$)

*from post-hoc analysis
MPC treated groups have significantly greater numbers of patients with treatment success at both 6 & 12 months.

Treatment Success Over 12 Months
50% VAS back pain reduction AND 15 point ODI improvement AND no intervention at the treated level at both 6 and 12 months*.

* from post-hoc analysis
Take away points from Mesoblast Study

- Allogeneic MPCs were well tolerated
  - No issues related to use of an allogenic product were identified
Take away points from Mesoblast Study

- Both MPC doses showed improvement relative to controls for pain and functional improvement and reduced interventions
- There appears to be a minimal number of cells needed to exert a physiologic response but more may not always be better.
Take away points from Mesoblast Study

• Radiographic evidence demonstrating decreased abnormal vertebral movement, suggesting improvement in disc structure and stability
If you are in a book store and cannot find the book for which you search, you are obviously in the.....
WONG FOOK HING BOOK STORE
Autologous MSC’s

BMC
Interventional Disc Repair by Autologous Mesenchymal Bone Marrow Cells: A Pilot Study

Luis Orozco,1 Robert Soler,1 Carlos Motero,1 Mercedes Alberca,1 Ana Sánchez,1 and Javier García-Sancho1,2

• 10 pts with chronic LBP with Lumbar DDD
• Mesenchymal stem cells harvested from iliac crest, cultured (21-28 d), injected into nucleus pulposus
• 9:10 pts improved
• Analgesic effect approaching 71% efficacy
• No change in disc height

Two-Year Results of the Use of Autologous Point-of-Care Bone Marrow Concentrate for the Treatment of Discogenic Low Back Pain

*International Orthopaedics* | 2015 | Kenneth Pettine, M.D.

**Stem Cells, 2015**
(1-yr results)

**Clinical Study**
- Failed conventional therapy >3 mo.
- Eligible for surgical relief
- IRB cleared protocol

<table>
<thead>
<tr>
<th></th>
<th>One-Level</th>
<th>Two-Lesl</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of Patients</td>
<td>13</td>
<td>13</td>
</tr>
<tr>
<td>Median Age</td>
<td>40 Range 25-51</td>
<td>37 Range 18-61</td>
</tr>
<tr>
<td>Average BMI</td>
<td>27.1</td>
<td>26.1</td>
</tr>
<tr>
<td>Cause of Injury</td>
<td>Trauma</td>
<td>7</td>
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<tr>
<td></td>
<td>Unknown</td>
<td>6</td>
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<td>Discs of Modified Pfirrmann Grade:</td>
<td></td>
<td></td>
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<tr>
<td>IV</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>V</td>
<td>3</td>
<td>6</td>
</tr>
<tr>
<td>VI</td>
<td>5</td>
<td>11</td>
</tr>
<tr>
<td>VII</td>
<td>3</td>
<td>8</td>
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</table>
Disc Injection Therapy

60cc BMA drawn from the posterior iliac crest

BMA centrifuged for 12 min. 6cc bone marrow concentrate (BMC) drawn

Total procedure time: 30-45 min.
Disc Injection Therapy

- Cell analysis was performed on samples from 20 patients
- No adverse events for bone marrow aspiration—no chronic pain (60 cc aspirated volume)
- No adverse events at the disc injection site(s)

<table>
<thead>
<tr>
<th>Group</th>
<th>Avg. MSC dose per disc</th>
<th>% improv. 12 mo. ODI</th>
<th>% improv. 12 mo. VAS</th>
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</thead>
<tbody>
<tr>
<td>All Patients</td>
<td>8,138</td>
<td>57%</td>
<td>58%</td>
</tr>
<tr>
<td>&lt; 40 years, &lt; 2,000 CFU-F/mL</td>
<td>3,852</td>
<td>65%</td>
<td>83%</td>
</tr>
<tr>
<td>&lt; 40 years, &gt; 2,000 CFU-F/mL</td>
<td>9,927</td>
<td>69%</td>
<td>64%</td>
</tr>
<tr>
<td>&gt; 40 years, &lt; 2,000 CFU-F/mL</td>
<td>4,433</td>
<td>34%</td>
<td>29%</td>
</tr>
<tr>
<td>&gt; 40 years, &gt; 2,000 CFU-F/mL</td>
<td>13,241</td>
<td>74%</td>
<td>74%</td>
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</table>
Disc Injection Therapy
3-year Survivor Cohort (n = 20)
Disc Injection Therapy
3-year Survivor Cohort

Effect of Progenitor Cell concentrations on pain reduction through 3 years
(ODI – solid lines, VAS – dashed lines, Lower cell counts – Red, Greater cell counts - Green)
Patient Outcomes (September 2015)

• 8 patients showed a single grade level improvement in their Pfirrmann score at 1-yr (40% of enrolled patients)

• 3 1-level and 3 2-level patients progressed to surgical repair by 3-yr (77% avoided surgery through three years)

• 2 Patients received a 2nd injection two years ago; no additional 2nd injections have occurred

• 73% average reduction in pain and 67% average improvement in ODI at 3-yr for the surviving 20 patients

• >2000 CFU-F patients: 86% improvement in ODI/90% reduction in VAS; <2000 CFU-F: 41% ODI/48% VAS
Disc Injection Therapy

