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

KENNETH A. PETTINE,[a] MATTHEW B. MURPHY,[b,c] RICHARD K. SUZUKI,[c] THEODORE T. SAND[c]

Key Words. Autologous cell therapy • Mesenchymal stem cells • Bone marrow concentrate • Intervertebral disc injection

[a]Rocky Mountain Associates in Orthopedic Medicine and the Orthopedic Stem Cell Institute, Johnstown, Colorado, USA; [b]Department of Biomedical Engineering, The University of Texas at Austin, Austin, Texas, USA; [c]Celling Biosciences, Austin, Texas, USA

Correspondence: Matthew B. Murphy, Ph.D., Department of Biomedical Engineering, The University of Texas at Austin, Austin, Texas, USA. Telephone: 512-637-2060; Fax: 512-637-2096; e-mail: mbmurphy@utexas.edu

ABSTRACT

Degenerative disc disease (DDD) induces chronic back pain with limited nonsurgical options. In this open label pilot study, 26 patients (median age 40 years; range 18–61) received autologous bone marrow concentrate (BMC) disc injections (13 one level, 13 two levels). Pretreatment Oswestry disability index (ODI) and visual analog scale (VAS) were performed to establish baseline pain scores (average 56.5 and 79.3, respectively), while magnetic resonance imaging was 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 ($p \leq .0001$). Eight of twenty patients improved by one modified Pfirrmann grade at 1 year. The average BMC contained 121×10^6 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, $p = .03$) and 70.6% (VAS, $p = .01$). This study provides evidence of safety and feasibility in the nonsurgical treatment of DDD with autologous BMC and indicates an effect of mesenchymal cell concentration on discogenic pain reduction. STEM CELLS 2015;33:146–156

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 proinflammatory molecules such as TNF-α 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

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)	37.4 (37)
		Range 25–51	Range 18–61
Males		5	6
Females		8	7
Average BMI		27.1	26.1
		Range 19–37	Range 20–34
Cause of injury	Trauma	7	5
	Unknown	6	8

Abbreviation: BMI, body mass index.

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 MSCs may result from interactions between the MSCs and nucleus pulposus cells in treating degenerative discs. For example, Sobajima et al. reported that bone marrow-derived MSCs cocultured with nucleus pulposus cells possessed a synergistic effect that yielded the greatest increase in proteoglycan synthesis and glycosaminoglycan 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, nonexpanded 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 visual analog scale (VAS) pain scores at 12 months post-treatment are reported.

Study Design and Clinical Protocol

This study is a prospective, open-label, nonrandomized, 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 nonoperative 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 nonpathologic disc; pretreatment baseline ODI score of at least 30 on the

100-point scale; and pretreatment baseline low back pain of at least 40 mm on the 100 mm 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 preinjection medical history and physical examination including ODI and VAS. These tests were repeated at 3, 6, and 12 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 12 months and assigned a modified Pfirrmann score (Grades 1–8; 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 (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 3 days and put on restricted physical activity for 2 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

Table 2. Average cell viability, 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 ($\pm$1.2) %	TNC/ml in BMC	121 ($\pm$11) $\times$ 10^6
Cell phenotype subpopulation	**% of TNC**	**Subpopulation Concentration in BMC (cells per milliliter)**	
CFU-F	0.0025%	2,713 ($\pm$491) per ml	
CFU-O	0.0027%	2,913 ($\pm$418) per ml	
Lineage$^-$ cells (CD 2$^-$/3$^-$/8$^-$/11b$^-$)	25.89%	31.5 $\times$ 10^6/ml	
Lineage$^-$/CD34$^+$	1.397%	1.69 $\times$ 10^6/ml	
Lineage$^-$/CD34^{High}/CD90+/CD105+	0.0007%	802/ml	
Lineage$^-$/CD34^{Low}/CD90+/CD105+	0.0040%	4,832/ml	
Lineage$^-$/CD34$^-$/CD90+/CD105+	0.0049%	5,914/ml	

Abbreviations: CFU-F, colony-forming unit-fibroblast; CFU-O, colony-forming unit-osteogenic; TNC, total nucleated cells.

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 (TNC) 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 (noncultured) BMC cells were stained with a series of rabbit anti-human monoclonal antibodies for a hematopoietic lineage-committed (nonprogenitor) 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 50,000–500,000 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 were 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

($\alpha = 0.05$). Multivariable data were evaluated by analysis of variance using JMP 9 statistical analysis software (SAS Institute, Cary, NC).

