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# Percutaneous Injection of Autologous Bone Marrow Concentrate Cells Significantly Reduces Lumbar Discogenic Pain through 12 Months

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**Key Words.** Autologous cell therapy • mesenchymal stem cells • bone marrow concentrate • intervertebral disc injection

## ABSTRACT

**Degenerative disc disease (DDD) induces chronic back pain with limited non-surgical options. In this open label pilot study, twenty-six patients (median age 40 years; range 18-61) received autologous bone marrow concentrate (BMC) disc injections (13 one level, 13 two levels). Pre-treatment Oswestry Disability Index (ODI) and Visual Analogue Scale (VAS) were performed to establish baseline pain scores (average 56.5 and 79.3 respectively), while MRI were independently scored according to the modified Pfirrmann scale. Approximately 1 mL of BMC was analyzed for total nucleated cell (TNC) content, colony forming unit-fibroblast (CFU-F) frequency, differentiation potential, and phenotype characterization. The average ODI and VAS scores were reduced to 22.8 and 29.2 at 3 months, 24.4 and 26.3 at 6 months, and 25.0 and 33.2 at 12 months, respectively ($p \leq 0.0001$). Eight of 20 patients improved by one modified Pfirrmann grade at one year. The average BMC contained $121 \times 10^6$ TNC/mL with 2,713 CFU-F/mL (synonymous with mesenchymal stem cells). Although all subjects presented a substantial reduction in pain, patients receiving greater than 2,000 CFU-F/mL experienced a significantly faster and greater reduction in ODI and VAS. Subjects older than 40 years who received fewer than 2,000 CFU-F/mL experienced an average pain reduction of 33.7% (ODI) and 29.1% (VAS) at 12 months, while all other patients' average reduction was 69.5% (ODI, p=0.03) and 70.6% (VAS, p=0.01). This study provides evidence of safety and feasibility in the non-surgical treatment of DDD with autologous BMC and indicates an effect of mesenchymal cell concentration on discogenic pain reduction.** STEM CELLS *2014; 00:000–000*

## INTRODUCTION

Degenerative disc disease is a progressive deterioration of intervertebral discs causing a loss of disc height and pain. Back pain affects millions of Americans and results in billions of dollars in lost income and medical expenses annually. In fact, degenerative changes in lumbar discs are so ubiquitous that they are considered "a normal aging process," as documented in several magnetic resonance imaging (MRI) scan studies [1–3]. However, the exact cause of disc degeneration is complicated. Various animal studies have been contradictory in directly correlating biomechanical stress and disc degeneration [4–11]. Likewise, published clinical studies have failed to link disc degeneration directly to mechanical factors such as labor-intensive [12,13]. 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 [12–19].

Disc degeneration on a cellular level also is complicated. Nutrients must travel through the capillary network in the vertebral body, then diffuse through the endplate into the extracellular matrix of the disc to reach the

 

nucleus pulposus cells [20–23]. Calcification of the endplates impairs nutrient flow such as glucose and oxygen [24]. Endplate calcification also exacerbates the hypoxic acidic environment further impairing disc cell metabolism [25–27]. Stress, trauma, or natural degeneration in the disc tissue results in production of pro-inflammatory molecules such as TNF-□ and interleukins (IL-1, 4, and 12), as well as a build up of local acidity. The combined effects of nutrient deprivation and inflammatory environments result in a decrease in proteoglycan synthesis and a cascade of nucleus pulposus cell death [28,29].

Recently, a regenerative medicine approach to the repair of damaged or chronically inflamed tissues has been sought as an alternative to invasive surgery or pharmaceuticals. Bone marrow concentrated cells (BMC) represent a possible biological option for use in regenerative medicine [30,31]. BMC contains a variety of stem and progenitor cells, including mesenchymal stem cells (MSC). The anti-inflammatory effects of MSCs have been demonstrated in numerous animal models of injury including myocardial infarction, renal ischemia and reperfusion injury, hepatic failure, autoimmune encephalomyelitis, burn wounds, and osteoarthritis [32–38]. *In vitro* studies suggest the regenerative potential of mesenchymal stem cells may result from interactions between the MSCs and nucleus pulposus cells in treating degenerative discs. For example, Sobjima et al. reported that bone marrow-derived MSCs co-cultured with nucleus pulposus cells possessed a synergistic effect that yielded the greatest increase in proteoglycan synthesis and glycosaminoglycan (GAG) content compared with cultures of nucleus pulposus cells or MSCs alone [39]. MSCs can be harvested from the iliac wing bone marrow, and concentrated at point-of-care thereby avoiding manipulation of the cells and minimizing the risk of contamination with infectious microbes or xeno-derived proteins (as commonly found in culture-expansion and cryopreservation medium).

The purpose of this study was to evaluate the use of autologous bone marrow concentrated cells (BMC) to treat moderate to severe discogenic low back pain in an attempt to avoid or delay progression to lumbar fusion or artificial disc replacement. This is the first report on the potential efficacy of treating discogenic low back pain with autologous, non-expanded bone marrow concentrated cells at point-of-care. Results from the cell analysis of the patient samples, as well as data from MRI, Oswestry Disability Index (ODI) and VAS pain scores at 12-months post-treatment are reported.

## Materials and Methods

### Study Design and Clinical Protocol

This study is a prospective, open-label, non-randomized, two-arm study conducted at a single center with an IRB approved clinical protocol. Patients were enrolled with informed consent as subjects in the study who presented with symptomatic moderate to severe discogenic low back pain as defined according to the following criteria: centralized chronic low back pain that increased with activity and lasted at least 6 months; undergone non-operative management for 3 months without resolution; shown a change in normal disc morphology as defined by MRI evaluation; have a modified Pfirrmann score of 4-7; have a Modic Grade II change or less; disc height loss of <30% compared to an adjacent non-pathologic disc; pre-treatment baseline ODI score of at least 30 on the 100-point scale; and pre-treatment baseline low back pain of at least 40 mm on the 100 mm Visual Analogue Scale (VAS). An intact annulus was not required to be in the study. Standard exclusion criteria include: an abnormal neurologic exam; symptomatic compressive pathology due to stenosis or herniation; spondylolysis or any spondylolisthesis. Within the study design, patients with less than 25% improvement in ODI or VAS at or beyond the 6 month evaluation point were eligible for a second BMC injection. All patients were eligible to opt out of the study and undergo surgery at any time.

