New Biomaterials for Degenerative Disc Disease

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13.1 Introduction

Degenerative disc disease is one of the main causes of chronic low back pain originating from degeneration of the intervertebral disc and accounts for more patients suffering than any other single cause [1]. Up to 80% of the population will experience low back pain at some point in their lives, and most of them will have pain on many occasions with nearly 20% of the population experiencing low back pain at any given time [2–4]. Back pain is the second leading cause of physician visits next to the common cold and is the greatest cause of disability and lost days from work worldwide [5, 6]. The cost of treating low back pain is extremely high with estimates ranging higher than 100 billion dollars annually [7].

A critically important step in the effective treatment of low back pain is to make an accurate anatomic diagnosis of the exact location of the pain generator. This can

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L. Manfrè, J. Van Goethem (eds.), *The Disc and Degenerative Disc Disease*,
New Procedures in Spinal Interventional Neuroradiology,
https://doi.org/10.1007/978-3-030-03715-4_13

be challenging but a combination of imaging and diagnostic interventional procedures can reveal the source of back pain in up to 90% of all patients [1].

Discogenic back pain is the most common cause affecting nearly half of the patients with low back pain [1]. Depalma et al. found the prevalence of pain related to the facet joints, sacroiliac joints, and lumbar discs was 31%, 18%, and 42%, respectively [1]. The pain originating from the disc is thought to be due to internal disc disruption (IDD) [8]. This produces the discogenic pain syndrome caused by disc degeneration not related to sciatica or nerve root referred pain. Internal disc disruption has been designated as a separate clinical entity and is thought to account for up to 42% of chronic low back pain [1, 8, 9]. It occurs primarily in younger patients and is separate from other types of degenerative disc entities that will produce pain such as lumbar degenerative disc disease (DDD), segmental instability, and disc herniations [10].

Internal disc disruption is seen as annular disruption on computed tomography (CT) scans after contrast injection or on magnetic resonance (MR) imaging as disc dehydration or high intensity zones, but the findings of disc degeneration along very often do not correlate with either the presence or the severity of the patient's pain [11]. Lumbar X-rays of IDD patients do not have any characteristic signs.

The diagnosis of painful IDD can be supported using provocative and/or anesthetic discography. Discography can be separated into objective anatomic findings on fluoroscopic exam during the procedure and post discogram CT or MRI after injection. Subjective findings constitute recreation of usual and customary pain during pressurization of the suspected disc or alleviation of symptoms after anesthetic infiltration. The use of discography as a method of diagnosing IDD is still debatable, but the authors have found through involvement in numerous intradiscal clinical trials that it is a valid method for assisting in the diagnosis of this anatomic disorder.

The natural history of low back pain due to IDD is chronic and incessant. The treatment for discogenic low back pain has typically been limited to nonsurgical management, disc arthroplasty, or interbody fusion [12]. Despite many treatments available to treat chronic low back pain, there is little consensus among physicians as to which treatment approach is best. Pharmacologic treatment typically includes nonsteroidal anti-inflammatory drugs (NSAIDs) and muscle relaxants, but the literature support for efficacy of treatment is not strong with only minimal improvements in pain and function [13, 14]. Chronic opioid therapy is only marginally effective and is associated with significant side effects and risk of addiction and overdose [14]. Physical therapy with core muscle strengthening along with manipulation has some temporary benefit but the long-term effects are unknown [14]. Epidural injections are performed for patients with discogenic low back pain and have been shown to produce fair results [15]. Intradiscal electrothermal annuloplasty (IDET) was used to treat discogenic low back pain starting in 1996, and a more recent meta-analysis of its effectiveness has shown that this also produces fair results [16]. Over the past two decades, surgical interbody fusion for discogenic back pain has increased significantly but the reported results are mediocre, the postoperative course is difficult, the complication rate is not trivial, and up to 20% of patients will undergo additional surgery within 4 years of lumbar fusion [17–19].

There are therapies that are focused on either treating the inflammatory pathways (i.e., steroid injections) or disrupting the nerve conduction from the painful disc (i.e., methylene blue, ozone, biaculoplasty, etc.) [11]. These types of therapies may be successful in reducing pain but do not have the ability to heal the disc or reverse the degenerative changes suspected to be responsible for the pain. Research efforts have been focusing more on the development of treatments that will repair or regenerate damaged intervertebral discs. Treatments have been focused on restoring the cellular health of the intervertebral disc and on reducing the pain associated with IDD [20].

The benefits of biologic treatments likely originate from tissue repair and changes in cytokine expression following injection of biologic material.