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 milliliter 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 Fig. S1). 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 Fig. S2). This indicates that not only do the samples possess a classic 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 Fig. S2B). 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$^+$

Table 3. Average pretreatment and post-treatment pain (ODI) and QOL (VAS, 0–100) scores

Patient population	Assessment	Pretreatment	3 months	6 months	12 months
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 $< 2,000$ ($n = 9$)	ODI	54.2	33.7***	36.3	26.3**
	VAS	80.4	46.4**	36.7**	34.5**
CFU-F per ml $> 2,000$ ($n = 11$)	ODI	59.3	14.8*,#	13.5*,#	17.6*
	VAS	82.0	17.5*,#	10.8*,##	25.5*

Statistically significant differences from pretreatment score: $p \leq .0001$ (*), $p < .005$ (**), $p < .01$ (***). Statistically significant differences between $<$ and $>2,000$ CFU-F/ml populations: $p < .005$ (#), $p < .01$ (##).
Abbreviations: ODI, Oswestry disability index; VAS, visual analog scale.

and Lineage$^-$/CD34$^-$/CD90$^+$/CD105$^+$ most closely correlated to CFU-F.

Postinjection 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 3, 6, and 12 months follow-up visits. Data were 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 pretreatment and post-treatment pain scores are reported in Table 3 as an overall series and by population subsets (one-level vs. 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 3, 6, and 12 months, respectively. Similarly, the average percentage of VAS reduction was 64.6%, 64.2%, and 58.0% after 3, 6, and 12 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 3 months. Two patients elected to undergo a second injection of BMC at 6 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 6 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 .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 3 and 6 months post-therapy ($p < .005$ for ODI at 3 and 6 months and VAS at 3 months, $p < .01$ for VAS at 6 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 ($\leq$ or $>$ median age of 40 years) and CFU-F concentrations greater or less than 2,000/ml of BMC revealed interesting differences in pain relief. Significant overall reduction of ODI and VAS scores (Fig. 1A, 1B) was observed in each cohort. As shown in Table 3, all patients with CFU-F concentrations greater than 2,000/ml in their BMC preparation, regardless of age, demonstrated a statistically significant improvement in pain scores over those below that MSC concentration at 3 and 6 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 $<$ and $>2,000$ CFU-F/ml cohorts were 24.6 (ODI, $p = .01$) and 33.4 (VAS, $p = .014$) at 3 months, 30.6 (ODI, $p = .006$) and 37.0 (VAS, $p = .02$) at 6 months, and 25.2 (ODI, $p = .025$) and 28.8 (VAS, $p = .03$) at 12 months. Among all patients with $<2,000$ CFU-F/ml in their BMC, there was a statistical difference based on age at 12 months (ODI $p = .02$, VAS $p = .03$). 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, 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.

Table 4. Patient-by-patient information, CFU-F concentration in BMC, number of grades improvement in mPS magnetic resonance imaging (MRI) scores according to blinded review, and percentage reduction in pain by ODI and VAS at 3, 6, and 12 months postinjection

Patient ID	Patient age	CFU-F/ml	No. of discs injected	Level	Initial mPS grade	12 months mPS grade	No. Grades improvement	% Reduction ODI			% Reduction VAS		
								3 months	6 months	12 months	3 months	6 months	12 months
<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[a,b]	36	1,114	2	L4-L5	6	Surgery		ND	44%	22%	ND	44%	18%
				L5-S1	6								
211[b,c]	38	938	2	L4-L5	7	7	0	24%	7%	93%	66%	51%	88%
				L5-S1	7	7							
212[b]	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[b]	33	3,268	1	L4-L5	5	4	1	72%	91%	81%	98%	99%	88%
110[b]	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[b]	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[c]	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[b]	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[b]	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[a]	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%

[a]Elected for spinal fusion surgery.
[b]Patient received discogram prior to BMC injection.
[c]Opted for second BMC injections after 6 months.
Abbreviations: CFU-F, colony-forming unit-fibroblast; mPS, modified Pfirrmann scale; ND, not determined; no f/u, no follow-up MRI; ODI, Oswestry disability index; VAS, visual analog scale.