All patients underwent a pre-injection medical history and physical examination including ODI and VAS. These tests were repeated at three, six, and twelve months following the procedure. All patients had a normal neurologic examination of the lower extremities, demonstrated a loss of lumbar range of motion and had pain to deep palpation over the symptomatic disc(s) with associated muscle spasm. Study patient demographics are listed in Table 1. Thirteen patients underwent an intradiscal injection of autologous BMC at a single symptomatic lumbar disc and thirteen subjects had two adjacent symptomatic disc levels injected. Discography was performed in four patients in the one-level group and four patients in the two-level group to ascertain the symptomatic disc. All other patients were injected based on MRI scanning. MRI scans were repeated at twelve months and assigned a modified Pfirrmann score (Grade 1 to 8; 1 = hydrated, healthy disc, 8 = dark, dehydrated disc) by a blinded independent reviewer.

### Bone Marrow Collection and Processing

Bone marrow aspirate (BMA, 55 mL) was collected over acid citrate dextrose-anticoagulant (ACD-A, 5 mL) from the patient's posterior iliac crest. The procedure was performed with IV sedation consisting of midazolam (Versed) and fentanyl. Positioning of the Jamshidi needle in the iliac wing was confirmed by fluoroscopy. BMA was collected in a 60 mL syringe in a series of discrete pulls on the plunger (targeting a collection of 5-10 mL per pull), with repositioning of the needle tip between pulls based on the reported enrichment of progenitor cells by Hernigou et al. [40]. The BMA was processed using the ART BMC system (Celling Biosciences, Austin, TX) to produce a bone marrow concentrated cell preparation via centrifugation for 12 minutes. Typically, a BMC volume of 7 mL (6 mL for injection and 1 mL for cell analysis) was drawn from the processed device and immediately transferred to the physician for injection.

 

### Intradiscal Injection

With the patient in a prone position, the injection site(s) was treated with local anesthetic (1% buffered lidocaine). BMC was percutaneously injected into the symptomatic disc(s) through a standard posterior lateral discogram approach with a two-needle technique. The injection point of the 22 gauge needle was verified with fluoroscopy with needle placement occurring simultaneously with BMC processing. Approximately 2-3 mL of BMC was used per symptomatic lumbar disc injection. Patients were prescribed pain medicine to be used as needed for three days and put on restricted physical activity for two weeks.

### Analysis of the Bone Marrow Concentrate

Cell analysis and characterization of 20 out of the 26 patients' BMC samples were performed. An aliquot (1 mL) of each subject's BMC was packed in a shipping container with 5°C cold packs and shipped overnight to the cell analysis laboratory (Celling Biosciences, Austin, TX). The samples were received and processed immediately to determine total nucleated cell count and viability using a NucleoCounter NC-100 (Chemometec, Denmark). The BMC was diluted in phosphate buffered saline (PBS, Invitrogen, Grand Island, NY) with 2% fetal bovine serum (FBS, HyClone human mesenchymal grade, Thermo Scientific, Waltham, MA) and subjected to a Ficoll-Paque (GE Healthcare Life Sciences, Piscataway, NJ) gradient separation (1:1 cell solution to Ficoll ratio by volume) in order to deplete red blood cells. Analysis of the recovered cells included performing colony forming unit-fibroblast and osteogenic (CFU-F and CFU-O, respectively) assays and phenotypic analysis by flow cytometry. For phenotype analysis, fresh (non-cultured) BMC cells were stained with a series of rabbit anti-human monoclonal antibodies for a hematopoietic lineage-committed (non-progenitor) panel of markers including CD2, 3, 8, and 11b (APC-Cy7), CD34 (PE), CD90 (FITC), and CD105 (APC), as well as appropriate isotype controls. Isotype, single color stain, and four-color stain samples were analyzed by a Guava EasyCyte 8HT (Millipore, Billerica, MA). The CFU-F assay was performed by creating a dilution series (in culture medium with 5% FBS and 1% antibiotics) of each cell preparation at concentrations of 50,000 to 500,000 total nucleated cells (TNC) per well in standard 12-well plates. The plates were placed in an incubator at 37°C, 5% $CO_2$ and 100% humidity for 72 hours when the medium was replaced. Medium was replaced every 3 days. After 9 days in culture, wells were gently washed with PBS, fixing the colonies/cells with methanol, staining the attached cells with Crystal Violet, rinsing with water and air-drying the plates. Visualization and counting of the colonies was done with an inverted microscope. Colonies containing 20 or more cells were scored as a CFU-F. The CFU-O assay was performed identically as CFU-F, but after 9 days the medium was changed to an osteogenic induction medium (AdvanceSTEM Osteogenic Differentiation Kit, HyClone, Logan, UT) for an additional 9 days with complete medium change every 3 days. On day 18, the wells were washed with PBS, then fixed for 15 minutes in 2% formalin solution and co-stained for alkaline phosphatase activity (Vector Blue ALP, Vector Labs, Burlingame, CA) and calcified extracellular matrix (0.5% Alizarin Red solution, Sigma-Aldrich, St. Louis, MO).

### Statistical Analysis

Univariable data comparisons (pain scores by time, patient age, number of levels injected, or CFU-F concentration; CFU-F frequency by patient age or CFU-O) were analyzed by two-tailed Student's t-test with a 95% confidence interval (□=0.05). Multivariable data were evaluated by analysis of variance (ANOVA) using JMP 9 statistical analysis software (SAS Institute, Cary, NC).

## RESULTS

### Bone Marrow Concentrate Cell Analysis

Fresh BMC aliquots were analyzed within 24 hours of the procedure. The average TNC concentration, cell viability, CFU-F frequency, CFU-O frequency, and CD marker phenotypic analyses are reported in Table 2. TNC and CFU-F per mL of BMC injectate yields were consistent with published manufacturer's data. The average CFU-O frequency and concentration were slightly higher, but within statistical error compared to CFU-F. All BMC samples yielded robust CFU-F formation after 9 days in culture with a virtually identical yield and frequency of CFU-O (Supporting Information Figure 1). Alkaline phosphatase activity is displayed in blue, while mineralization resulted in red coloration of colonies. The statistical correlation between CFU-F and CFU-O demonstrates that 18 of the 20 CFU-O samples analyzed fall within the 95% confidence interval of CFU-F (Supporting Information Figure 2). This indicates that not only do the samples possess a classical characteristic of MSCs (CFU-F in primary *in vitro* culture), but they also have the capacity to differentiate at nearly a 1-to-1 correlation with CFU-F.

