

### Treating Discogenic Pain with Mesenchymal Stem Cell Exosomes: What Is the Biologic Mechanism of Action

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Received Date: 02-18-2019

Accepted Date: 02-20-2019

Published Date: 02-24-2019

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#### Abstract

Over the last several years it has become increasingly understood by researchers and clinicians that the clinical efficacy of utilizing Mesenchymal Stem Cells to treat discogenic pain is not dependent on the cells differentiating into Nucleus Pulposus cells but entirely on their paracrine release of growth factors and exosomes. Living MSCs are not required to accomplish the release of GFs and exosomes into a disc. The purpose of this paper is to introduce the concept of using acellular MSC exosome products and the rationale of why acellular will replace all current cellular therapies both autogenous and allogeneic for the treatment of discogenic pain.

**Keywords:** Mesenchymal Stem Cells; Stem cells; Cell-based therapy; Bone Marrow Concentrate; Exosomes

#### Abbreviations

MSCs: Mesenchymal Stem Cells; GF: Growth Factors; NP: Nucleus Pulposus; BMC: Bone Marrow Concentrate; NDI: Neck Disability Index; PTEN: Phosphatase and Tensin Homolog.

#### What Causes Discogenic Pain in the Spine?

The exact cause of disc degeneration and subsequent cervical and lumbar discogenic pain is complicated. The primary etiology for early disc degeneration must be from a genetic predisposition. Various animal studies have been contradictory in directly correlating biomechanical stress and disc degeneration [1, 2]. Likewise, published clinical studies have failed to link disc degeneration directly to mechanical factors such as labor [3]. As a further complication, the perception of pain in humans is complex, related to psychosocial factors, environmental factors, and one's perception of life's satisfaction

[4]. Disc degeneration on a cellular level also is complicated. Nutrients must travel through the capillary network in the vertebral body, and then diffuse through the endplate into the extracellular matrix of the disc to reach the nucleus pulposus cells [5-7]. Calcification of the end-plates impairs nutrient flow such as glucose and oxygen [8]. Endplate calcification also exacerbates the hypoxic acidic environment further impairing disc cell metabolism [9-12]. Stress, trauma, or natural degeneration in the disc tissue results in the production of proinflammatory molecules such as TNF- $\alpha$  and interleukins (IL-1, 4,12) as well as a build-up of local acidity. The combined effects of nutrient deprivation and inflammatory environment result in a decrease in proteoglycan synthesis and a cascade of nucleus pulposus cell death [13, 14].

### **What are the Published Results of Treating Discogenic Pain in the Cervical and Lumbar Spine with Bone Marrow Concentrate?**

The senior author has currently published six papers on the clinical results of treating cervical and lumbar discogenic pain utilizing bone marrow concentrate (BMC) [15-20]. The lumbar published results are on 146 adults seeking a surgical consult for discogenic back pain opting instead for a disc injection of BMC. All were surgical candidates based on FDA criteria.

#### **Methods**

These were IRB approved non-randomized prospective studies. All study patients met strict inclusion/ exclusion criteria with a diagnosis of discogenic low back pain. Either one or two symptomatic discs were injected with 2 to 3 mL of BMC with the addition of glucose and bicarbonate in four published papers. The fifth paper consisted of patients with multi-level disc pathology. In this group, an average of 3.6 discs was injected. Clinical outcomes were based on pain (VAS) and Function (ODI), subsequent surgery, opioid use, and MRI pre and post procedure out to five years.

#### **Results**

One-year MRI indicated 40% of patients improved

in disc hydration. No radiographs or MRIs worsened. See Table one for the follow-up pain and function scores.

**Table 1:** Average Improvement from baseline in PAIN and FUNCTION

	<b>Baseline</b>	<b>1 Year</b>	<b>2 Year</b>	<b>3 Year</b>	<b>5 Year</b>
ODI	56.5	66%	67.7%	69.4%	75.9%*
VAS	79.3	58.2%	71.2%	72.4%	74.1%

\*The difference between the three and five-year ODIs  $p < 0.003$

#### **Conclusions**

The average patient improved 70% in pain and 73% in function from baseline to 5 year follow up. All changes in VAS and ODI had a p-value of  $< 0.001$ . No patient had surgery between the 3 and 5 year follow up. There was no difference in clinical result comparing age, gender, BMI or number of disc levels injected. The cervical paper is a prospective non-randomized study of 182 patients with two-year follow-up. These patients had multi-level cervical disc pathology. The average patient had 2.44 discs injected with BMC. The clinical results were based on comparing pre-treatment VAS and Neck Disability Index (NDI) to various time points out to two years post-treatment.