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 3 and 6 months (Table 4). Most of these patients sustained >40% pain reduction at 12 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 ($p \leq .03$), 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 12 months after treatment according to the modified Pfirrmann scale. Figure 3 illustrates
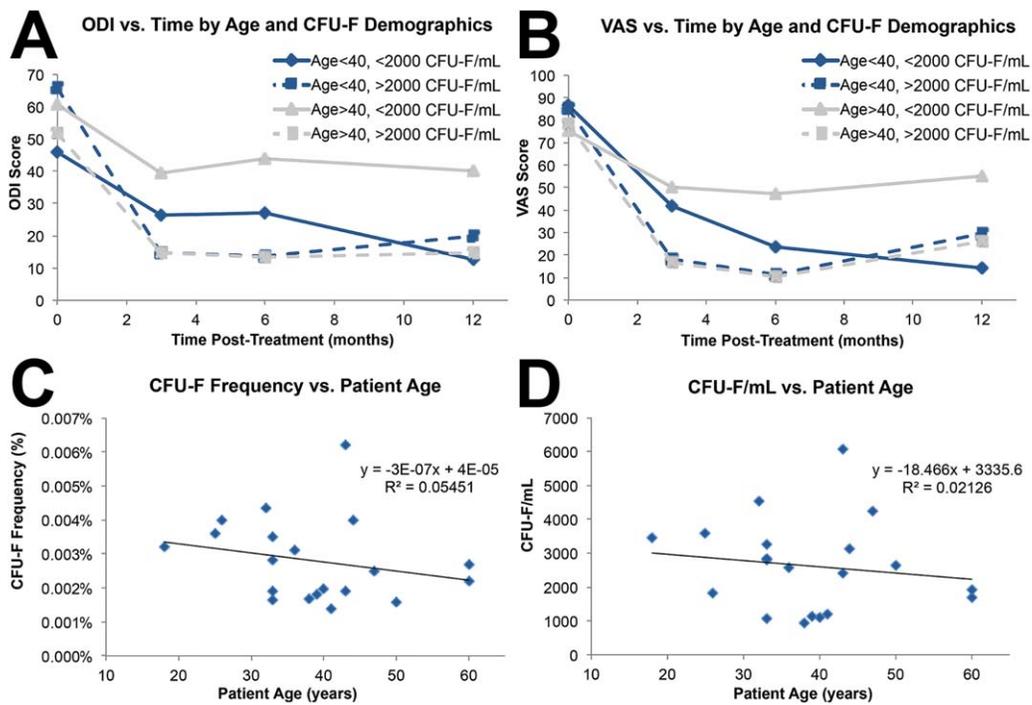
Figure 1. Average ODI **(A)** and VAS **(B)** scores versus time segregated by patient age and CFU-F concentration in BMC injectate. Age division was greater or less than the study median age of 40 years. Blue lines denote average of patients ≤40 years and gray lines represent patients older than 40. 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. Abbreviations: CFU-F, colony-forming unit-fibroblast; ODI, Oswestry disability index; VAS, visual analog scale.

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 twenty-six 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 6-month evaluation, the patient was eligible for reinjection of the disc(s) at their and the physician's discretion. Five of the twenty-six patients met reinjection criteria at 6 months: two underwent reinjection (one at 7 months, one at 8 months) and were significantly improved clinically at 1 year (average 94% reduction from initial ODI and VAS scores); two patients underwent surgery after 6 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 postinjection time points showed statistically significant sustained pain relief.
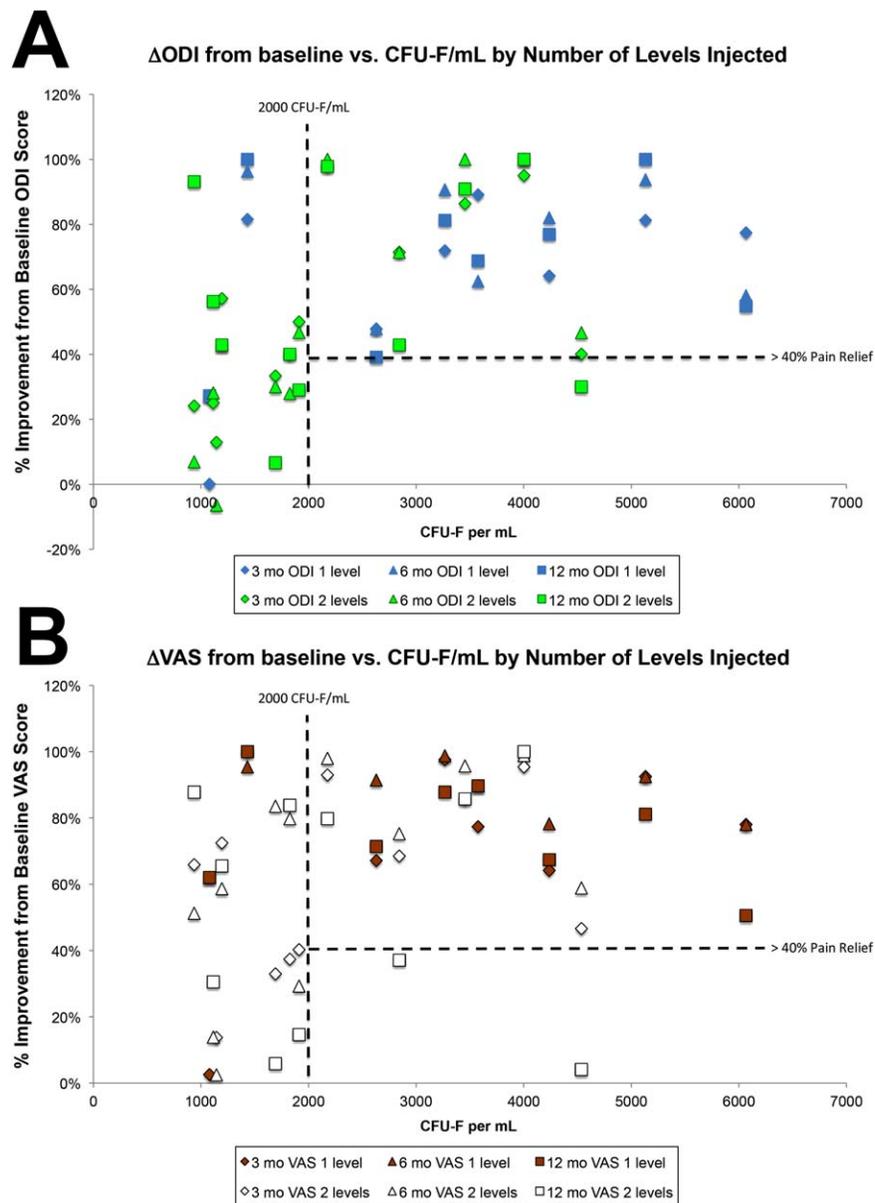
Figure 2. Percentage pain score improvement from baseline (pretreatment) of raw ODI **(A)** and VAS **(B)** scores versus CFU-F concentration in BMC by individual patient, comparing one-level and two-level injection subjects. Natural segregations occurred at 2,000 CFU-F/ml and 40% reduction in pain. Abbreviations: CFU-F, colony-forming unit-fibroblast; ODI, Oswestry disability index; VAS, visual analog scale.