A substantial fraction of lineage$^-$ (cells not committed or differentiated toward a hematopoietic lineage) cells were positive for CD90, CD105 and CD34, which are common markers for mesenchymal and hematopoietic stem cells. CD34 expression was observed as three distinct populations: CD34$^{Bright}$, CD34$^{Dim}$, and CD34$^-$. As MSCs have been reported universally to express both CD90 and CD105, the percentages of cells from each of the three CD34 subpopulations that were also Lineage$^-$/CD90$^+$/CD105$^+$ were compared to the CFU-F frequency for each individual BMC sample in an attempt to define a phenotypic population of interest. As listed in Table 3, the average CFU-F frequency was 0.0025%, or 25 per million TNC. The Lineage$^-$/CD34$^{Bright}$/CD90$^+$/CD105$^+$ population represented only 0.0007% of nucleated cells, while the Lineage$^-$/CD34$^{Dim}$/CD90$^+$/CD105$^+$ (0.0040%) and Lineage$^-$/CD34/CD90$^+$/CD105$^+$ (0.0049%) populations exceeded the CFU-F frequency and could

 

encompass the MSC population. A linear regression was performed on CFU-F frequency versus Lineage$^-$/CD90$^+$/CD105$^+$ phenotypes by CD34 expression (Supporting Information Figure 2B). Although none of the populations fit to the CFU-F unity line within statistical error ($R^2$ > 0.9), the linear fit of Lineage$^-$/CD34$^{Dim}$/CD90$^+$/CD105$^+$ and Lineage$^-$/CD34$^-$/CD90$^+$/CD105$^+$ most closely correlated to CFU-F.

## Post-injection Pain Relief and Decreased Impairment

There were no reported adverse events at the aspiration site (iliac crest) or injection site (disc) for any patient in the study. At 12 months, MRI provided no evidence of new or increased herniation related to the injection with the 22 gauge needle, nor signs of osteophyte or other heterotopic tissue formation. Patients' pain scores were determined by ODI and VAS pain indices prior to treatment and at three, six, and twelve month follow up visits. Data was collected on all enrolled patients. Generally, patients reported moderate discomfort for 24-48 hours after injections followed by relief of pain below baseline values. The average pre- and post-treatment pain scores are reported in Table 3 as an overall series and by population subsets (one-level versus two-level, older or younger than the median age (40 years), and greater or less than 2,000 CFU-F/mL). The "by patient" pain scores and MRI scoring are reported in Table 4. The average percentage of ODI reduction was 58.1%, 55.5%, and 56.8% after three, six, and twelve months, respectively. Similarly, the average percentage of VAS reduction was 64.6%, 64.2%, and 58.0% after three, six, and twelve months, respectively. Only five patients, three of whom received two-level injections and two who received one-level injections, did not improve by at least 25% in ODI and VAS by three months. Two patients elected to undergo a second injection of BMC at six months and are statistically improved at 12 months. One of these patients (age 19) underwent a second injection at 7 months, with score improvements from 20 to 2 (ODI) and 59 to 0 (VAS) between 6 and 12 months. The other patient (age 38) received a second BMC injection at 8 months and demonstrated improvements from 54 to 4 (ODI) and 40 to 10 (VAS) between 6 and 12 months. Two patients elected to undergo surgery (one single-level anterior lumbar interbody fusion, one two-level lumbar posterior fusion) within six months after the BMC injection.

Subjects were divided into subpopulations of interest based on levels (number of discs) injected, age, gender, and CFU-F concentration to determine statistically significant impacts on pain scores. There was no statistical effect of gender on pain score reduction for any subdivided demographic. Although there was statistically significant reduction in ODI and VAS scores at all post-treatment time points for all demographics (p-values ranging from 0 to 0.01), there were not significant differences in pain scores or percentage of improvement over baseline based on patient age or number of levels injected. The effect of CFU-F (or MSC) concentration on pain relief was statistically significant at three and six months post-therapy ($p<0.005$ for ODI at three and six months and VAS at three months, $p<0.01$ for VAS at six months).

## Effect of Patient Age on Pain Relief and CFU-F Frequency and Concentration

As described in the previous section, there were no statistically significant differences in raw or percentage change of ODI or VAS scores based on age. However, separating cohorts based on both age (≤ or > median age of 40 years) and CFU-F concentrations greater or less than 2,000 per mL of BMC revealed interesting differences in pain relief. Significant overall reduction of ODI and VAS scores (Fig. 1A and 1B) was observed in each cohort. As shown in Table 3, all patients with CFU-F concentrations greater than 2,000 per mL in their BMC preparation, regardless of age, demonstrated a statistically significant improvement in pain scores over those below that MSC concentration at three and six months. Interestingly, those differences are greater when the < 2,000 CFU-F/mL group is fractionated by age. In younger patients (≤ 40 years), there were no significant differences in pain scores at any time point based on CFU-F concentration. For patients > 40 years, however, the differences in average ODI and VAS scores between the < or > 2,000 CFU-F/mL cohorts were 24.6 (ODI, p=0.01) and 33.4 (VAS, p=0.014) at three months, 30.6 (ODI, p=0.006) and 37.0 (VAS, p=0.02) at six months, and 25.2 (ODI, p=0.025) and 28.8 (VAS, p=0.03) at twelve months. Among all patients with < 2,000 CFU-F/mL in their BMC, there was a statistical difference based on age at twelve months (ODI p=0.02, VAS p=0.03). No such difference exists for patients with > 2,000 CFU-F/mL. There was no strong correlation between patient age and CFU-F frequency or concentration (Fig. 3C and 3D). There was no correlation between age and CFU-F %, but there was evidence of a generally decreasing trend with increased age. There was no significant correlation between CFU-F concentration and patient age due to the variation of CFU-F frequency by patient as well as variation in the TNC concentration.

## Effect of CFU-F Concentration on Pain Scores at 3, 6, and 12 months

The statistically significant effects of CFU-F concentration based upon the 2,000 CFU-F/mL threshold were reported in Table 3 and Figure 1. The seemingly arbitrary 2,000 CFU-F/mL value originated from analysis of percent-wise improvements in ODI and VAS scores versus CFU-F concentration by patient (Fig. 2). Regardless of age, gender, or number of levels injected, all patients who received > 2,000 CFU-F/mL reported > 40% reduction in ODI and VAS scores at three and six months (Table 4). Most of these patients sustained > 40% pain reduction at twelve months (10/11 ODI, 9/11 VAS). It should be noted that both patients who dropped below

 

40% pain improvement received a two-level injection. Among patients whose BMC contained < 2,000 CFU-F/mL, there was a variation in pain reduction at all time points. There was a mildly significant effect in this population based on age ($p \leq 0.03$), but not for number of levels injected.