#### **Methods**

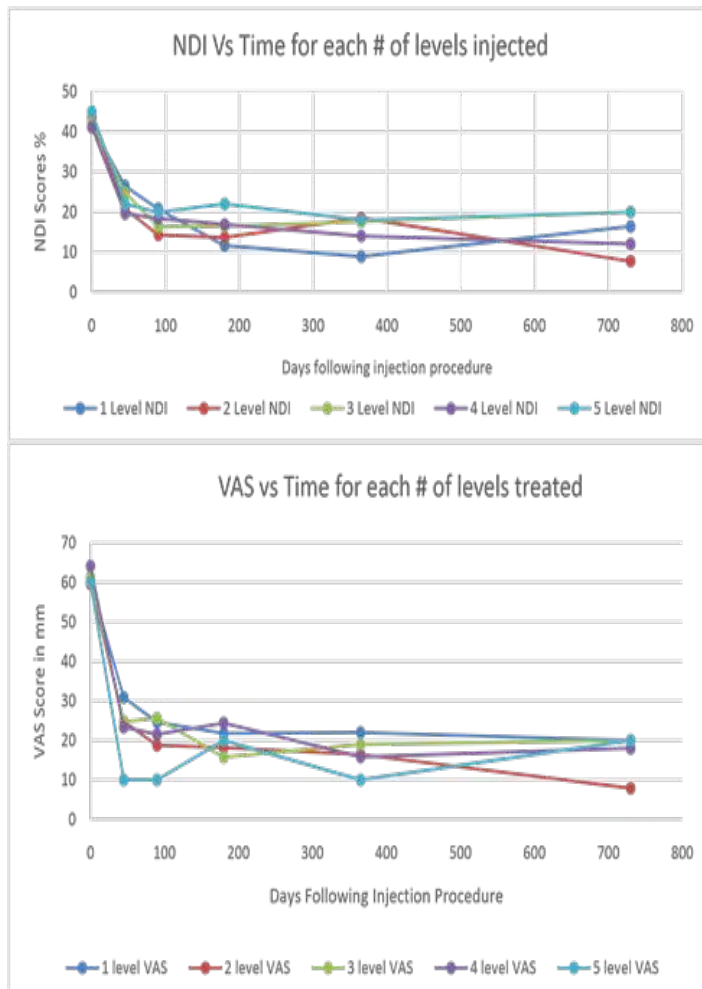
There were 182 patients (97 male, 85 female) with an average age of 54.5 (range 18 to 80). The 30-minute procedure involved aspirating 55 ml of bone marrow from the iliac wing, concentrating this via centrifugation to a volume of 3ml, and then injecting 0.5ml of the bone marrow concentrate into each abnormal cervical disc. The procedure was performed with IV sedation. Number of levels injected was: one level=33 patients, two levels=60 patients, three levels=45 patients and four levels=44 patients. The average number of levels injected was 2.44. Pre-procedure Neck Disability Index (NDI) was 44.5 (range 12-100), and Visual Analog. The cervical paper is a prospective non-randomized study of 182 patients with two-year follow-up. These

patients had multi-level cervical disc pathology. The average patient had 2.44 discs injected with BMC. The clinical results were based on comparing pre-treatment VAS and Neck Disability Index (NDI) to various time points out to two years post-treatment.

**Results**

Six-month follow-up NDI and VAS were 17.4 and 22.5. One-year NDI and VAS were 15.8 and 21.4. Two-year follow-up NDI and VAS were 16.5 and 20.7. All scores had a P-value of less than 0.001. There was no difference in the clinical results comparing one, two, three, or four-disc levels injected. There were no injection complications, and no patient had surgery during the study. The clinical findings are detailed in Tables 2 and 3.

**Table 2: Clinical Findings (NDI, VAS)**



**Conclusions**

These results indicate a bone marrow concentrate injection may be a reasonable non-surgical option for patients with symptomatic degenerated cervical discs.

**What was the Cell Count Data from the BMC Patients?**

Average cell viability, TNC, total and frequency of CFU-F/CFU-O, and CD marker phenotypes in fresh bone marrow concentrate.

