Mesoblast Cascade trial: use of stem cells to treat lumbar discogenic pain - FDA Phase III RCT

J. Scott Bainbridge, M.D. (Principal Investigator – Colorado site)

www.DenverBackPainSpecialists.com
Currently Enrolling 2016

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REVIEW

Diversity of intervertebral disc cells: phenotype and function

Girish Patappai, Zhen Li, Marianna Peroglio, Nadine Wismer, Mauro Alini and Sibylle Grad

AO Research Institute Davos, Davos, Switzerland

Table 2: Summary of characteristics of human IVD cells.

<table>
<thead>
<tr>
<th></th>
<th>NP cells</th>
<th>AF cells</th>
<th>CEP cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Morphology</td>
<td>Rounded</td>
<td>Elongated</td>
<td>Rounded</td>
</tr>
<tr>
<td>Gene expression</td>
<td>Type II collagen, Cytokeratin-19, FOX-F1, CA12, Pax-1, Brachyury</td>
<td>Type I collagen, type V collagen, tenomodulin</td>
<td></td>
</tr>
<tr>
<td>ECM proteins</td>
<td>Collagen type II, Aggrecan, hyaluronan</td>
<td>Collagen type II, Aggrecan, elastin</td>
<td>Type II collagen, Aggrecan, Hyaluronan</td>
</tr>
</tbody>
</table>

Fig. 1. Schematic representation of an intervertebral disc (IVD). The cartilaginous endplate (CEP) and the adjacent vertebral (VB) are visualized in the sagittal section of the disc (A). The annulus fibrosus (AF) is formed by structured lamellae which encapsulate the central nucleus pulposus (NP) (B).

Fig. 1. Characteristics of the IVD specific niche. The vasculature that nourishes the IVD mainly consists of the capillary network of the endplate, while a minor portion comes from a small number of capillaries that penetrate only a few millimeters into the outermost annulus fibrosus (AF). Nutrients and metabolites can reach the centre of the disc essentially by fluid flow or diffusion through the vertebral endplates and the AF. As a result, oxygen tension within the IVD is significantly reduced towards the centre of the nucleus pulposus (NP) and the disc cell metabolism is partly anerobic, leading to high concentrations of lactic acid and low pH conditions.

AF: Vascularity: Slightly vascularised in the outermost layer; otherwise avascular. Hypoxia: Becomes hypoxic in a gradient of outer to inner and distance from the end plate. Dense lamellar structure made of type I collagen withstands biomechanical stress.

NP: Avascular, hypoxia, low pH, low nutrition, low cellularity, high GAG content (negative charge) and type II collagen

End plate: Vascularity in capillaries: avascular, hypoxia, low pH, low nutrition, low cellularity, high GAG content (negative charge)
Fig. 5 (A) An adaptation from Crock & Yoshizawa (1976), demonstrating the vascularly seen across a sagittal cross-section of a lumbar vertebra. (B) A nerve density graph from our L4 vertebra correlating the vascular arterial clusters from Crock & Yoshizawa (1976) to high density nerve regions in the center portion of the nerve density graph. (C) An image of a cluster of innervated vessels from a coronal section taken of the center of the vertebral body, verifying the presence of the central arterial clusters within the L4 vertebra.


FIGURE 100.2 A sketch of a transverse section of a lumbar intervertebral disc showing the structural elements that are targets of some intradiscal therapies. The disc is innervated laterally and anteriorly by branches of the sympathetic trunk and grey ramus communicantes (1), and posteriorly by the sinuvertebral nerves (2). Nerve endings are normally found within the outer third of the annulus fibrosus. Radial fissures (3) attract a neoinnervation in which nerve fibers extend deeper into the disc. Treatments can be directed at the degraded nucleus, the radial and circumferential fissures, or the nerve endings that mediate discogenic pain.

Yin/Bogduk; Bonica’s Management of Pain, 4th Edition
DDD is multifactorial; Genetics may account for 70% of risk (Battie et al)

Loss of water absorption of nucleus due to proteoglycan breakdown and conversion of cell and collagen type within the nucleus alters mechanics of disc; annular tearing leads to neurovascular ingrowth
Intradiscal conditions of early degeneration stimulate mesenchymal stem cells towards migration, paracrine function, proliferation and chondrogenic differentiation.

Illustration of homeostatic imbalance of disc leading to MSC mediated tissue repair response.
Complexity of anabolic vs. catabolic balance of disc.

Findings in the degenerated disc:

- Loss of structural integrity of the annulus
- Annular tears with ingrowth of pain fibers with associated pain (Peng)
  - Annular MRI T2 high intensity zone (HIZ) may be associated with pain (April, Kahn)
- Endplate pathology (Kepler) with Modic changes on MRI and associated pain (Mok; Fields)
- Degree of expression of connective tissue growth factor (CTGF) and resulting fibrotic changes in the nucleus correlate with pain (Peng)
- Sensory nerve ingrowth is stimulated by degenerative changes in the nucleus (De –G-Aggregans), brain derived growth factor (BDGF), and nerve growth factor (NGF) (Garcia-Cosamalon)
REVIEW

Intervertebral disc, sensory nerves and neurotrophins: who is who in discogenic pain?