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 months) and sustained (6 and 12 months) 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 3 months and between 3 and 6 months. This result of a critical CFU-F concentration (2,000/ml)

may be analogous to the clinical findings of Hernigou et al. that tibial nonunion 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 indicate a clinical success of 45.1% at 2-year
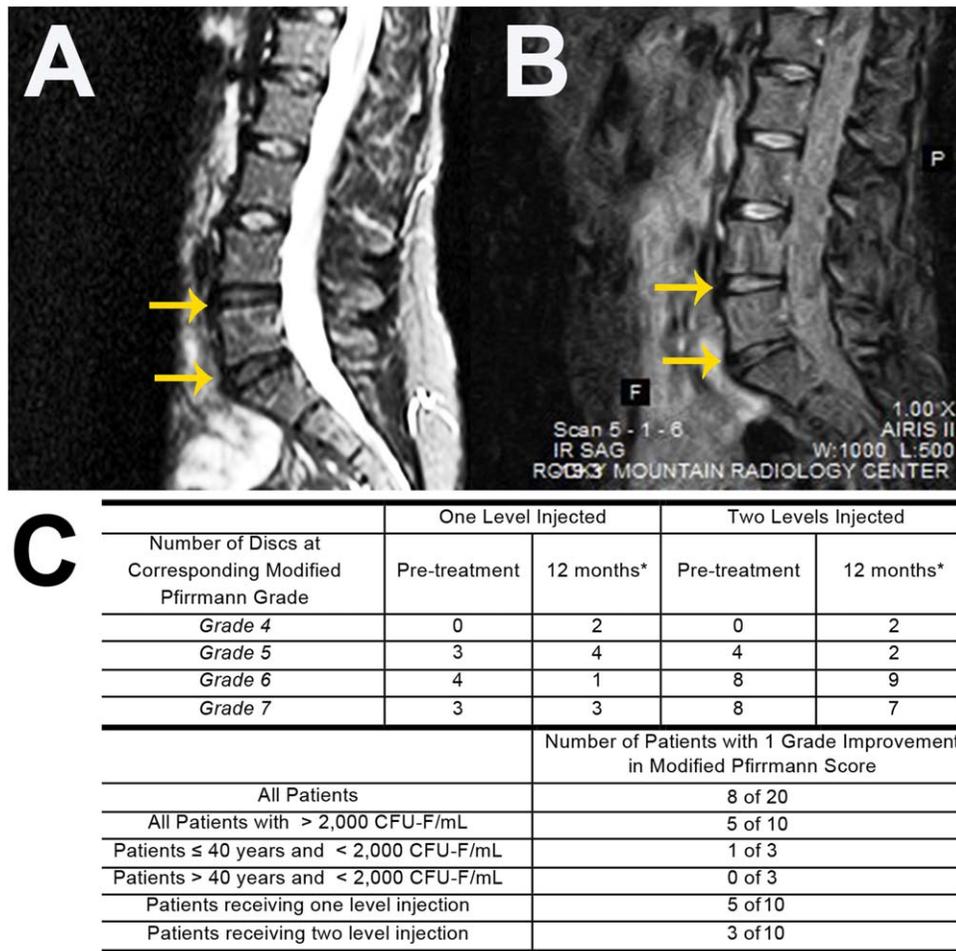
Figure 3. Magnetic resonance image (MRI) before pretreatment **(A)** and 12 months after injection **(B)** of autologous BMC into L4-L5 and L5-S1 intervertebral discs. L4-L5 improved from grade 5 to grade 4 while L5-S1 remained at grade 6. **(C):** Pretreatment and 12-month modified Pfirrmann MRI scores 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 nonsurgical patients did not receive 12-month follow-up MRI). Abbreviation: CFU-F, colony-forming unit-fibroblast.