**Table 3: Clinical Findings**

<b>Cell viability at 24 hours</b>	98.1 (±1.2)%	TNC/ml in BMC  121 (±11) x 10 <sup>6</sup>
<b>Cell phenotype subpopulation</b>	% of TNC	Subpopulation Concentration in BMC (cells per milliliter)
<b>CFU-F</b>	0.0025%	2,713 (±491) per ml
<b>CFU-O</b>	0.0027%	2,913 (±418) per ml
<b>Lineage<sup>-</sup> cells (CD 2<sup>+</sup>/3<sup>+</sup>/8<sup>+</sup>/11b<sup>-</sup>)</b>	25.89%	31.5 x 10 <sup>6</sup> /ml
<b>Lineage<sup>-</sup>/CD34<sup>+</sup></b>	1.397%	1.69 x 10 <sup>6</sup> /ml
<b>Lineage<sup>-</sup>/CD34<sup>High</sup>/CD90+/CD105+</b>	0.0007%	802/ml
<b>Lineage<sup>-</sup>/CD34<sup>Low</sup>/CD90+/CD105+</b>	0.0040%	4,832/ml
<b>Lineage<sup>-</sup>/CD34<sup>-</sup>/CD90+/CD105+</b>	0.0049%	5,914/ml

The results of the BMC cell analysis showed the average number of MSCs/cc of BMC was a minuscule number of less than 3,000 MSCs/cc. This represents only 0.0025% of the 121,000,000 Total Nucleated Cells/cc.

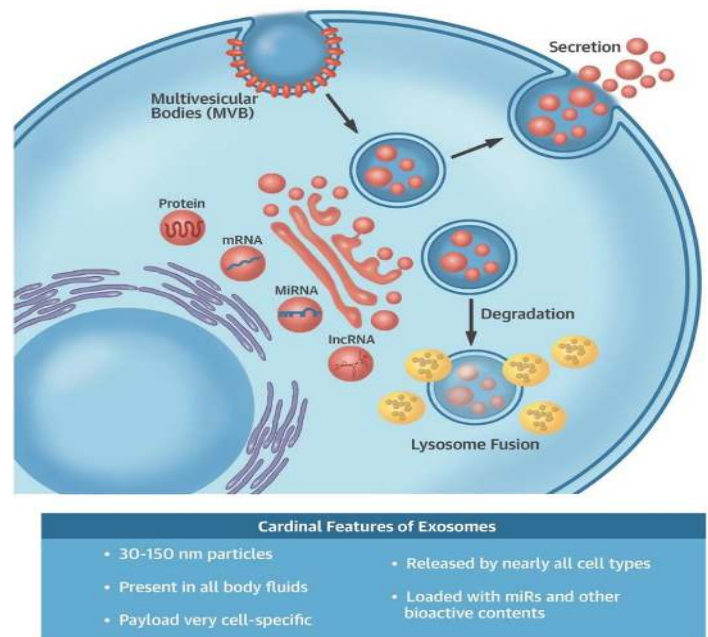
**The Question Is:** How Can this Incredibly Small Number of MSCs Result in Such a Statistically Huge Improvement in Clinical Results Even Out to Five Years?

### What Are Mesenchymal Stem Cells (MSC) Exosomes?

The MSC has always been the primary cell for orthopedics because only it can become a chondroblast, osteoblast or fibroblast. Recently we have begun to appreciate and realize the MSC may be the most critical cell in your body because of what it releases to communicate with other cells. The MSC modulates your immune system to control inflammation by releasing exosomes, secretomes, growth factors, cytokines, and chemokines. These proteins are what are essential in regenerative medicine, NOT the MSC itself. Caplan even suggests changing the name to Medicinal Signaling Cells [21].

Arnold Caplan, the Ph.D. responsible for naming the mesenchymal stem cell (MSC) states, "Now that mesenchymal stem cells (MSCs) have been shown to be perivascular in vivo, the existing traditional view that focuses on the multipotent differentiation capacity of these cells should be expanded to include their equally interesting role as cellular modulators that brings them into a broader therapeutic scenario. We discuss existing evidence that leads us to propose that during local injury, MSCs are released from their perivascular location, become activated, and establish a regenerative microenvironment by secreting bioactive molecules and regulating the local immune response. These trophic and immunomodulatory activities suggest that MSCs may serve as site-regulated "drugstores" in vivo [22]. Extensive researches have shown Caplan to be correct. The MSC produces numerous growth factor proteins to treat orthopedic pathology. But the most crucial paracrine method by which the MSC functions may be by the creation of the acellular structure named the exosome [23-26]. The exosome is a tiny 30 to 150 nanometer-sized bi-phospholipid membrane-enclosed structure created by the Golgi body or apparatus. An MSC is 1,000 times larger than an exosome. The diameter of a hair is 80,000 nanometers. Exosomes contain growth factors, signaling lipids, and micro and messenger RNA. The RNA contents in exosomes mediate most of their anti-inflammatory effects. The RNA is placed into an