José García-Cosmalón,1 Miguel E. del Valle,2 Marta G. Calavia,2 Olivia García-Suárez,2 Alfonso López-Muñiz,2 Jesús Otero3 and José A. Vega2,4

Fig. 2. Schematic of factors that are stimulatory (+) or inhibitory (−) for neural ingrowth into the posterior intervertebral disc (IVD). Stimulatory factors include nerve growth factor (NGF), brain-derived growth factor (BDGF), and de-glycosylated aggrecan (“De-G Aggrecan”). Normal aggrecan inhibits neural ingrowth.

Fig. 6

Schematic representation of the possible mechanisms involved in the genesis of the discogenic pain. (A) Inflammation causes release of proinflammatory cytokines in the intervertebral disc (IVD), which act on mast cells and macrophages to trigger secretion of NGF. Cells in the IVD upregulate expression of NGF and substance P (SP) during inflammation. Increased levels of NGF can be retrogradely transported to dorsal root ganglia (DRGs) or stimulate mastocytes and macrophages locally initiating a positive feedback loop. (B) Increased levels of NGF reaching the DRGs act on TrkA-expressing neurons inducing expression of peptides that mediate pain [SP and calcitonin gene-related peptide (CGRP)]. The increased levels of NGF in the IVD, as well as the breakdown of the IVD aggrecan, result in ingrowth of nociceptive nerve fibers and, presumably, in anterograde transport to the IVD, which maintains pain. (C) Synaptic transmission in lamina I and II of the dorsal horn of the spinal cord is mediated by SP and CGRP. In addition, brain-derived neurotrophic factor (BDNF) produced in DRGs projects to the same spinal cord laminae and modulates pain transmission. (D) These complex networks are able to originate and maintain pain of IVD origin.
Discogenic LBP Clinical Presentation

* Pain with increased intradiscal pressure
  * Sitting
  * Bend/lift
  * Valsalva (cough, sneeze)
  * Mornings (due to increased water absorption overnight)
* Midline disc may hurt with extension
* May include instability symptoms of catch/shift/crepitus, pain with arising from flexion and transitional movements
  * Hx of persistent pain between acute episodes, loss of extension, “vulnerability” in neutral zone
* Centralization of pain with McKenzie evaluation
  * Sensitivity 40%, specificity 94%, positive likelihood ratio 6.9

Differentiating Source of Axial LBP

<table>
<thead>
<tr>
<th>Structure</th>
<th>Image</th>
<th>History (P=pain)</th>
<th>Exam</th>
</tr>
</thead>
<tbody>
<tr>
<td>Disc</td>
<td>MRI: HIZ, Modic Changes</td>
<td>P arising from sit, midline; P w bend, lift, Valsalva</td>
<td>Centralization (McKenzie), flexion</td>
</tr>
<tr>
<td>LZJ</td>
<td>DDD/DJD common (not predictive)</td>
<td>P standing, better walking, sitting; age &gt; 52</td>
<td>P w combined extension / rotation (absent = negative predictor)</td>
</tr>
<tr>
<td>SIJ</td>
<td>Not predictive or sensitive/ specific but rule out fracture, stress response, tumor, inflammation</td>
<td>P arising from sit, P unilateral at or below PSIS</td>
<td>Fortin finger (pt. points to SIJ as P location; Gillet test; 3 of 5 positive: pelvic distraction, compression, FABER, thigh thrust, Gaenslen’s</td>
</tr>
</tbody>
</table>
Diagnostic Confirmation: Provocation Discography and Imaging

- Diagnose with provocative discography with manometry using [I]SIS / IASP criteria
  - Concordant >6/10 pain at <20psi above opening pressure
  - Grade III or worse annular tear on modified Dallas discogram scale (tear to outer third of annulus fibrosis)
  - Control level with non-concordant pain <6/10
- Correlation with Modic and high intensity zone (HIZ) MRI findings

Figure 9.38 - A. Sagittal MR image, T2 fast spin echo. Mild disc degeneration at L3-4 and L4-5 is seen as decreased disc signal intensity. Sign of increased signal intensity involve the posterior disc margins at L3-4 and L4-5, compatible with annular tears (arrow). B. Lateral radiograph. Curved contrast media is seen centrally in the disc, and curvilinear contrast bridges the posterior disc margin (arrows), compatible with annular tear.
3.9.3. Modified Pfirrmann Score

The Modified Pfirrmann Score will be graded as: Grade I, Grade II, Grade III, Grade IV, Grade V, Grade VI, Grade VII or Grade VIII in accordance with the following definitions from Griffith et al.:  