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% to 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 1 year after surgery vary considerably. Rodgers et al. reported patients undergoing lateral lumbar interbody fusion with β-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, this study did not use allogeneic cells, culture-expanded cells, nor genetically modified cells. The rabbit study also used an artificial model for degenerative disc disease (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 BMA. 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 × 10^3 cells per milliliter of injectate), while hematopoietic stem cells are present in the CD34$^+$/lineage$^-$ fraction (1.55 × 10^6 cells per milliliter) [72–74]. Few studies have reported the results of using 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 3 months, 6 months, and 1 year. The average number of MSCs available for injection was 23 ± 5 × 10^6 cells, with an average viability of the cells at the time of injection of 83% ± 5%. For comparison, the patients in this study received an average of only 8.3 × 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 nonrandomized FDA Phase I study [76]. Three of the fifteen enrolled patients went on to surgery after the injection. Both of these studies (Orozco et al., Coric et al.) used cell therapies regulated by the FDA as a drug.

This study differs from Orozco et al. and Coric and Pettine using autologous bone marrow concentrated cells in a single treatment, point-of-care, 30-minute procedure. The use of autologous, noncultured 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 < .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 twenty-six 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 nonsurgical interventions (physical therapy, epidural steroids, non-steroidal anti-inflammatory drugs, and opioids) [51, 79].

CONCLUSIONS

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 twenty patients according to MRI in conjunction with sustained pain relief through 12 months represents promise for the use of this regenerative medicine approach. The data suggest that there might be a critical dose concentration of fresh, noncultured 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 coadministration of biomaterials used with cells to improve healing of degenerated or herniated discs.

AUTHOR CONTRIBUTIONS

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

DISCLOSURE OF POTENTIAL CONFLICTS OF INTEREST

K.A.P. reports no conflicts of interest concerning this article. 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.

REFERENCES

1 Boden SD, Davis DO, Dina TS et al. Abnormal magnetic-resonance scans of the lumbar spine in asymptomatic subjects. A prospective investigation. J Bone Joint Surg Am 1990;72:403–408.

2 Powell MC, Wilson M, Szypryt P et al. Prevalence of lumbar disc degeneration observed by magnetic resonance in symptomless women. Lancet 1986;2:1366–1367.

3 Weishaupt D, Zanetti M, Hodler J et al. MR imaging of the lumbar spine: Prevalence of intervertebral disk extrusion and sequestration, nerve root compression, end plate abnormalities, and osteoarthritis of the facet joints in asymptomatic volunteers. Radiology 1998;209:661–666.

4 Ching CTS, Chow DHK, Yao FYD et al. The effect of cyclic compression on the mechanical properties of the inter-vertebral disc: An in vivo study in a rat tail model. Clin Biomech 2003;18:182–189.

5 Hutton WC, Ganey TM, Elmer WA et al. Does long-term compressive loading on the intervertebral disc cause degeneration? Spine 2000;25:2993–3004.

6 Iatridis JC, Mente PL, Stokes IA et al. Compression-induced changes in intervertebral disc properties in a rat tail model. Spine 1999;24:996–1002.

7 Kroeber MW, Unglaub F, Wang H et al. New in vivo animal model to create intervertebral disc degeneration and to investigate the effects of therapeutic strategies to stimulate disc regeneration. Spine 2002;27:2684–2690.

8 Lotz JC, Chin JR. Intervertebral disc cell death is dependent on the magnitude and duration of spinal loading. Spine 2000;25:1477–1483.

9 Lotz JC, Colliou OK, Chin JR et al. Compression-induced degeneration of the intervertebral disc: An in vivo mouse model and finite-element study. Spine 1998;23:2493–2506.