- The Degenerative disc contains:
  - Elevated levels of matrix metalloproteinases
  - Elevated levels of IL-1
- Bone marrow contains:
  - alpha-2-Macroglobulin (inhibitor of MMP’s)
  - IL-1RAP (Interleukin-1 receptor accessory protein - reduces the pain associated with IL-1)
Thus BMC is a multi-modal therapeutic agent
It contains:
  • Biochemical Modifiers
  • MSC’s, EPC’s (endothelial progenitor cells),
    HSC’s and other progenitor cells
• Takes control of the “pro-inflammatory”
  environment in the disc

Disc Injection Therapy
PRP DATA
Lumbar Intradiscal Platelet Rich Plasma Injections: A Prospective, Double-Blinded Randomized Controlled Study

Yetsa A. Tuakli-Wosornu, M.D., M.P.H. ¹, Alon Terry, M.D. ¹, Kwadwo Boachie-Adjei, B.S., C.P.H. ¹, Caitlin Gribbin, B.A. ¹, Elizabeth E. LaSalle, B.S. ¹, Julian Harrison, B.S.¹, Joseph Nguyen, M.P.H. ¹, Jennifer Solomon, M.D. ¹, Gregory Lutz, M.D. ¹

Funding: PR&EF
Harvest Technologies

¹ Physiatry Department, Hospital for Special Surgery – New York, NY
Determine if a single intradiscal PRP injection at time of discography has therapeutic value compared to control (contrast) injection

MRI imaging of study subject prior to study participation in 2011 (left) and post study participation in 2013 (right) (a) L5-S1 T2 axial image of individual in 2011 (b) L5-S1 T2 axial image of individual in 2013 with resolution of the HIZ (c) T2 Sagittal image 1 of individual in 2011 (d) T2 Sagittal image of individual in 2013 with 2 resolution of the HIZ at L5-S1. Of note is that the HIZ at L4-5 is the same or larger 3 despite also being treated with PRP.

Purpose

Determine if a single intradiscal PRP injection at time of discography has therapeutic value compared to control (contrast) injection.
Methodology

- 2:1 Randomization PRP vs contrast
- Double-blinded/crossover at 8 weeks
- Lumbar discogram 25G needle 3cc syringe
- Independent observer
- Injection of contrast first (0.5-1.5 cc)
- Followed by 1-2 cc contrast (control) or PRP (treatment)
PRP vs. Control Evaluation
NRS... 

![Graph showing NRS changes over time with statistically significant change compared to baseline.](image-url)
PRP Longitudinal Evaluation
SF-36

- statistically significant change compared to baseline
Progress through 24 mo. Post-PRP

Pain Right Now: -6 points
Pain at Best: -4 points
Pain at Worst: -6 points

24 month △

NRS Pain

Time (weeks)
Summary

• Intradiscal PRP is a readily available, safe, cost-effective treatment for IDD
Stem Cell Treatment for Disc Pain
What are the issues?
• Source of Cells
• Autologous (your cells) vs. Allogeneic (someone else's cells)
• Cellular induction
• Method of Transplantation
• Carrier
• Risks
Source of Cells

- Abundance
- Ease to obtain
- Capacity to differentiate into chondrocyte-like cells
- Viable in the hypoxic and hypoglycemic, and low pH environment
- Minimal or no immune response
- No risk for tumor growth
Source of Cells

- Bone Marrow and Adipose derived MSC’s
- Other sources are being developed commercially
Autologous vs. Allogeneic

- Allogeneic cells
  - Off-the-shelf availability
  - Specific dose (cell count known and optimized)
  - Cell behavior well studied
- Autologous Cells
  - Requires an invasive procedure to obtain
  - Contains much more than just MSC
Cellular Induction

• Induction techniques can increase the yield of differentiation of MSC’s toward chondrocyte-like cells
  • Co-culture MSC’s with NP cells
  • Stimulation factors IGF1, TGF Beta1, GDF5, BMP (PRP)
    • Hypoxia
    • Dexamethasone
    • Gene therapy sox-9
Method of Transplantation

• Disc is avascular - injection is necessary
• Successful transplantation by direct injection has been demonstrated in animal models
Carrier

- Characteristic of a favorable carrier or scaffold
  - Deter extravasation of injectate
  - Provide 3D environment for cellular proliferation
  - Enhance cell survival, proliferation and proper differentiation
- Collagen gel, hydrogel, hyaluran gel
- Fibrin Sealant
Risks

- Disk space infection
- Worsening of disc degeneration
- Tumorigenesis
MSC’s Intradiscal

- MSC’s have the capacity to repair degenerative discs
  - differentiation toward chondrocyte-like cells
  - producing proteoglycans and type II collagen
  - Supportive animal and human data

*after Gou et al Am J Phys Med Rehab 2014*
PRP Intradiscal

• Positive effects of PRP have been published in in-vitro studies of animal and human disc cells.
• Disc cells cultured with PRP demonstrate improved proteoglycan synthesis and annulus cell proliferation.
The End
Thank you for your time.

Aaron Calodney, M.D.
AaronCalodney@me.com