- Grade I: Nucleus and inner fibers of annulus uniformly hypointense and equal to cerebrospinal fluid, distinction between annulus fibers and posterior aspect of disc, normal disc height.
- Grade II: Nucleus and inner fibers of annulus hypointense (> presacral fat) + hypointense linear nuclear cleft, distinction between annulus fibers and posterior aspect of disc, normal disc height.
- Grade III: Nucleus and inner fibers of annulus hypointense though < presacral fat, distinction between annulus fibers and posterior aspect of disc, normal disc height.
- Grade IV: Nucleus and inner fibers of annulus mildly hypointense (slightly > outer fibers of annulus), no distinction between annulus fibers and posterior aspect of disc, normal disc height.
- Grade V: Nucleus and inner fibers of annulus hypointense (= outer fibers of annulus), no distinction between annulus fibers and posterior aspect of disc, normal disc height.
- Grade VI: Nucleus and inner fibers of annulus hypointense, no distinction between annulus fibers and posterior aspect of disc, < 30% reduction of disc height.
- Grade VII: Nucleus and inner fibers of annulus hypointense, no distinction between annulus fibers and posterior aspect of disc, < 60% reduction of disc height.
- Grade VIII: Nucleus and inner fibers of annulus hypointense, no distinction between annulus fibers and posterior aspect of disc, < 80% reduction of disc height.
- Transplantable biomaterials
  - Scaffolds of natural and synthetic materials (PRP, amniotic fluid, and fibrin sealant)
  - Maintain cell viability and promote tissue ingrowth
  - Promote regeneration

- Gene therapy
  - Growth factors (e.g., OP-1, BMP-2, GDF-5, FGF) are short lived
  - Gene can be inserted into cells
  - Viral or non-viral vector
  - In vivo or ex vivo gene transduction
  - Concerns of immunogenicity, toxicity, and mutagenesis of viral and low transfection efficiency of non-viral vectors, no establishment of long term efficacy
  - Not cost effective

- Stem cell therapy (most research and promise for human disc repair to date)
  - Co-culture of MSCs and NP cells can upregulate activity of NP cells (in vitro)
  - Cartilage endplate-derived stem cells (CESCs) may be more potent for chondrogenesis than MSCs from other source tissue (e.g., BMA)
Mesenchymal stem cells in regenerative medicine: Focus on articular cartilage and intervertebral disc regeneration

MSC Restorative and Regenerative Properties:
- Secrete macromolecules with immunoregulatory properties
- Structure regenerative microenvironments
- Differentiate into end-stage cell types

1. Pre-chondrogenic stage
   - Main ECM components: hyaluronan, collagen type I and II, and others

2. Aggregation and condensation into pre-cartilage nodules
   - Main ECM components: hyaluronan, collagen type I and II, fibronectin, versican, and thrombospondin

3. Overt chondrocyte differentiation
   - Main ECM components: hyaluronan, collagen types II and IX, and others

Transcription factors: Sox9, Sox5, Sox6, Runx2, BMP-2, BMP-4, BMP-7 (Bmp7), Msx1 and 2, Gli-1, Smads, Lef1, ZEB1, AP-2 and SPI-1, CREB

Extracellular signaling molecules: FGF, Hedgehog, TGF-β1, 2 and 3, BMPs, PDGF, IGF, RA, Wnt, WNT-1, 2, 3, 4, 5, 9

Protein kinase/phosphoprotein phosphatases: MAPKs (ERK2, JNK), PD, PKA, PKC, NDKB and R, PKC, PKA, R, P2K, P2R, PKB (Akt, mTOR)

Cell adhesion and junctions:
- n-CAM, E-cadherin, gap junction

Migration, proliferation and aggregation

Differentiation, changes in morphology

Chondrogenic condensation

Differentiated chondrocytes

Fig. 1. Schematic summarizing the key stages of chondrogenic differentiation including the transcription factors, signaling molecules and protein kinases/phosphoprotein phosphatases involved.
FDA Regulation of Cells as Drugs

- FDA creates regulations that classify allogeneic cultured cells, or more than "minimally manipulated" cells as drugs
  - 1990’s
  - Prior to that, cells regulated as devices or transplant tissue
  - Biologics License Application (BLA) process

- 21 CFR 1271 (cellular product regulations)
  - Allows for harvest and concentration of cells
  - Point of care applications
  - Cannot combine or expand or manipulate cells without FDA oversight of research
  - FDA trials

- Centeno, Bashir PMR 2015
Intra-discal MSCs – Human Studies (Allogeneic)
Mesoblast FDA Phase II RCT