Some of the biologic materials that have been injected into the intervertebral disc include fibrin sealant, isolated growth factors, juvenile chondrocytes, platelet-rich plasma, and mesenchymal stem cells (MSCs) [20, 21]. There have been multiple clinical trials testing MSCs in patients with painful IDD but most of these have had small sample sizes. Despite a lack of firm evidence on the efficacy of stem cell therapy, the trials that have been published suggest a substantial improvement in pain and function [22, 23]. The provisional results of one large phase 2/3 prospective randomized control trial using bone marrow-derived expanded allogeneic MSCs injected into painful lumbar discs has demonstrated significant improvements in pain and function [24].

13.2 Intradiscal Biologic Treatments

As mentioned above, there have been many biologic treatments for the intervertebral disc including fibrin adhesives, disc restorative solution, chondrocytes, platelet-rich plasma, bone morphogenic protein, transforming growth factor, disc chondrocytes, and autologous and allogeneic mesenchymal stem cells. Many biologic treatments have been studied and have been found to lack significant efficacy over saline or a placebo. Some of these failed attempts at biologic treatments of the disc have provided guidance for current and future RCTs in this space. Initial studies were focused on MRI changes and 6-month outcomes. We have since learned that MRI changes are not consistent, 6-month results may be too early, and saline treatment to the disc is not a placebo but has up to a 40% responder rate [24]. Biologic therapies still hold significant promise and continue to be studied.

Recent biologic studies for IDD involve injecting directly into the nucleus of the lumbar disc. Risk of discitis has been a concern historically but was found to be between 1 and 4% after discography procedures, but after the introduction of antibiotics the rate of discitis is negligible [25, 26]. Consequently, the use of antibiotics is recommended when injecting any substance into the intervertebral disc.

Discs normally break down their matrix with enzymes such as metalloproteinases, and this degradation is mitigated and/or reversed by certain growth factors such as bone morphogenetic protein-2 (BMP-2), BMP-7 (also known as osteogenic protein-1; OP-1), growth differentiation factor-5 (GDF-5), transforming growth

factor-β (TGF-β), insulin-like growth factor-1 (IGF-1), and others [27, 28]. Studies evaluating the use of BMP-7 (OP-1), GDF-5, alpha-2-macroglobulin (A2M), and platelet-rich plasma (PRP) have been conducted to determine the safety and efficacy of these growth factors in treating symptoms from IDD.

13.2.1 Fibrin Adhesives

Injection into the disc with fibrin adhesives involves fibrinogen combined with thrombin just prior to injection into the nucleus pulposis with enough volume to fill the potential space of the nucleus and extend into and seal the annular defects from inside (Fig. 13.1). This has been proven in vivo and in vitro [27, 28], and a randomized investigation comparing non-autologous fibrin versus normal saline has shown that significant discal repair occurred along with improvement of the disc's biochemical environment [27]. Although the fibrin sealant initially showed promise, a prospective randomized control trial comparing the fibrin sealant Biostat with a saline injection procedure failed to show statistically significant better results in patients injected with the sealant versus those patient injected when measured at 6 months. This was a rigorous phase III trial with 260 patients including 220 one treatment level subjects and 40 two treatment level subjects who were randomized in a 3:1 ratio. The study has not yet been published.