10 Puustjärvi K, Lammi M, Helminen H et al. Proteoglycans in the intervertebral disc of young dogs following strenuous running exercise. Connect Tissue Res 1994;30:225–240.

11 Yamazaki S, Weinhold PS, Graff RD et al. Annulus cells release ATP in response to vibratory loading in vitro. J Cell Biochem 2003;90:812–818.

12 Deyo RA, Bass JE. Lifestyle and low-back pain. The influence of smoking and obesity. Spine 1989;14:501–506.

13 Heliövaara M. Risk factors for low back pain and sciatica. Ann Med 1989;21:257–264.

14 Adams MA, Freeman BJ, Morrison HP et al. Mechanical initiation of intervertebral disc degeneration. Spine 2000;25:1625–1636.

15 Allan DB, Waddell G An historical perspective on low back pain and disability. Acta Orthop Scand Suppl 1989;234:1–23.

16 Elfering A, Semmer N, Birkhofer D et al. Risk factors for lumbar disc degeneration: A 5-year prospective MRI study in asymptomatic individuals. Spine 2002;27:125–134.

17 Elfering A, Semmer NK, Schade V et al. Supportive colleague, unsupportive supervisor: The role of provider-specific constellations of social support at work in the development of low back pain. J Occup Health Psychol 2002;7:130–140.

18 Sandover J. Dynamic loading as a possible source of low-back disorders. Spine 1983;8:652–658.

19 Hiyama A, Mochida J, Iwashina T et al. Transplantation of mesenchymal stem cells in a canine disc degeneration model. J Orthop Res 2008;26:589–600.

20 Crock HV, Yoshizawa H. The blood supply of the lumbar vertebral column. Clin Orthop Relat Res 1976;6–21.

21 Eyre D, Benya P, Buckwalter J et al. Intervertebral disk: Basic science perspectives. In New Perspectives on Low Back Pain. Park Ridge, IL: American academy of orthopaedic surgeons 1989;147–214.

22 Maroudas A, Stockwell RA, Nachemson A et al. Factors involved in the nutrition of the human lumbar intervertebral disc: Cellularity and diffusion of glucose in vitro. J Anat 1975;120:113–130.

23 Urban J. Diffusion of small solutes into the intervertebral disc: As in vivo study. Biorheology 1978;15:203–221.

24 Roberts S, Menage J, Eisenstein SM. The cartilage end-plate and intervertebral disc in

scoliosis: Calcification and other sequelae. J Orthop Res 1993;11:747–757.

25 Bartels EM, Fairbank JC, Winlove CP et al. Oxygen and lactate concentrations measured in vivo in the intervertebral discs of patients with scoliosis and back pain. Spine 1998;23:1–7; discussion 8.

26 Holm S, Maroudas A, Urban JP et al. Nutrition of the intervertebral disc: Solute transport and metabolism. Connect Tissue Res 1981;8:101–119.

27 Ohshima H, Urban JP. The effect of lactate and pH on proteoglycan and protein synthesis rates in the intervertebral disc. Spine 1992;17:1079–1082.

28 Bibby SRS, Urban JPG. Effect of nutrient deprivation on the viability of intervertebral disc cells. Eur Spine J 2004;13:695–701.

29 Horner HA, Urban JP. 2001 Volvo Award Winner in Basic Science Studies: Effect of nutrient supply on the viability of cells from the nucleus pulposus of the intervertebral disc. Spine 2001;26:2543–2549.

30 Murphy MB, Moncivais K, Caplan AI. Mesenchymal stem cells: Environmentally responsive therapeutics for regenerative medicine. Exp Mol Med 2013;45:e54.

31 Murphy MB, Blashki D, Buchanan RM et al. Adult and umbilical cord blood-derived platelet-rich plasma for mesenchymal stem cell proliferation, chemotaxis, and cryo-preservation. Biomaterials 2012;33:5308–5316.

32 Black LL, Gaynor J, Gahring D et al. Effect of adipose-derived mesenchymal stem and regenerative cells on lameness in dogs with chronic osteoarthritis of the coxofemoral joints: A randomized, double-blinded, multicenter, controlled trial. Vet Ther 2007;8:272–284.

33 Black LL, Gaynor J, Adams C et al. Effect of intraarticular injection of autologous adipose-derived mesenchymal stem and regenerative cells on clinical signs of chronic osteoarthritis of the elbow joint in dogs. Vet Ther 2008;9:192–200.

34 Ohnishi S, Yanagawa B, Tanaka K et al. Transplantation of mesenchymal stem cells attenuates myocardial injury and dysfunction in a rat model of acute myocarditis. J Mol Cell Cardiol 2007;42:88–97.