### Rehydration of Degenerated Intervertebral Discs

Physiological changes to injected discs were observed by MRI and scored by a blinded independent reviewer evaluation of images prior to treatment and at twelve months after treatment according to the modified Pfirrmann scale. Figure 3 illustrates representative MRI images of L4-L5 and L5-S1 discs prior to and 12 months after BMC injection. Twelve month MRI data show an improvement of at least one Pfirrmann grade in 5 of 10 one-level patients and 3 of 10 two-level patients (Fig. 3B). Six of the 26 patients did not undergo a 12-month MRI (two went on to surgery, four did not schedule follow-up MRI). Of the 20 patients whose cells were analyzed, there was an overall average improvement in modified Pfirrmann score of 0.27 per disc and 8 subjects improved one grade from baseline. Based on the cohorts identified by pain scores (CFU-F > or < 2,000/mL and patient age > or ≤ 40 years), similar trends were observed. Patients with BMC containing greater than 2,000 CFU-F/mL, regardless of age, demonstrated an average improvement of 0.58 in modified Pfirrmann and 5 of 10 patients improved by a grade. Younger patients (≤ 40 years) with below 2,000 CFU-F/mL also showed improvement, albeit of 0.17 grades per disc in modified Pfirrmann. Patients older than 40 years with fewer than 2,000 CFU-F/mL demonstrated an overall regression on average of 0.17 per disc, although the changes in MRI scores were not statistically significant for any cohort.

## DISCUSSION

The clinical severity of the 26 patients enrolled in this study should be emphasized (average ODI was 56.5 and VAS was 79.3). All patients enrolled in the study experienced moderate to severe discogenic pain and were surgical candidates for spinal fusion or artificial disc replacement. Five published clinical studies comparing fusion with artificial disc replacement reported similar pain scores for enrolled patients [41–45]. The patients' pretreatment modified Pfirrmann MRI scores were 4 or greater, indicative of moderate to severe disc dehydration and degeneration. The typical patient in this study reported significant relief of their low back symptoms within 14 days following injection of the BMC into the nucleus pulposus of the symptomatic disc(s). The immediate relief may be secondary to a potential placebo effect and primarily due to the reported anti-inflammatory properties of the MSCs [30]. Eight patients received discograms prior to treatment. Among those patients, there were no statistical differences in CFU-F concentration, MRI improvement, or reduction in ODI or VAS compared to patients who did not undergo discography or the entire patient population. As a part of the clinical study design, if a patient's ODI or VAS was not reduced by 25% at the six-month evaluation, the patient was eligible for re-injection of the disc(s) at their and the physician's discretion. Five of the 26 patients met re-injection criteria at six months: two underwent re-injection (one at 7 months, one at 8 months) and were significantly improved clinically at one year (average 94% reduction from initial ODI and VAS scores); two patients underwent surgery after six months. The ODI and VAS improvement between 6 and 12 months for the two patients who received a second BMC injection were among the top three for all patients in the study. One can speculate that the first injection may have partially remodeled the disc tissue and microenvironment (i.e. reducing inflammation), making the effect of the second injection more substantial. This result also might indicate that 6 months is the duration in which most pain reduction is achieved and merit a second injection in patients with low or no improvement.

The ODI and VAS data obtained at all post-injection time points showed statistically significant sustained pain relief. These data indicated no statistically significant difference in the clinical benefit of the bone marrow concentrate injection whether the patient had a traumatic versus unknown etiology to their discogenic low back pain. Patient age had little effect on pain scores or CFU-F frequency. Although the youngest (18 years) and oldest patient (61 years) had the greatest and least reduction in ODI and VAS, respectively, outcomes were statistically variable. However, mesenchymal cell concentration had a positive affect on short term (3 month) and sustained (6 and 12 month) pain relief. Comparing patient populations with less than 2,000 CFU-F/mL (n=9) and greater than 2,000 CFU-F/mL (n=11), patients with greater progenitor cell concentrations demonstrated statistically significantly greater improvements in pain scores between treatment and three months and between three and six months. This result of a critical CFU-F concentration (2,000 per mL) may be analogous to the clinical findings of Hernigou et al. that tibial non-union fractures and supraspinatus tendon (rotator cuff) tear repairs require greater than 1,500 CFU-F/mL of BMC injectate to heal [46,47]. No correlation could be established between CFU-F concentration and patient age due to the inherent variability of CFU-F frequency and TNC concentration between patients based on health and bone marrow aspiration technique.

Cell-based therapies to treat lumbar disc degeneration offer an attractive solution to current conservative and especially surgical interventions [48]. Lumbar fusion is an accepted surgical technique in patients with demonstrated lumbar instability such as degenerative spondylolisthesis, infectious conditions of the spine, progressive spinal deformities, and traumatic injuries

 

[49–54]. Lumbar fusion for discogenic back pain remains controversial. Class I data published from the ProDisc-L study indicates a clinical success of 45.1% at two-year follow-up for fusion in these patients. The clinical success rates following lumbar fusion are generally reported to be 50-70% [51,55–59]. An additional morbidity associated with lumbar fusion is the development of adjacent level degeneration. The reported incidence of accelerated adjacent level degeneration ranges from 2-15% per year with 3.9% of patients per year undergoing an additional surgery [60]. Lumbar fusion surgery also is expensive and associated with long recovery time and permanent impairments. Reported ODI and VAS after lumbar spinal fusion one year after surgery vary considerably. Rodgers et al. reported patients undergoing lateral lumbar interbody fusion with □-tricalcium phosphate had initial ODI and VAS of 52 and 81, respectively, with improvements to 38 (ODI) and 50 (VAS) by 3 months, which was sustained through 12 months [61]. A meta-analysis of lumbar fusions using recombinant human bone morphogenetic protein 2 (rhBMP-2) indicated no statistical improvement in pain scores over conservative care or iliac crest bone grafts, and an associated increase in the incidence of cancer [62]. Arts et al. reported unsatisfactory outcomes in terms of pain relief in 65% of subjects undergoing spinal fusion with BMP-2, in addition to other complications reported in the literature including retrograde ejaculation and retroperitoneal ossification [63–66]. Conversely, there were zero reported adverse events at the injection site, aspiration site, or systemically in all 26 subjects in this study. There was no observed formation of osteophytes in or around any of the injected discs via MRI after 12 months [66]. Unlike the rabbit disc injection study performed by Vadala et al., which found evidence of osteophyte formation in some animals, the present study did not utilize allogeneic cells, culture-expanded cells, nor genetically modified cells. The rabbit study also employed an artificial model for DDD (multiple 16 gauge needle stabs) that may have contributed to cell leakage and/or osteophyte formation. In terms of cancer risk, Hernigou et al. reported in 2013 that there is no increased incidence of cancers in a study of 1,873 patients receiving autologous BMC injections up to 22 years post-treatment compared to the general population [67].