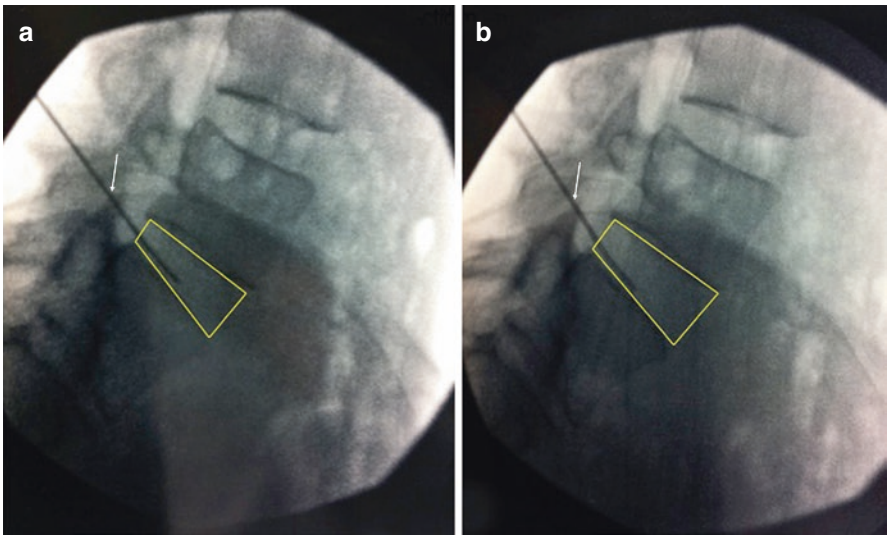


Fig. 13.1 (**a**, **b**) Lateral fluoroscopic views showing the needle (white arrows in **a** and **b**) in the intervertebral disc. The outline of the disc (yellow lines in **a** and **b**) shows increased disc height after the injection into the nucleus pulposis and adjacent annular fissures (**b**) as compared to the pre-injection disc height (**a**)

13.2.2 Bone Morphogenic Protein

A 12-site, double-blind, placebo-controlled RCT with a 3:1 randomization comparing BMP-7 to saline was conducted with 100 patients and a maximum follow-up time of 2 years. The study had patients with less back pain, improved activity levels, better sitting tolerance, and a low crossover to surgery rate; but despite these positive findings, the patients injected with BMP-7 failed to show statistical significance in the primary end points of pain and function improvement. Magnetic resonance imaging of the intervertebral discs also showed no evidence of anatomic improvements. This negative study has not been published at the time of the preparation of this chapter.

13.2.3 Growth Differentiation Factor

A phase III trial sponsored by Advanced Technologies and Regenerative Medicine (a Johnson & Johnson affiliate, Raynham, Massachusetts) with 150 patients and 15 clinical sites, including two international sites compared intradiscal GDF-5 with saline. This double-blinded RCT was randomized with half of the patients receiving saline and half receiving GDF-5. The follow-up time points were at 6 months and 1 year but the study was stopped after interim analysis due to lack of efficacy. This study has also not yet been published.

13.2.4 Alpha-2-Macroglobulin

In addition to growth factors, alpha-2-macroglobulin (A2M) has been injected into the intervertebral disc [29]. It has been shown that A2M reduces a cartilage degradation product called fibronectin-aggrecan complex (FAC), which has been found in patients with DDD.

A prospective cohort trial with 24 patients with low back pain and MR imaging-concordant DDD were injected with A2M and the Oswestry disability index (ODI) and visual analog scores (VAS) were noted at baseline and at the 3- and 6-month time points [29]. The FAC was also measured in each patient to see if this correlated with the response to A2M injection. It was shown that the patients with FAC-positive in assays were significantly more likely to show pain and functional improvement. The mean VAS improvement in FAC-positive patients was 4.9 ± 0.9 and 4.0 ± 1.0 at 3- and 6-month, compared to 1.5 ± 1.2 and 2.3 ± 1.3 in those with negative FAC ($p < 0.0001$). The ODI also improved significantly with an average of 37 ± 9.3 and 28 ± 14 points at 3- and 6-month in FAC-positive patients compared to 9.4 ± 11.9 and 12.6 ± 11.8 points at 3- and 6-month in FAC-negative patients ($p < 0.0001$). The authors concluded that A2M may be an important treatment for the pain and dysfunction associated with DDD provided that the FAC biomarker is present. The study was rigorous as the authors used a definition of clinical improvement that was in excess of the minimal clinically important difference (MCID) and an outcome measure that was a combination of the VAS and ODI.

13.2.5 Platelet-Rich Plasma

The use of intradiscal PRP has substantially more data than nearly all the other growth factor studies combined (Table 13.1), and all of the observational studies were considered to be of moderate quality as assessed by the Interventional Pain Management Techniques–Quality Appraisal of Reliability and Risk of Bias Assessment for Nonrandomized Studies (IPM-QRBNR) criteria [30]. One RCT of

Table 13.1 Recent study details and outcomes of the use of Platelet Rich Plasma (PRP) in intervertebral disc degeneration