<table>
<thead>
<tr>
<th>Author/Methods</th>
<th>Method</th>
<th>n</th>
<th>End point</th>
<th>POM</th>
<th>Success</th>
<th>SAE</th>
</tr>
</thead>
</table>
| Mesoblast Phase 2 DePalma SIS ASM 2014 | Blind, RCT | 100 | 12 mo | VAS, % success | 1. % > 50% drop in VAS  
2. % with VAS < 2.1 | |
| Safety        | Control | Saline 20 |       |           |                                      | 1 fatigue |
| Efficacy (VAS, ODI, MRI, x-ray F/E) | Control | Hyaluronic acid HA 20 | N=40 |       | 1. 33% (95%CI: 19,48)  
2. 18% (95%CI: 6.1,30) | 1 severe LBP |
| Treat         | MPC 6M N=30 |       |       |           |                                      | 2 (1LBP and 1 infection) |
| Treat         | MPC 18M N=30 |       |       |           | 1. 69% (95%CI: 53,86)  
2. 52% (95%CI: 34,70) | 2 (mod and sev LBP) |

Mesoblast phase II
Control n=40
Treat n=60

Figure 1: Percent Reduction in Lower Back Pain VAS at 12 Months (Pooled Controls)

Source: MSB-DR001 Clinical Study Report
Discs became “stiffer”

Mesoblast Cascade trial: use of stem cells to treat lumbar discogenic pain - FDA Phase III RCT

J. Scott Bainbridge, M.D. (Principal Investigator – Colorado site)

www.DenverBackPainSpecialists.com
“A Prospective, Multicenter, Randomized, Double-blind, Placebo-Controlled Study to Evaluate the Efficacy and Safety of a Single Injection of rexlemestrocel-L alone or Combined with Hyaluronic Acid (HA) in Subjects with Chronic Discogenic Lumbar Back Pain Through 24 Months”

Protocol Amendment v.5.0, dated 2016

Currently Enrolling

Figure 14: Clinical Examples of Modic Changes on T1- and T2-weighted MRI

Modic I and II
Included in trial

Modic III
Excluded from trial
Mesoblast Intra-discal Phase 3 Trial

Inclusion

- VAS ave 24 hour LBP >= 40mm
  - (max 90mm)
- VAS ave 24 hour LEP <= 20mm
- ODI 30 or more
- Chronic (6 mo) LBP; 6 mo PT ex, Rx
- Pfirrmann 3-6
- Modic 2 or less
- Single symptomatic disc – see clinical disc selection algorithm below

Exclusion

- Pfirrmann >= 7 (30% disc height loss)
- Modic 3
- 2 symptomatic discs on discography
- Scoliosis >= 15 degree Cobb angle
- Prior treatment of disc (inj, surg, disc RF)
- Morphine equivalent >75mg/d
- BMI >40
- Osteoporosis
- Facet or SIJ pain generators
Decision Matrix for Selecting Index Level

NOTE:
Invasive procedures, such as the medial branch block to rule out facet pain, SI joint injections (if deemed necessary), or discography to confirm that the index level is painful, should be performed after other criteria have been met.

3.1 Overview of Study Design

This is a prospective, multicenter, randomized, double-blind, placebo-controlled Phase 3 study designed to evaluate the safety and efficacy of Mosoblast’s exenestrol-L alone or combined with hyaluronic acid (HA) in subjects with chronic low back pain (>6 months) not adequately controlled by conservative measures and associated with moderate radiographic degenerative changes of a disc.
Efficacy Endpoint

Primary Efficacy Endpoint

Overall Treatment Success at both 12 AND 24 months: Measured as subjects meeting each of the following criteria at both 12 AND 24 months:

- At least a 50% reduction from baseline in low back pain VAS score (average pain over 24 hours); AND
- At least a 15 point decrease from baseline in ODI score; AND
- No interventions affecting the treated disc (interventions include: [a] surgical intervention [eg, discectomy, intervertebral fusion, or disc replacement]; [b] injections for alleviation of pain at the treated disc [eg, epidural corticosteroid injection, or transforaminal injection]).

Secondary Outcomes: EQ-5D, iPCQ (work impairment), MRI and F/E xray changes, Self assessment of treatment (SAT), safety measures

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Supplemental Materials

Intra-discal Scaffold / PRP – Human Studies

<table>
<thead>
<tr>
<th>Author</th>
<th>Method</th>
<th>n</th>
<th>End point</th>
<th>POM</th>
<th>Success</th>
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<tbody>
<tr>
<td>Fibrin Sealant - Biostat</td>
<td>Phase III IND RCT, saline</td>
<td>220</td>
<td>26 weeks</td>
<td></td>
<td>33.5% Biostat 39.3% saline</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3:1</td>
<td></td>
<td>50% of each group had MCSD VAS and RMDQ</td>
<td></td>
</tr>
<tr>
<td>PRP Tuakli-Worsornu PMR 2016</td>
<td>Blind(2) RCT Control (disco) ID PRP</td>
<td>N=18</td>
<td>8 weeks</td>
<td>FRI, NRS, PF SF-36 MNASS DQ</td>
<td>FRI, Best pain, NASS Statistically Sig improvement FRI held to one year</td>
</tr>
<tr>
<td></td>
<td></td>
<td>N=29</td>
<td></td>
<td>FRI=Functional Rating Index</td>
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Lumbar Intradiscal Platelet-Rich Plasma (PRP) Injections: A Prospective, Double-Blind, Randomized Controlled Study


Abstract

Objective: To determine whether single injections of autologous platelet-rich plasma (PRP) into symptomatic degenerative intervertebral discs will improve participant-reported pain and function.