In addition to safety, an important advantage of autologous BMC therapy seems to be a complex combination of immunosuppressive and anti-inflammatory effects with a capacity to coordinate tissue repair [68]. The cell concentration device was very consistent in capturing the TNC and mononuclear (MNC) fraction from whole bone marrow aspirate. The average CFU-F frequency of 25 per $10^6$ TNC was comparable to previously reported values [69–71]. The MSC population is reported to be present within the $CD90^+/CD105^+/lineage^-$ subpopulation ($12 \times 10^3$ cells per mL of injectate), while HSCs are present in the $CD34^+/lineage^-$ fraction ($1.55 \times 10^6$ cells per mL) [72–74]. Few studies have reported the results of utilizing cell-based biologics in the treatment of chronic discogenic low back pain. Orozco et al. injected culture expanded autologous bone marrow MSCs into the nucleus pulposus of 10 patients with chronic discogenic low back pain [75]. A single level was injected in eight patients and two levels in two patients. For 9 of the 10 patients, there was a slight statistically significant improvement in this group in terms of VAS and ODI at three months, six months and one year. The average number of MSCs available for injection was $23 \pm 5 \times 10^6$ cells, with an average viability of the cells at the time of injection of 83% ± 5%. For comparison, the patients in the current study received an average of only $8.3 \times 10^3$ CFU-Fs (technically analogous to MSCs) in the injectate with an average viability greater than 98%. Another study by Coric and Pettine injected $10^7$ allogeneic juvenile chondrocytes in a non-randomized FDA Phase I study [76]. Three of the 15 enrolled patients went on to surgery after the injection. Both of these studies (Orozco et al., Coric et al.) utilized cell therapies regulated by the FDA as a drug.

This study differs from Orozco et al. and Coric and Pettine by utilizing autologous bone marrow concentrated cells in a single treatment, point-of-care, 30 minute procedure. The use of autologous, non-cultured cells reduces the risks of infection, disease transmission, sample mismatch, and cost compared to culture-expanded autologous or allogeneic cells. The metabolism and effect of culture-expanded cells may vary drastically *in vivo* based on the *in vitro* culture conditions (e.g. media reagents, oxygen concentration, pH, etc.). The present results show statistically significant improvement in Oswestry and VAS to a p-value < 0.0001. These results were true whether one disc or two discs were injected and whether the etiology of the discogenic low back pain was trauma or unknown. Only two of the 26 patients in this study have undergone a surgical procedure after injection of autologous BMC. This study, while highly supportive of using a patient's autologous bone marrow concentrate in an intradiscal injection to treat lumbar discogenic pain, has several limitations. The study included only 26 patients who split between one-level and two-level disc treatments. Furthermore, there was a wide range of ages in the patient population. The expected variability of TNC and CFU-F concentrations was confirmed in this study as well, but this inherent variability might have an influence on the interpretation of the reported results. Although there was no placebo group in this pilot study, the placebo effect is unlikely or limited given the statistical correlation between MSC concentration in BMC and patient outcomes (ODI/VAS and MRI improvement), that both the patients and physician were blinded to each patient's cell analysis, and the duration of pain relief (beyond 12 months). A placebo effect is not usually associated with long-term improvements in pain scores or to objective clinical outcome measurements (e.g. MRI) [77,78]. Spontaneous recovery from DDD has not been reported using non-surgical interventions

 

(physical therapy, epidural steroids, NSAIDs, and opioids) [51,79].

## Conclusion

Increased understanding of disc biology and pathophysiology, combined with better knowledge surrounding cell-based therapy has motivated researchers to pursue human studies on the use of autologous BMC to treat chronic discogenic low back pain. Statistically significant improvement in pain scores and impairment was demonstrated in 21 of 26 patients, with the most dramatic improvement in patients with higher CFU-F concentrations. Rehydration of the discs in eight of 20 patients according to MRI in conjunction with sustained pain relief through 12 months represent promise for the use of this regenerative medicine approach. The data suggests that there might be a critical dose concentration of fresh, non-cultured MSCs (2,000/mL), which is significantly less than dosages explored in similar studies using allogeneic or culture-expanded MSCs. These results are promising and encourage larger clinical trials with an expanded patient population and the co-administration of biomaterials used with cells to improve healing of degenerated or herniated discs.

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## Disclosure of Potential Conflicts of Interest

K.A.P. reports no conflicts of interest concerning this paper. M.B.M, R.K.S., and T.T.S. are employees of Celling Biosciences, who provided the bone marrow concentration devices used in the course of this study.

## Author Contributions

K.P.: Conception and design, provision of study patients, collection of data, data analysis and interpretation, manuscript writing, final approval of manuscript; M.M.: Conception and design, collection and assembly of data, data analysis and interpretation, manuscript writing; R.S.: Collection of data; T.S.: Conception and design, provision of study materials, data analysis and interpretation

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**Figure 1.** Average ODI (A) and VAS (B) scores versus time segregated by patient age and CFU-F concentration in BMC injectate. Age division was greater or less than the study median age of 40 years. Blue lines denote average of patients ≤ 40 years and gray lines represent patients older than 40. Solid lines refer to populations with cell concentrations < 2,000 CFU-F/mL while dashed lines indicate > 2,000 CFU-F/mL in the BMC injectate. Individual CFU-F frequencies (C) and concentrations (D) were variable with no strong correlation to age.