Study details	Chronicity of injury and biologic used	Follow-up period	Conclusions
Tuakli-Wosornu et al., 2016 (277) Lumbar discogenic pain Prospective, double-blind, randomized controlled study, $n = 47$	Chronic PRP injections	1 year	Intradiscal injections of PRP ×1 showed significant improvement at 8-week follow-up, with maintained improvement compared to controls at 1-year follow-up
Monfett et al., 2016 (276) Lumbar discogenic pain, lumbar disc degeneration Prospective trial, $n = 29$	Chronic PRP injections	2 years	Intradiscal PRP injections show continued safety and improvements in pain and function at 2 years post-procedure
Navani et al., 2018 (274) Lumbar discogenic pain Prospective case series, $n = 20$	Chronic PRP, single injection, 2 mL injected up to 3 disc levels	18 months	At 18 months, 15 patients remained for survey compared to 18 patients surveyed at 6 months: >50% relief in VAS in 93% of patients at 18 months ($n = 14/15$) and in 94% of patients ($n = 17/18$) at 6 months [2]. Improvement in SF-36 scores in 93% of patients at 18 months ($n = 14/15$) compared to 100% ($n = 18/18$) at 6 months
Akeda et al., 2017 (279) Lumbar discogenic pain Preliminary clinical trial, $n = 14$	Chronic PRP injections	12 months	Intradiscal injection of autologous PRP releasate in patients with low back pain was safe with no adverse events observed during follow-up The results showed reduction in mean pain scores at 1 month sustained throughout the observation periods of 6 and 12 months
Levi et al., 2016 (275) Lumbar discogenic pain Prospective trial, $n = 8$	Chronic PRP, single injection	6 months	Single or multiple levels (up to 5) of discogenic pain injected with PRP showed encouraging improvement, with more patients developing improvement over time. Cohort up to 6 months

(continued)

Table 13.1 (continued)

Study details	Chronicity of injury and biologic used	Follow-up period	Conclusions
Kirchner and Anitua, 2016 (278) Lumbar disc degeneration Observational retrospective pilot study, $n = 86$	Chronic PRGF-Endoret	6 months	Fluoroscopy-guided infiltrations of intervertebral discs and facet joints with PRGF in patients with chronic low back pain resulted in significant pain reduction assessed by VAS The results showed reduction of the VAS over time. The study ended at 6 months with 91% of the patients showing an excellent score, 8.1% showing moderate improvement, and 1.2% showing lack of response

Adapted from: Navani A, Manchikanti L, Albers SL, Latchaw RE, Sanapati J, Kaye AD, Atluri S, Jordan S, Gupta A, Cedeno D, Vallejo A, Fellows B, Knezevic NN, Pappolla M, Diwan S, Trescot AM, Soin A, Kaye AM, Aydin SM, Calodney AK, Candido KD, Bakshi S, Benyamin RM, Vallejo R, Watanabe A, Beall D, Stitik TP, Foye PM, Helander EM, Hirsch JA. Responsible, Safe, and Effective Use of Biologics in the Management of Low Back Pain: American Society of Interventional Pain Physicians (ASIPP) Guidelines. Pain Physician. 2019 Jan;22(1S):S1-S74
PRP platelet-rich plasma, *PRGF* plasma rich in growth factors, *VAS* Visual Analog Scale, *SF-36* 36-item Short Form Survey

47 patients followed for 1 year concluded that a single intradiscal injection of PRP showed significant improvement in pain beginning at an 8-week follow-up that was maintained when compared to control patients at the 1-year follow-up. A meta-analysis including all other studies in Table 13.1 with a pooled patient number of 171 was analyzed, five of the studies showed decrease in pain scores following injection of PRP [31–36]. The combined mean difference in pain scores at the 6-month follow-up was 40.29 ± 13.76 points (95% CI: −67.25 to −13.33, $p < 0.001$, I^2 93.3%). Heterogeneity across all of the studies was high ($I^2 = 98\%$). The 12-month follow-up evaluation had three studies with 63 patients and showed a decrease in post-injection pain scores [31–36]. The combined mean difference in pain scores from baseline to 12-month follow-up was 34.405 ± 6.879 points (95% CI: −47.88 to −20.92, $p < 0$ 0.013, I^2 77.2%). Heterogeneity across the studies at the 12-month follow-up was also high ($I^2 = 77\%$). Due to differences in functional measurement and a lack of detailed data meta-analysis results of functional improvement data were not possible.

13.2.6 Mesenchymal Stem Cells/Medicinal Signalling Cells

As mentioned, the use of MSCs in the intervertebral disc has been characterized by small clinical trials and RCTs with small sample sizes. A recent meta-analysis by Wu et al. [22] conducted a random effects model analysis to assess outcomes. The initial search identified 1393 articles but only six studies were appropriate for review. The characteristics of these studies are shown in Table 13.2. Three of these studies used MSCs [37–39] and three used chondrocytes [40–42] with five of the six

Table 13.2 Characteristics of the studies on cell-based therapy for the treatment of discogenic low back pain