Design: Prospective, double-blind, randomized controlled study.

Setting: Outpatient physical spine practice.

Participants: Adults with chronic (4–6 months), moderate-to-severe lumbar discogenic pain that was unresponsive to conservative treatment.

Methods: Participants were randomized to receive intradiscal PRP or contrast agent after provocative discography. Before pain, physical function, and participant satisfaction were collected at 1 week, 4 weeks, 8 weeks, 6 months, and 1 year. Participants in the control group who did not improve at 8 weeks were offered the option to receive PRP and subsequently followed.

Main Outcome Measures: Functional Rating Index (FRI), Numeric Rating Scale (NRS) for pain, the pain and physical function domains of the 36-Item Short Form Health Survey, and the Modified North American Spine Society (MNSS) Outcome Questionnaire were used.

Results: Forty-seven participants (29 in the treatment group, 18 in the control group) were analyzed by an independent observer with a 20% follow-up rate. Over 8 weeks of follow-up, there were statistically significant improvements in participants who received intradiscal PRP with regard to pain (NRS best pain; p = .02), function (FRI; p = .01), and patient satisfaction (MNSS Outcome Questionnaire; p = .01) compared with controls. No adverse events of disk space infection, neurologic injury, or progression foraminal were reported following the injection of PRP.

Conclusions: Participants who received intradiscal PRP showed significant improvements in FRI, NRS best Pain, and MNSS patient satisfaction scores over 8 weeks compared with controls. Those who received PRP maintained significant improvements in PRP scores through at least 1 year of follow-up. Although these results are promising, further studies are needed to define the subset of patients most likely to respond to biologic intradiscal treatment and the ideal cellular characteristics of the intradiscal PRP injections.

Review Article

Cell-Based Therapies Used to Treat Lumbar Degenerative Disc Disease: A Systematic Review of Animal Studies and Human Clinical Trials

David Oehme,1 Tony Goldschlager,2 Peter Ghosh,3,4 Jeffrey V. Rosenfeld,3,4 and Graham Jenkins

![Diagram]

**Figure 2:** Flow diagram demonstrating the systematic analysis process.
Human cell based trials.

**Table 4: Clinical studies utilising cell-based therapies to treat human lumbar disc degeneration.**

<table>
<thead>
<tr>
<th>Author</th>
<th>Clinical details</th>
<th>Cells transplanted</th>
<th>Method of cell administration</th>
<th>Results</th>
<th>Level of evidence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hacke et al. [65]</td>
<td>10 patients with low back pain due to degenerative disc disease</td>
<td>Autologous bone marrow haematopoietic precursor stem cells (HSCs)</td>
<td>Percutaneous injection with concurrent hyperbaric oxygen therapy</td>
<td>(i) No improvement in back pain in any patient (ii) 80% of patients underwent surgical intervention within 1 year</td>
<td>(i) 3</td>
</tr>
<tr>
<td>Meisel et al. [15]</td>
<td>28 patients undergoing microdiscectomy with back pain (EuroDISC study)</td>
<td>Autologous culture expanded disc derived chondrocytes</td>
<td>Percutaneous injection 12 weeks following microdiscectomy</td>
<td>(i) Patients receiving cell transplantation had reduced back pain at 2 years (ii) Increased MRI T2 signal of treated and adjacent discs</td>
<td>(i) 1</td>
</tr>
<tr>
<td>Orezzo et al. [22]</td>
<td>10 patients with low back pain and radiological evidence of degenerative disc disease</td>
<td>Autologous MSCs</td>
<td>Percutaneous injection</td>
<td>(i) Clinical improvement in back pain, leg pain and disability (ii) Increased MRI T2 signal (iii) Disc height not recovered</td>
<td>(i) 3</td>
</tr>
<tr>
<td>Yoshikawa et al. [23]</td>
<td>2 patients with back pain and sciatica, with radiological evidence of lumbar spinal stenosis and disc disease</td>
<td>Autologous bone marrow MSCs</td>
<td>Percutaneous injection within collagen sponge</td>
<td>(i) Increased MRI T2 signal (ii) Less instability (iii) Clinical improvement in both patients</td>
<td>(i) 3</td>
</tr>
</tbody>
</table>

HSCs: haematopoietic precursor stem cells. MSCs: mesenchymal stem cells.