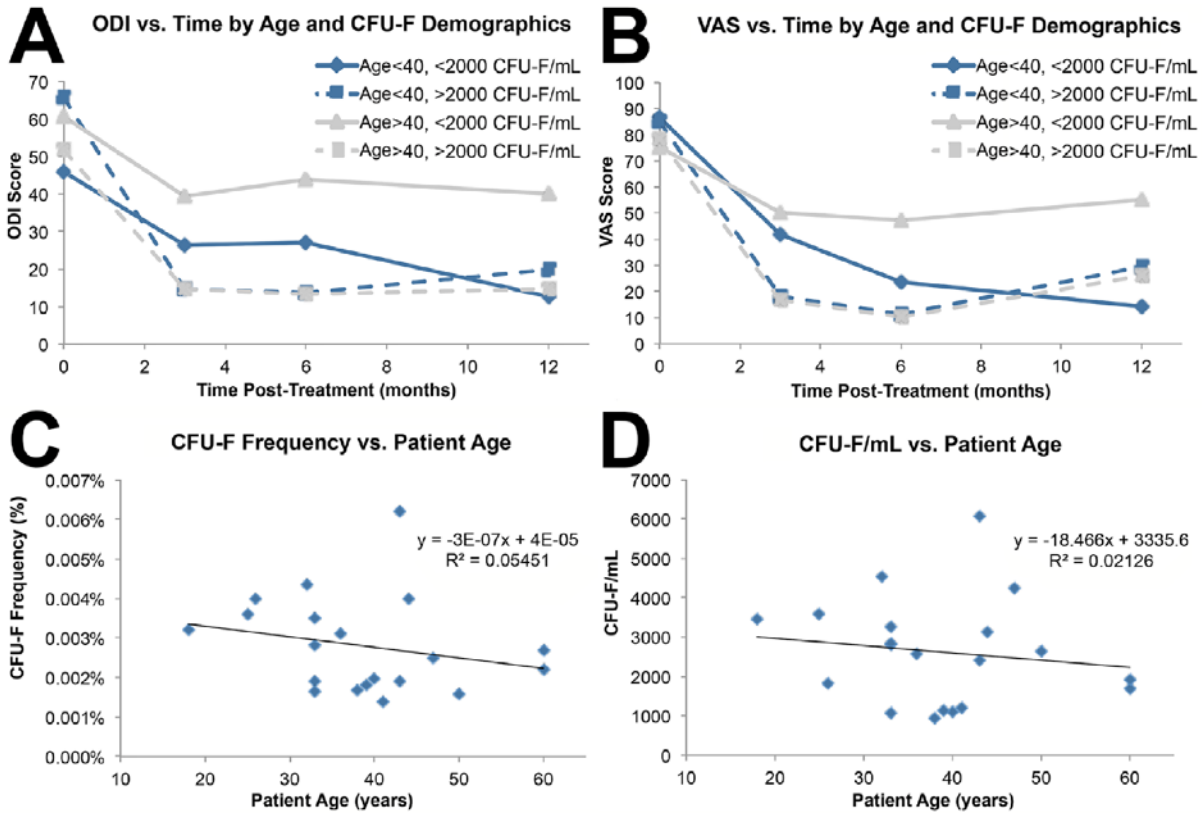


**Figure 2.** Percentage pain score improvement from baseline (pre-treatment) of raw ODI (A) and VAS (B) scores versus CFU-F concentration in BMC by individual patient, comparing one-level and two-level injection subjects. Natural segregations occurred at 2,000 CFU-F/mL and 40% reduction in pain.

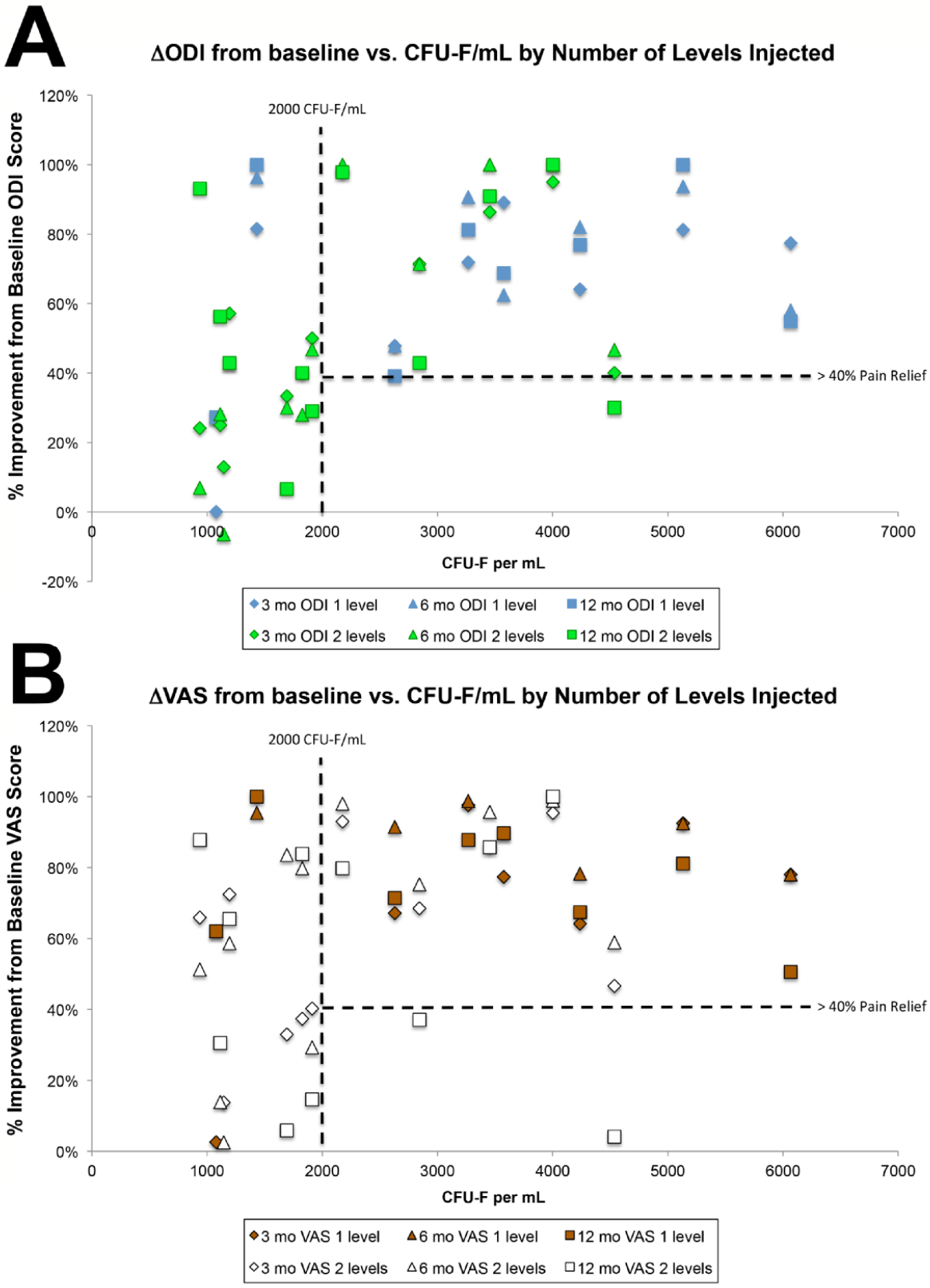
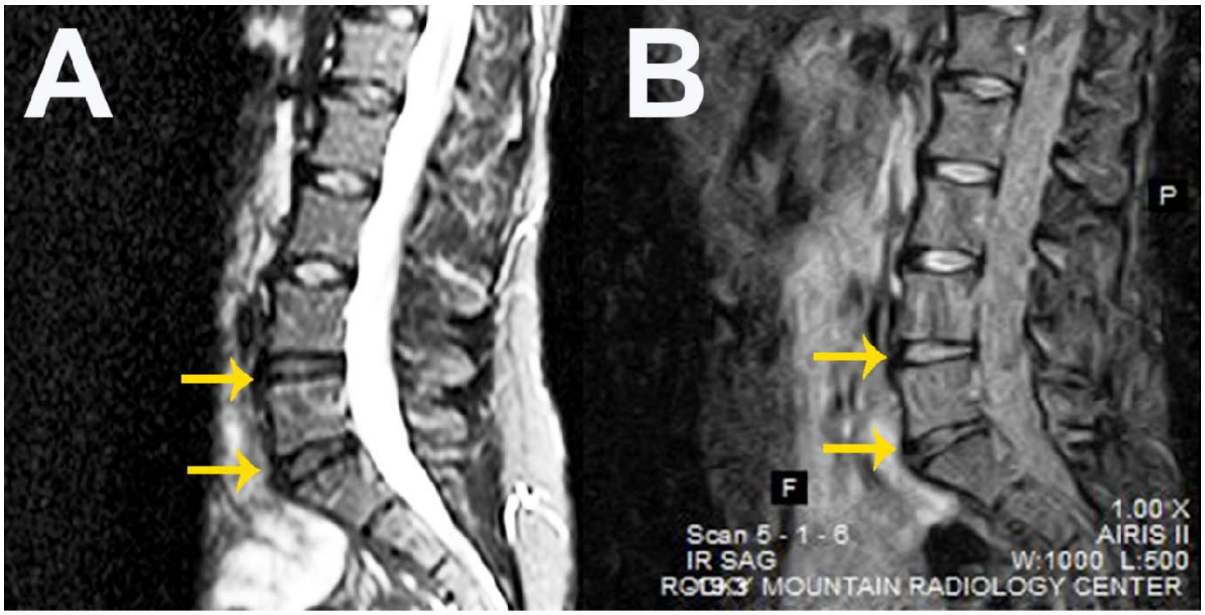