35 Semedo P, Wang PM, Andreucci TH et al. Mesenchymal stem cells ameliorate tissue damages triggered by renal ischemia and reperfusion injury. Transplant Proc 2007;39:421–423.

36 Parekkadan B, van Poll D, Suganuma K et al. Mesenchymal stem cell-derived molecules reverse fulminant hepatic failure. PLoS One 2007;2:e941.

37 Gerdoni E, Gallo B, Casazza S et al. Mesenchymal stem cells effectively modulate pathogenic immune response in experimental autoimmune encephalomyelitis. Ann Neurol 2007;61:219–227.

38 Rasulov MF, Vasilenko VT, Zaidenov VA et al. Cell transplantation inhibits inflammatory reaction and stimulates repair processes in burn wound. Bull Exp Biol Med 2006;142:112–115.

39 Sobajima S, Vadala G, Shimer A et al. Feasibility of a stem cell therapy for intervertebral disc degeneration. Spine J 2008;8:888–896.

40 Hernigou P, Homma Y, Henri C et al. Benefits of small volume and small syringe

for bone marrow aspirations of mesenchymal stem cells. Int Orthop 2013;37:2279–2287.

41 Sasso RC, Foulk DM, Hahn M. Prospective, randomized trial of metal-on-metal artificial lumbar disc replacement: Initial results for treatment of discogenic pain. Spine 2008;33:123–131.

42 Gornet MF, Dryer RF, Peloza JH et al. Lumbar disc arthroplasty with MAVERICK disc versus stand-alone interbody fusion. Spine 2011;36:E1600–E1611.

43 Blumenthal S, McAfee PC, Guyer RD et al. A prospective, randomized, multicenter Food and Drug Administration investigational device exemptions study of lumbar total disc replacement with the CHARITE artificial disc versus lumbar fusion: Part I: Evaluation of clinical outcomes. Spine 2005;30:1565–1575.

44 Zigler J, Delamarter R, Spivak JM et al. Results of the prospective, randomized, multi-center Food and Drug Administration investigational device exemption study of the ProDisc-L total disc replacement versus circumferential fusion for the treatment of 1-level degenerative disc disease. Spine 2007;32:1155–1162.

45 Guyer RD, McAfee PC, Hochschuler SH et al. Prospective randomized study of the Charite artificial disc: Data from two investigational centers. Spine J 2004;4:252S–259S.

46 Hernigou P, Poignard A, Beaujean F et al. Percutaneous autologous bone-marrow grafting for nonunions. J Bone Joint Surg Am 2005;87:1430–1437.

47 Hernigou P, Flouzat Lachaniette CH, Delambre J et al. Biologic augmentation of rotator cuff repair with mesenchymal stem cells during arthroscopy improves healing and prevents further tears: A case-controlled study. Int Orthop 2014;38:1811–1818.

48 Richardson SM, Hoyland JA. Stem cell regeneration of degenerated intervertebral discs: Current status. Curr Pain Headache Rep 2008;12:83–88.

49 Herkowitz H, Kurz L. Degenerative lumbar spondylolisthesis with spinal stenosis. A prospective study comparing decompression with decompression and intertransverse process arthrodesis. J Bone Joint Surg Am 1991;73:802–808.

50 Anderson PA, Tribus CB, Kitchel SH. Treatment of neurogenic claudication by interspinous decompression: Application of the X STOP device in patients with lumbar degenerative spondylolisthesis. J Neurosurg Spine 2006;4:463–471.

51 Weinstein JN, Lurie JD, Tosteson TD et al. Surgical versus nonsurgical treatment for lumbar degenerative spondylolisthesis. N Engl J Med 2007;356:2257–2270.

52 Hanley EN, David SM. Current concepts review—Lumbar arthrodesis for the treatment of back pain*. J Bone Joint Surg Am 1999;81:716–730.

53 Juratli SM, Franklin GM, Mirza SK et al. Lumbar fusion outcomes in Washington State workers' compensation. Spine 2006;31:2715–2723.

54 Watters WC, Baisden J, Bono CM et al. Antibiotic prophylaxis in spine surgery: An evidence-based clinical guideline for the use of prophylactic antibiotics in spine surgery. Spine J 2009;9:142–146.

55 Brox JI, Reikerås O, Nygaard Ø et al. Lumbar instrumented fusion compared with cognitive

intervention and exercises in patients with chronic back pain after previous surgery for disc herniation: A prospective randomized controlled study. Pain 2006;122:145–155.

56 Maghout Juratli S, Franklin GM, Mirza SK et al. Lumbar fusion outcomes in Washington State workers' compensation. Spine 2006;31:2715–2723.