Oehme et al 2015

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**Treatment of discogenic back pain with autologous bone marrow concentrate injection with minimum two year follow-up**

Kenneth Petline¹ · Richard Suzuki² · Theodore Sand² · Matthew Murphy¹,³

---

**Figure 1** Average ODI and VAS scores by time for all non-surgery patients (ODI blue, VAS green). 238×162 mm (300×300 DPI)

**Figure 2** Average ODI and VAS scores of patients through 24 months according to CFU-F concentrations <2,000 (blue) or >2,000 (green) CFU-F/mL in BMC: 238×162 mm (300×300 DPI)
5 lost to fusion – not included
Cash pay

Intra-discal MSCs – Human Studies (Autologous – Expanded)

<table>
<thead>
<tr>
<th>Author</th>
<th>Method</th>
<th>n</th>
<th>End point</th>
<th>POM</th>
<th>Success</th>
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<tr>
<td>Oroszco</td>
<td>BMA, expanded</td>
<td>10</td>
<td>12 mo</td>
<td>VAS, ODI, MRI H2O content %</td>
<td>VAS 69&gt;20 ODI 25&gt;7.4 MRI .62&gt;.72</td>
</tr>
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</table>
Intra-discal MSCs – Human Studies (Allogeneic juvenile chondrocytes)

<table>
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<th>Author</th>
<th>MSCs Type</th>
<th>Species</th>
</tr>
</thead>
<tbody>
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<td>Acosta 2011</td>
<td>Juvenile chondrocytes</td>
<td>Porcine</td>
</tr>
<tr>
<td>Coric 2012</td>
<td>Juvenile chondrocytes</td>
<td>Human</td>
</tr>
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</table>

Prospective study of disc repair with allogeneic chondrocytes

Presented at the 2012 Joint Spine Section Meeting

Clinical article

DOMAGKO CORC, M.D.,1 KENNETH PETTINE, M.D.,2 ANDREW SUNIC, M.D.,3
AND MARGARET O. BOLLES, R.N.4
1Carolina Neurosurgery and Spine Associates; 2Department of Neurosurgery, Carolina Medical Center;
Charlotte, North Carolina; and 3Loveland Orthopedic Clinic, Loveland, Colorado

Object. The purpose of the study was to evaluate the safety and initial efficacy of NoQo allogeneic juvenile chondrocyte delivered percutaneously for the treatment of lumbar spondylolisthesis with mechanical low-back pain (LBP). NoQo is a cell-based biological therapy for disc repair. The authors report the results at 12 months of the NoQo Phase I investigational new drug (IND) single-arm, prospective feasibility study for the treatment of LBP for single-level degenerative disc disease (Pfirrmann Grades III–IV) at L3–5.

Methods. Fifteen patients (6 women and 9 men) were enrolled at 2 sites. Institutional review board approval was obtained, and all patients signed a study-specific informed consent. All patients have completed a minimum of 1 year of follow-up.

Patients were evaluated pre-treatment and at 1, 3, 6, and 12 months post-treatment. Evaluations included routine neurological examination, serum liver and renal function studies, MRI, Oswestry Disability Index (ODI), the Numerical Rating Scale (NRS), and the 36-item Short Form Health Survey (SF-36).

Results. Fifteen patients were treated with a single percutaneous delivery of NoQo juvenile chondrocytes. The mean patient age was 46 years (19–74 years). Each treatment consisted of 1–2 ml (mean injection 1.3 ml) of juvenile chondrocytes (approximately 10^{6} chondrocyte cells/ml with fibrin carrier). The mean peak pressure during treatment was 87.6 psi. The treatment time ranged from 5 to 31 seconds.

The mean ODI (baseline 53.3, 12-month 20.3; p = 0.0001), NRS (baseline 5.7, 12-month 3.1; p = 0.0025), and SF-36 physical component summary (baseline 35.3, 12-month 46.9; p = 0.0002) scores all improved significantly from baseline. At the 6-month follow-up, 13 patients underwent MRI (one patient underwent CT imaging and another refused imaging). Ten (77%) of those 13 patients exhibited improvements on MRI. Three of these patients showed improvement in disc contour or height. High-intensity zones (HIz), consistent with posterior annular tears, were present at baseline in 9 patients. Of those, the H Ez was either absent or improved in 8 patients (89%) by 6 months. The H Ez was improved in the ninth patient at 3 months, with no further MRI follow-up. Of the 10 patients who exhibited radiologic improvement at 6 months, findings continued to improve or were sustained in 8 patients at the 12-month follow-up. No patient experienced neurological deterioration. There were no disc infections, and there were no serious or unexpected adverse events. Three patients (20%) underwent total disc replacement by the 12-month follow-up due to persistent, but not worse than baseline, LBP.