**Figure 3.** Magnetic resonance image (MRI) before pre-treatment (A) and 12 months after injection (B) of autologous BMC into L4-L5 and L5-S1 intervertebral discs. L4-L5 improved from grade 5 to grade 4 while L5-S1 remained at grade 6. (C) Pre-treatment and 12 month modified Pfirrmann MRI scores for one-level and two-level intervertebral disc injections by blinded independent reviewer and number of patients who showed improvement of one grade among the 20 patients completed 12 month MRI (* Two patients elected for surgical intervention and four non-surgical patients did not receive 12 month follow-up MRI).




C

| | One Level Injected | | Two Levels Injected | |
|---|---|---|---|---|
| Number of Discs at Corresponding Modified Pfirrmann Grade | Pre-treatment | 12 months* | Pre-treatment | 12 months* |
| *Grade 4* | 0 | 2 | 0 | 2 |
| *Grade 5* | 3 | 4 | 4 | 2 |
| *Grade 6* | 4 | 1 | 8 | 9 |
| *Grade 7* | 3 | 3 | 8 | 7 |

| | Number of Patients with 1 Grade Improvement in Modified Pfirrmann Score |
|---|---|
| All Patients | 8 of 20 |
| All Patients with > 2,000 CFU-F/mL | 5 of 10 |
| Patients ≤ 40 years and < 2,000 CFU-F/mL | 1 of 3 |
| Patients > 40 years and < 2,000 CFU-F/mL | 0 of 3 |
| Patients receiving one level injection | 5 of 10 |
| Patients receiving two level injection | 3 of 10 |

**Table 1.** Demographics of Study Patients by number of discs (levels) injected, age, gender, BMI, and cause of injury.

| | | **One-Level Injection** | **Two-Level Injection** |
|---|---|---|---|
| **Number of Patients** | | 13 | 13 |
| **Average (Median) Age** | | 38.0 (40)*range 25-51* | 37.4 (37) *range 18-61* |
| **Males** | | 5 | 6 |
| **Females** | | 8 | 7 |
| **Average BMI** | | 27.1 *range 19-37* | 26.1 *range 20-34* |
| **Cause of Injury** | ***Trauma*** | 7 | 5 |
| | ***Unknown*** | 6 | 8 |

**Table 2**. Average Cell Viability, Total Nucleated Cells (TNC), Total and Frequency of CFU-F/CFU-O and CD Marker Phenotypes in fresh Bone Marrow Concentrate.

| **Cell Viability at 24 hours** | 98.1 (± 1.2) % | **TNC/mL in BMC** | 121 (± 11) x $10^6$ |
|---|---|---|---|
| **Cell Phenotype Subpopulation** | **% of TNC** | **Subpopulation Concentration in BMC (cells/mL)** | |
| **CFU-F** | 0.0025% | 2,713 (± 491) per mL | |
| **CFU-O** | 0.0027% | 2,913 (± 418) per mL | |
| **Lineage$^-$ Cells (CD 2$^-$/3$^-$/8$^-$/11b$^-$)** | 25.89% | 31.5 x $10^6$ per mL | |
| **Lineage$^-$/CD34$^+$** | 1.397% | 1.69 x $10^6$ per mL | |
| **Lineage$^-$/CD34$^{High}$ /CD90+/CD105+** | 0.0007% | 802 per mL | |
| **Lineage$^-$/CD34$^{Low}$ /CD90+/CD105+** | 0.0040% | 4,832 per mL | |
| **Lineage$^-$/CD34$^-$/CD90+/CD105+** | 0.0049% | 5,914 per mL | |

**Table 3.** Average Pre- and Post-treatment Pain (Oswestry Disability Index, ODI) and QOL (Visual Analogue Scale, VAS, 0-100) Scores. Statistically significant differences from Pre-treatment score: $p \leq 0.0001$ ($^{*}$), $p < 0.005$ ($^{**}$), $p < 0.01$ ($^{***}$). Statistically significant differences between < and > 2000 CFU-F/mL populations: $p < 0.005$ ($^{\#}$), $p < 0.01$ ($^{\#\#}$).

| Patient Population | Assessment | Pre-treatment | 3 month | 6 month | 12 month |
|---|---|---|---|---|---|
| *All Subjects (n=26)* | ODI | 56.5 | 22.8$^{*}$ | 24.4$^{*}$ | 25.0$^{*}$ |
| | VAS | 79.3 | 29.2$^{*}$ | 26.3$^{*}$ | 33.2$^{*}$ |
| *One-Level Injection (n=13)* | ODI | 56.5 | 18.4$^{*}$ | 19.8$^{*}$ | 26.2$^{**}$ |
| | VAS | 78.5 | 23.8$^{*}$ | 20.2$^{*}$ | 31.4$^{*}$ |
| *Two-Level Injections (n=13)* | ODI | 55.5 | 27.4$^{**}$ | 29.3$^{**}$ | 22.7$^{*}$ |
| | VAS | 79.4 | 34.8$^{*}$ | 32.7$^{*}$ | 33.0$^{*}$ |
| *Age ≤ 40 (n=14)* | ODI | 57.1 | 18.2$^{*}$ | 20.6$^{*}$ | 25.1$^{**}$ |
| | VAS | 83.4 | 24.6$^{*}$ | 23.5$^{*}$ | 32.3$^{*}$ |
| *Age > 40 (n=12)* | ODI | 55.8 | 27.8$^{**}$ | 28.5$^{**}$ | 24.8$^{**}$ |
| | VAS | 74.8 | 34.2$^{**}$ | 29.2$^{**}$ | 34.5 |
| *CFU-F per mL < 2000 (n=9)* | ODI | 54.2 | 33.7$^{***}$ | 36.3 | 26.3$^{**}$ |
| | VAS | 80.4 | 46.4$^{**}$ | 36.7$^{**}$ | 34.5$^{**}$ |
| *CFU-F per mL > 2000 (n=11)* | ODI | 59.3 | 14.8$^{*,\#}$ | 13.5$^{*,\#}$ | 17.6$^{*}$ |
| | VAS | 82.0 | 17.5$^{*,\#}$ | 10.8$^{*,\#\#}$ | 25.5$^{*}$ |