57 Brox JI, Sørensen R, Friis A et al. Randomized clinical trial of lumbar instrumented fusion and cognitive intervention and exercises in patients with chronic low back pain and disc degeneration. Spine 2003; 28:1913–1921.

58 Deyo RA, Gray DT, Kreuter W et al. United States trends in lumbar fusion surgery for degenerative conditions. Spine 2005;30: 1441–1445; discussion 1446–1447.

59 Turner JA, Ersek M, Herron L et al. Surgery for lumbar spinal stenosis. Attempted meta-analysis of the literature. Spine 1992; 17:1–8.

60 Ghiselli G, Wang JC, Bhatia NN et al. Adjacent segment degeneration in the lumbar spine. J Bone Joint Surg Am 2004;86-A: 1497–1503.

61 Rodgers WB, Gerber EJ, Rodgers JA. Clinical and radiographic outcomes of extreme lateral approach to interbody fusion with β-tricalcium phosphate and hydroxyapatite composite for lumbar degenerative conditions. Int J Spine Surg 2012;6: 24–28.

62 Fu R, Selph S, McDonagh M et al. Effectiveness and harms of recombinant human bone morphogenetic protein-2 in spine fusion: A systematic review and meta-analysis. Ann Intern Med 2013;158:890–902.

63 Arts MP, Kols NI, Onderwater SM et al. Clinical outcome of instrumented fusion for the treatment of failed back surgery syndrome: A case series of 100 patients. Acta Neurochir 2012;154:1213–1217.

64 Carragee EJ, Mitsunaga KA, Hurwitz EL et al. Retrograde ejaculation after anterior lumbar interbody fusion using rhBMP-2: A cohort controlled study. Spine J 2011;11:511–516.

65 Deutsch H. High-dose bone morphogenetic protein-induced ectopic abdomen bone growth. Spine J 2010;10:e1–4.

66 Vadala G, Sowa G, Hubert M et al. Mesenchymal stem cells injection in degenerated intervertebral disc: Cell leakage may induce osteophyte formation. J Tissue Eng Regen Med 2012;6:348–355.

67 Comer GC, Smith MW, Hurwitz EL et al. Retrograde ejaculation after anterior lumbar interbody fusion with and without bone morphogenetic protein-2 augmentation: A 10-year cohort controlled study. Spine J 2012; 12:881–890.

68 Hernigou P, Homma Y, Flouzat-Lachaniette CH et al. Cancer risk is not increased in patients treated for orthopaedic diseases with autologous bone marrow concentrate. JBJS 2013;95:2215–2221.

69 English K. Intervertebral disc repair: Mesenchymal stem cells to the rescue? Transplantation 2011;92:733–734.

70 Simmons P, Torok-Storb B. Identification of stromal cell precursors in human bone marrow by a novel monoclonal antibody, STRO-1. Blood 1991;78:55–62.

71 Jones EA, Kinsey SE, English A et al. Isolation and characterization of bone marrow multipotential mesenchymal progenitor cells. Arthritis Rheum 2002;46:3349–3360.

72 Wexler SA, Donaldson C, Denning-Kendall P et al. Adult bone marrow is a rich source of human mesenchymal "stem" cells but umbilical cord and mobilized adult blood are not. Br J Haematol 2003;121:368–374.

73 Kern S, Eichler H, Stoeve J et al. Comparative analysis of mesenchymal stem cells from bone marrow, umbilical cord blood, or adipose tissue. Stem Cells 2006;24:1294–1301.

74 Deschaseaux F, Gindraux F, Saadi R et al. Direct selection of human bone marrow mesenchymal stem cells using an anti-CD49a antibody reveals their CD45med,low phenotype. Br J Haematol 2003;122:506–517.

75 Orozco L, Soler R, Morera C et al. Intervertebral disc repair by autologous mesenchymal bone marrow cells: A pilot study. Transplantation 2011;92:822–828.

76 Coric D, Pettine KA, Sumich A et al. Prospective study of disc repair with allogeneic chondrocytes. J Neurosurg Spine 2012;1–11.

77 Meisel HJ, Siodla V, Ganey T et al. Clinical experience in cell-based therapeutics: Disco chondrocyte transplantation. A treatment for degenerated or damaged intervertebral disc. Biomol Eng 2007;24:5–21.

78 Peng B, Pang X, Wu Y et al. A randomized placebo-controlled trial of intradiscal methylene blue injection for the treatment of chronic discogenic low back pain. Pain 2010;149:124–129.

79 Deyo RA, Mirza SK, Turner JA et al. Overtreating chronic back pain: Time to back off? J Am Board Family Med 2009;22: 62–68.

See www.StemCells.com for supporting information available online.