Conclusions. This is a 12-month report of the clinical and radiographic results from a US IND study of cell-based therapy (juvenile chondrocytes) in the treatment of lumbar spondylosis with mechanical LBP. The results of this prospective cohorts are promising and warrant further investigation with a prospective, randomized, double-blinded, placebo-controlled study design. Clinical trial registration no.: NCT01985431.

http://www.neurosurgery.org/doi/10.3171/2012.10.SPINE11241


Expanded juvenile chondrocytes
cadaveric, expanded
N=15, no control
10M+ chondrocytes

3 required fusion

<table>
<thead>
<tr>
<th>VAS</th>
<th>5.7 &gt; 3.1</th>
</tr>
</thead>
<tbody>
<tr>
<td>ODI</td>
<td>50 &gt; 20</td>
</tr>
<tr>
<td>SF-36</td>
<td>35 &gt; 47</td>
</tr>
</tbody>
</table>
Oehme, et al. 2015

Animal Studies

MSCs:
1. survive > 6 months
2. Increase disc height and hydration
3. Increase proteoglycans
4. Improve AF histology
5. Transform into disc chondrocytes

Intra-discal MSCs – Animal Studies

Huang, et al
Transplantable biomaterials
- Scaffolds of natural and synthetic materials (PRP, amniotic fluid, and fibrin sealant)
- Maintain cell viability and promote tissue ingrowth
- Promote regeneration

Gene therapy
- Growth factors (e.g. OP-1, BMP-2, GDF-5, FGF) are short lived
- Gene can be inserted into cells
- Viral or non-viral vector
- In vivo or ex vivo gene transduction
- Concerns of immunogenicity, toxicity, and mutagenesis of viral and low transfection efficiency of non-viral vectors; no establishment of long term efficacy
- Not cost effective

Stem cell therapy (most research and promise for human disc repair to date)
- Co-culture of MSCs and NP cells can upregulate activity of NP cells (in vitro)
- Cartilage endplate-derived stem cells (CESCs) may be more potent for chondrogenesis than MSCs from other source tissue (e.g. BMA)

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### Supplemental Table 1: Biomaterials for NP regeneration

<table>
<thead>
<tr>
<th>Biomaterials</th>
<th>Cell type</th>
<th>Model</th>
<th>Approach</th>
<th>Outcome</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alginate, chitosan- PLGA scaffolds</td>
<td>Human NP cells</td>
<td>in vivo</td>
<td>NP cells were grown in alginate scaffold.</td>
<td>Cell number was greater in alginate scaffold than that in chitosan-PLGA scaffolds.</td>
<td>83</td>
</tr>
<tr>
<td>EDC</td>
<td>Human ADSCs</td>
<td>in vivo</td>
<td>ADSCs cells were grown in EDCs. In vivo study, EDC hydrogel was placed into subcutaneous pocket.</td>
<td>Hydrogel exhibited shape-memory properties and supported cell viability and differentiation towards NP cell phenotype.</td>
<td>64</td>
</tr>
<tr>
<td>PEID</td>
<td>Pig NP cells</td>
<td>in vivo</td>
<td>Laminin functionalized PEID hydrogel containing NP cells was injected into IVD explant.</td>
<td>NP cell retention in cultured IVD explants was significantly higher over 14 days for cells in PEID-laminin hydrogel compared to cells in fluid suspension.</td>
<td>65</td>
</tr>
<tr>
<td>Polylactide</td>
<td>Rabbit NP cells</td>
<td>in vitro</td>
<td>Cells were seeded on surface of scaffold.</td>
<td>Scaffolds promoted NP cell adhesion, migration, and biosynthesis.</td>
<td>9</td>
</tr>
<tr>
<td>Silk, fibrin, and HA</td>
<td>Human chondrocyte</td>
<td>in vitro</td>
<td>Chondrocytes cells were cultured in hydrogel with 15% serum.</td>
<td>Cells in fibrin/hyaluron with 15% silk showed superior expression of type II collagen, Sox-9, and aggrecan.</td>
<td>49</td>
</tr>
<tr>
<td>Type II collagen and HA</td>
<td>Cell NP cells</td>
<td>in vitro</td>
<td>Cells were grown in hydrogel cross-linked with PEID.</td>
<td>Gel was not toxic to cells and supported cell growth.</td>
<td>3</td>
</tr>
<tr>
<td>Type II collagen and HA</td>
<td>Rat MSCs</td>
<td>in vitro</td>
<td>Cells were injected into hydrogels.</td>
<td>Hydrogels cross-linked with EDC (8% w/w) improved cellular viability and cell proliferation rate.</td>
<td>42</td>
</tr>
<tr>
<td>Collagen gel</td>
<td>Human MSCs</td>
<td>in vitro</td>
<td>Cells were grown in Collagen gel.</td>
<td>MSCs grown in Collagen hydrogel showed differentiation of MSCs to a phenotype similar to both articular chondrocytes and NP cells.</td>
<td>51</td>
</tr>
<tr>
<td>Nontissue scaffold, amelogenin-fibrin scaffold</td>
<td>Human MSCs</td>
<td>in vitro</td>
<td>Cells were seeded in nontissue scaffold.</td>
<td>Time-dependent development of chondrodifferentiation phenotype of seeded cells was observed.</td>
<td>46</td>
</tr>
<tr>
<td>Hydrogel carrier</td>
<td>Human AF and NP cells</td>
<td>in vivo</td>
<td>Cells were seeded into hydrogel.</td>
<td>Cell metabolism was improved. QnAg expression of collagen I, II, and X aggrecan, and Sox-9 was maintained.</td>
<td>47</td>
</tr>
<tr>
<td>PGA–hyaluronic acid</td>
<td>in vivo</td>
<td>Constructed hydrogel scaffold was inserted into degenerated disc of rabbit.</td>
<td>After 12 months of implantation, histological analysis showed cell infiltration into the defect and formation of repaired tissue.</td>
<td>Survival and proliferation of NP cells in PGA of bovine model were observed after 8 weeks.</td>
<td>48</td>
</tr>
<tr>
<td>PDLA</td>
<td>Beagle dog NP cells</td>
<td>in vivo</td>
<td>PDLA scaffold with NP cells were implanted into disc.</td>
<td></td>
<td>49</td>
</tr>
</tbody>
</table>
Recombinant DNA (plasmid)
Host cell (clone) cultured
Used to produce protein
OR
Produce copies of gene for transduction
Gene Therapy