**Table 4.** Patient-by-Patient information, CFU-F concentration in BMC, number of grades improvement in modified Pfirrmann Scale (mPS) MRI scores according to blinded review, and % reduction in pain by ODI and VAS at 3, 6, and 12 months post-injection. (ND: not determined, no f/u: no follow-up MRI, * Opted for second BMC injections after 6 months, ** Elected for spinal fusion surgery, # Patient received discogram prior to BMC injection).

| Patient ID | Patient [illegible] | CFU-F/mL | # of discs [illegible] | Level | Initial mPS Grade | 12 mo. mPS | # Grades Improve | 3 months | 6 months | 12 months | 3 months | 6 months | 12 months |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | % Reduction ODI | | | % Reduction VAS | | |
| **<40 years, <2,000 CFU-F/mL** | | | | | | | | | | | | | |
| 104 | 33 | 1,433 | 1 | L4-L5 | 6 | 5 | 1 | 81 % | 96% | 100 % | 100 % | 95% | 100 % |
| 107 | 33 | 1,080 | 1 | L4-L5 | 4 | No f/u | | 0% | 27% | 27% | 3% | 62% | 62% |
| 202 | 26 | 1,825 | 2 | L4-L5 | 4 | No f/u | | 40 % | 28% | 40% | 37% | 80% | 84% |
| | | | | L5-S1 | 6 | | | | | | | | |
| 214** # | 36 | 1,114 | 2 | L4-L5 | 6 | Surgery | | ND | 44% | 22% | ND | 44% | 18% |
| | | | | L5-S1 | 6 | | | | | | | | |
| 211*# | 38 | 938 | 2 | L4-L5 | 7 | 7 | 0 | 24 % | 7% | 93% | 66% | 51% | 88% |
| | | | | L5-S1 | 7 | 7 | | | | | | | |
| 212 # | 39 | 1,144 | 2 | L4-L5 | 6 | 6 | 0 | 13 % | -6% | ND | 14% | 3% | ND |
| | | | | L5-S1 | 7 | 7 | | | | | | | |
| **<40 years, >2,000 CFU-F/mL** | | | | | | | | | | | | | |
| 105 # | 33 | 3,268 | 1 | L4-L5 | 5 | 4 | 1 | 72 % | 91% | 81% | 98% | 99% | 88% |
| 110 # | 25 | 3,575 | 1 | L5-S1 | 5 | 5 | 0 | 89 % | 63% | 69% | 77% | 90% | 90% |
| 201 | 33 | 2,845 | 2 | L4-L5 | 5 | No f/u | | 71 % | 71% | 43% | 69% | 75% | 37% |
| | | | | L5-S1 | 5 | | | | | | | | |
| 205 | 32 | 4,536 | 2 | L4-L5 | 5 | 4 | 1 | 40 % | 47% | 30% | 47% | 59% | 4% |
| | | | | L5-S1 | 6 | 6 | | | | | | | |
| 206 | 18 | 3,455 | 2 | L4-L5 | 6 | 6 | 1 | 86 % | 100 % | 91% | 86% | 96% | 86% |
| | | | | L5-S1 | 7 | 6 | | | | | | | |
| 209 | 36 | 2,175 | 2 | L4-L5 | 5 | 5 | 0 | 98 % | 100 % | 98% | 93% | 98% | 80% |

| | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | L5-S1 | 6 | 6 | | | | | | | |
| **<40 years, No cell analysis** | | | | | | | | | | | | | |
| 101 # | 34 | | 1 | L4-L5 | 4 | No f/u | | 66% | 17% | 0% | 77% | 18% | 0% |
| 109 | 29 | | 1 | L5-S1 | 7 | 7 | 0 | 92% | 100% | -5% | 89% | 100% | -7% |
| 203* | 19 | | 2 | L4-L5 | 5 | 4 | 1 | 68% | 47% | 95% | 81% | 31% | 100% |
| | | | | L5-S1 | 5 | 5 | | | | | | | |
| **>40 years, <2,000 CFU-F/mL** | | | | | | | | | | | | | |
| 207 | 60 | 1,692 | 2 | L4-L5 | 7 | 7 | 0 | 33% | 30% | 7% | 33% | 84% | 6% |
| | | | | L5-S1 | 7 | 7 | | | | | | | |
| 208 | 60 | 1,911 | 2 | L4-L5 | 6 | 6 | 0 | 50% | 47% | 29% | 40% | 29% | 15% |
| | | | | L5-S1 | 6 | 6 | | | | | | | |
| 213 | 41 | 1,194 | 2 | L4-L5 | 6 | 6 | 0 | 57% | 43% | 43% | 72% | 59% | 66% |
| | | | | L5-S1 | 7 | 7 | | | | | | | |
| **>40 years, >2,000 CFU-F/mL** | | | | | | | | | | | | | |
| 102 | 44 | 5,131 | 1 | L5-S1 | 7 | 7 | 0 | 81% | 94% | 100% | 92% | 92% | 81% |
| 106 | 43 | 6,066 | 1 | L5-S1 | 6 | 6 | 0 | 77% | 58% | 55% | 78% | 78% | 51% |
| 108 # | 47 | 4,238 | 1 | L4-L5 | 5 | 4 | 1 | 64% | 82% | 77% | 64% | 78% | 67% |
| 112 | 50 | 2,630 | 1 | L5-S1 | 6 | 5 | 1 | 48% | 48% | 39% | 67% | 91% | 71% |
| 204 # | 43 | 4,003 | 2 | L4-L5 | 6 | 6 | 0 | 95% | 100% | 100% | 95% | 99% | 100% |
| | | | | L5-S1 | 7 | 7 | | | | | | | |
| **>40 years, No cell analysis** | | | | | | | | | | | | | |
| 103** | 42 | | 1 | L5-S1 | 6 | Surgery | | 38% | 15% | ND | 42% | -36% | ND |
| 111 | 41 | | 1 | L5-S1 | 7 | 7 | 0 | 42% | 46% | 73% | 32% | 57% | 75% |
| 113 | 45 | | 1 | L5-S1 | 6 | 5 | 1 | ND | 77% | ND | ND | 89% | ND |
| Study Avg. | 38 | 2,713 | 1.5 | | 5.9 | 5.8 | 0.4 | 60% | 57% | 57% | 65% | 66% | 59% |