exosome along with numerous peptide growth factors and signaling lipids by the Golgi bodies within the donor MSC. The exact type and amount of growth factor proteins, signaling lipids, and RNA placed into an exosome are dependent on the surrounding microenvironment of the MSC. The exosome is released into the extracellular matrix and taken up by a receptor cell. The exosome RNA is then taken into the receptor cell ribosome where the RNA is translated to create numerous anti-inflammatory growth factors, chemokines, cytokines, and secretomes. This process is illustrated in **Figure 1**.



**Figure 1:** (Top) Schematic of exosome biogenesis. (Bottom) Cardinal features of exosomes.

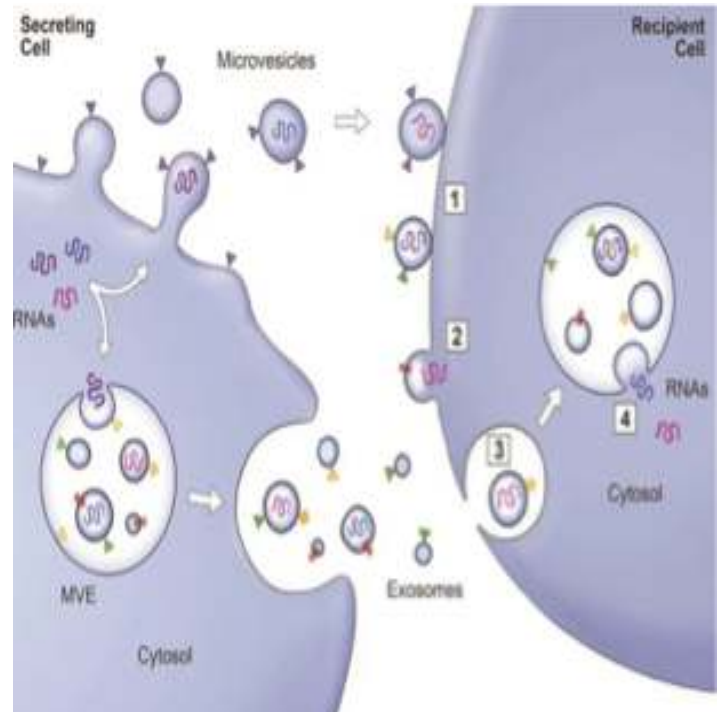
Exosomes arise from the fusion of surface membrane invaginations (multivesicular bodies) and the products of the Golgi apparatus. The resulting vesicles are either degraded by lysosomes or secreted as exosomes. Exosomes do not elicit acute immune rejection, and there is no risk for tumor formation. The effect of exosome RNA may last months or longer as the receptor cell ribosomes continue to translate the donor RNA [27-32]. BEES MAKE HONEY AND MSCs MAKE EXOSOMES [33].

The amount of scientific research on exosomes has dramatically increased since 2008 when it was discovered exo-

somes contain DNA. There were 28 scientific citations on exosomes in 1996 and 24,765 in 2016.<sup>33</sup> Exosome research has created a renaissance in our understanding of cellular communication. Cells communicate near and far by a dynamic of exosome secretion and uptake. This is illustrated in Figure two. “Think of the extracellular space as a sea containing trillions of messages in a bottle, quickly read and answered, always turning over, and you begin to get a sense of what is going on inside us every moment of every day [33].”

### What is the Biologic Mechanism of Action of BMC?

Bone marrow concentrate (BMC) contains on average only about 2,700 MSCs per cc. Despite the incredibly small number of MSCs found in BMC; there is extensive literature reporting clinical efficacy in animals and humans using BMC for the treatment of discogenic pain. This effect cannot be dependent upon BMC/MSK cell survival or differentiation. The efficacious effect must be from the release of acellular paracrine factors. The future of the biologic treatment of discogenic pain will be the utilization of acellular MSC derived growth factors, secretomes, chemokines, cytokines, and especially exosomes. These paracrine factors can be placed into the degenerated disc in concentrations of 100,000 or more times that of any cellular MSC treatment. One dose of an acellular MSC derived product will contain over TWO BILLION exosomes. These proteins and exosomes will function in a paracrine fashion to, directly and indirectly, alter the inflammatory environment of the degenerated disc to a normal non-painful physiologic environment. This is illustrated in **Figure 2**.