Growth factors (e.g. OP-1, BMP-2, GDF-5, FGF) are short lived Gene can be inserted into cells Viral or non-viral vector

Concerns of immunogenicity, toxicity, and mutagenesis of viral, and low transfection efficiency of non-viral vectors

No establishment of long term efficacy

Not cost effective

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**Supplemental table 3. Gene therapy in NP regeneration.**

<table>
<thead>
<tr>
<th>Gene</th>
<th>Delivery system</th>
<th>Cell type</th>
<th>Model</th>
<th>Approach</th>
<th>Outcome</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>LIFR</td>
<td>Lentivirus</td>
<td>Human NP cells</td>
<td>In vivo</td>
<td>Cultured human NP cells were transduced with viral vector</td>
<td>Telomerase activity and delayed cell senescence were restored. Collagen II andaggrocalcinogen increased.</td>
<td>93</td>
</tr>
<tr>
<td>GDF-5</td>
<td>Electroporation</td>
<td>Human MECs</td>
<td>In vivo</td>
<td>Transfected cells were cultured in alginate. Transfected cells were injected into nude agar, degeneration organ culture system</td>
<td>Expression of aggrecan and SOX 9 for cells in alginate gel was up-regulated. Human DNA was for 100 days degeneration organ culture system with dermal was partially recovered.</td>
<td>92</td>
</tr>
<tr>
<td>BMP-1</td>
<td>AAV</td>
<td>Human NP cells</td>
<td>In vivo</td>
<td>Cultured NP cells were transduced with virus encoding BMP-1 or AAV-BMP-1</td>
<td>Synthesis of proteoglycan increased.</td>
<td>55</td>
</tr>
<tr>
<td>BNP</td>
<td>AAV</td>
<td>Bone-derived NP cells</td>
<td>In vivo</td>
<td>Cultured NP and AF cells were transduced with virus encoding BMP-7 or BMP-7</td>
<td>Transduced NP and AF cells expressed appropriate gene to maintain their phenotype.</td>
<td>94</td>
</tr>
<tr>
<td>h-BMP-2</td>
<td>AAV</td>
<td>Dog NP cells</td>
<td>In vivo</td>
<td>MDA of beagle dogs were injected with NCA expressing h-BMP-2</td>
<td>MDA injected with NCA expressing h-BMP-2 showed senor degeneration.</td>
<td>95</td>
</tr>
<tr>
<td>h-AZ</td>
<td>Adenovirus</td>
<td>Rabbit NP cells</td>
<td>In vivo</td>
<td>Cultured NP cells were transduced with h-p34c. AZ was injected into rabbit lumbar FD</td>
<td>In vivo and in vitro studies demonstrated nucleus pulposus cells were efficiently transduced by AAV in vitro.</td>
<td>96</td>
</tr>
<tr>
<td>BNP</td>
<td>AAV</td>
<td>Rabbit NP cells</td>
<td>In vivo</td>
<td>Diced were punctured and injected with AAV vector encoding either BNP2 or BMP-2</td>
<td>Tracked discs showed less degeneration than untreated discs.</td>
<td>54</td>
</tr>
<tr>
<td>GAP, SOX-9</td>
<td>AAV</td>
<td>Rabbit NP cells</td>
<td>In vivo</td>
<td>Mixture of AAV-DNA and AAV-SOX-9 was injected into rabbit MDs</td>
<td>Disc height and expression of disc proteoglycans and Type II collagen increased because of the combined effect of GAP and SOX-9.</td>
<td>97</td>
</tr>
<tr>
<td>TGF</td>
<td>Adenovirus</td>
<td>In vivo</td>
<td>Adenoviral vector encoding TGF was injected into disc lumbar disc</td>
<td>Proteoglycan synthesis increased.</td>
<td>91</td>
<td></td>
</tr>
</tbody>
</table>
CRISPR-Cas9

- J. Craig Venter
  - Co-mapped the human genome

- Jennifer Doudna
  - Key role in developing CRISPR
  - UC-Berkeley

- Kathy Niakan
  - Will test CRISPR on viable human embryos
  - Francis Crick Institute (London)