**Figure 2:** Exosome Secretion and Uptake.

### What Is the Future for Using MSC Exosomes to Treat Discogenic Pain?

MSCs direct the anti-inflammatory function of other cells by releasing exosomes into the ECM. The future of using MSCs to treat discogenic pain will be to expand the MSCs in a defined growth media. The cells are then subjected to 48 hrs. of “stress” conditions of hypoxia, low glucose and low pH to maximize their release of anti-inflammatory exosomes. The growth media is then collected, and the exosomes separated and stored for future use [34].

The future of regenerative medicine is the use of ACCELLULAR vs. cellular products. Acellular MSC derived exosomes can provide a consistent product that can have proteomic analysis and RNA sequencing. Every growth factor can be identified and quantified. Every micro and messenger RNA can be characterized. Think of acellular exosomes as a bio-pharmacological quality product that can be standardized and tested regarding dose and biological activity.

None of this is possible with a cellular product. Perhaps most important is that an acellular product will not in-

roduce extensive foreign DNA into the recipient patient that an allogeneic cellular source does. No one knows the long-term effects of having foreign DNA. Is it carcinogenic? Replacing the administration of live cells with acellular exosomes will mitigate the safety concerns and limitations associated with the transplantation of viable replicating cells [35].

MSC derived exosomes do not have any of the immunogenic concerns related to the administration of allogeneic cellular products. Acellular exosome 'off-the-shelf' products have no immunogenicity [36]. There have been measured to be  $10^{10}$  exosomes per cc of conditioned growth media [37]. Exosomes can be stored at room temperature for up to three years with no loss to their biological activity [38, 39]. In contrast to cell-based therapy, MSC derived exosomes provide an 'off-the-shelf' therapeutic product that has safety and may have clinical efficacy superior to any autogenous or allogeneic MSC treatment for orthopedics and spine [40].

### **The Acellular MSC Paracrine Treatment for Discogenic Pain**

The future acellular treatment for DDD will involve a two-front attack. First, highly concentrated anti-inflammatory MSC derived growth factors are injected into the degenerated disc. These growth factors will enter the nucleus of the recipient nucleus pulposus cell (NPC). The donor growth factors will then stimulate DNA transcription of mRNA containing instructions for the production of continuous anti-inflammatory secretomes, chemokines, and cytokines. These will be released from the recipient NPC into the nucleus. Second, the highly concentrated donor exosomes will enter recipient nucleus pulposus cells to deliver their mRNA. This delivered mRNA will directly undergo translation in the recipient NPC ribosomes to produce anti-inflammatory secretomes, cytokines, and chemokines. Cheng et al. have recently published the results of a brilliant series of experiments that describes how exosome RNA returns the degenerated disc to a physiologic state [41]. MSC-derived exosomes prevent NPCs from apoptosis and alleviate IVD degeneration, at least partly, via the miR-21 RNA contained in MSC exosomes. Exosomal miR-21 restrains phosphatase

and tensin homolog (PTEN) and thus activates the PI3K/Akt pathway in NPCs. The activated Akt pathway dramatically decreases NPC apoptosis. Their work explains a promising therapeutic strategy for treating IVD degeneration.

These salubrious effects could last months or years. This acellular biologic treatment can all be achieved with a single disc injection, not requiring the morbidity and cost of obtaining autogenous MSCs. The future of regenerative medicine in orthopedics and spine may well be the utilization of highly concentrated acellular MSC derived growth factors and especially exosomes [42-46].

### **Conclusion**

The etiology of discogenic pain is multifactorial. MSCs create intracellular exosomes that are filled with hundreds of various anti-inflammatory growth factors along with micro and messenger RNA. High concentrations of these paracrine secretomes can be collected from the growth media of expanded allogeneic MSCs, encapsulated and lyophilized to create a powder with a three-year shelf life. This powder can be reconstituted in normal saline and injected into any disc. Recent research has identified exosome miR-21 RNA inhibits NP cell apoptosis. Autogenous cellular treatments have no consistency between donors. Allogeneic cellular products introduce large amounts of foreign DNA and after cell death, unwanted cellular debris including membranes, mitochondria, Golgi bodies, cytoplasm, etc., all within the disc creating inflammation. Acellular products can be produced to have a consistently known quantity of GFs and exosomes along with the proteomic analysis of the GF proteins and genetic analysis of the RNA. This represents a bio-pharmacological quality product. Acellular MSC exosomes deliver the positive aspects of cellular therapy without all the negative aspects of either autogenous or allogeneic cellular therapy.

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