Study	Sample size	Patients age (years)	Population	Cell type	Cell dose and deliver pathway	Follow up (months)	Main evaluation index
Mochida	9	20–29 years	Patients with Pfirrmann grade III disc degeneration and posterior lumbar intervertebral fusion	Autologous cultured nucleus pulposus chondrocytes that cocultured with MSCs	One million activated autologous NP cells were injected into the degenerated disc 7 days after fusion surgery	36 months	JOA and MRI
Kenneth Pettine 2015	26	18–61 years (median 40)	Patients presented with symptomatic moderate-to-severe discogenic low back pain	Autologous bone marrow concentration (non-expanded)	2–3 ml of bone marrow concentrate was injected in lumbar disc (1.66×10^6 ml^{-1})	24 months	ODI, VAS, and MRI
Xiao Dong Pang 2014	2	41.5 ± 3.5 (mean ± SD)	Patients with low back pain >2 years without lower leg pain and provocative discography (+)	Cultured human umbilical cord tissue-derived mesenchymal stem cells	1–2 ml of cultured HUC-MSCs (1×10^7 ml^{-1}) injected into lumbar disc immediately following discography	24 months	ODI and VAS scores
Domagoj Coric 2013	15	19–47 years (median 40)	Patients with single-level, symptomatic lumbar DDD from L3 to S1 and medically refractory low back pain	Expanded allogeneic juvenile chondrocyte cells	Mean 1.3 ml (1–2 ml, 10^7 ml^{-1}) cells solution was injected in the center of the disc space	12 months	ODI and NRS scores, 36-item short form health survey and MRI
Lluis Orozco 2011	10	35 ± 7 (mean ± SD)	Patients with degenerative disc disease and persistent low back pain (>6 months; decrease of disc height >50%)	Autologous expanded bone marrow-derived mesenchymal stem cells	$23 \pm 5 \times 10^6$ autologous expanded BMSCs was injected into the nucleus pulposus area	12 months	ODI and VAS scores and MRI
Hans Joerg Meisel 2006	12	18–75 years	Patients with discogenic pain after repeat discograms. Patients were treated cell therapy at least 3 months post endoscopy	Autologous cultured disc-derived chondrocytes (from surgical treatment of their disc prolapsed)	Cells are injected into disc approximately 12 weeks following discectomy. The cell dose was not mentioned	24 months	ODI, VAS scores, and MRI

Adapted from: Wu, T, Song, HX, Dong, Y, Li, JH. Cell-based therapies for lumbar discogenic low back pain: systematic review and single-arm meta-analysis. *Spine*. 2018; 43: 49–57

studies expanding the number of cells. The number of cells injected into the lumbar intervertebral discs varied widely ranging from 1 to 23 million cells ±5 million cells and the follow-up averaged 22 months, ranging from 12 to 36 months.

The VAS or numerical rating scale (NRS) of the studies had a prominent combined mean statistically significant difference in pain from baseline to follow-up of 44.2 points (95% CI: −61.8 to −26.5, $p < 0.001$, $I^2 = 99.4\%$). The ODI also demonstrated a profoundly positive difference with a pooled mean difference from baseline to follow-up of 32.2 points decreased (95% CI: −41.6 to −22.9, $p < 0.001$, $I^2 = 99.5\%$).

A subgroup analysis that evaluated potential heterogeneity in pain scores in respect to the injected cell type (stem cell versus chondrocyte) or the follow-up time period demonstrated that there was no difference in pain scores between either group in the follow-up times. These results, however, showed that the stem cells were more effective than the chondrocytes in giving rise to significantly less pain ($p < 0.001$).

The meta-analysis of Wu et al. also examined the MR imaging evaluation of patients undergoing cell therapy of the intervertebral disc and found some improvements in the contour and height of the disc along with an increase in the signal within the disc at the 12-month follow-up [22]. The increased signal noted at the final follow-up was initially not there at the 6-month follow-up. In the study by Mochida et al., when the degree of degeneration of the treated disc was less than a Pfirrmann grade III, there were no cases where the disc degeneration worsened at the time of the final 3-year follow-up.

The cellular therapy is designed to augment the existing cells within the intervertebral disc. The disc is a relatively acellular tissue with a cell density of only 5.8×10^3 cells/mm^3, and the cell number decreased prominently with age [43]. These cells play an important role in the production of matrix and in the maintenance of health of the intervertebral disc [43]. The process of DDD involves the loss of matrix and nucleus pulposis (NP) cells, so the therapeutic strategy of cellular therapy is to augment this cell population in an attempt to restore the functioning cell population and the matrix that would follow. The cell types that have been investigated include both autologous and allogeneic chondrocytes and MSCs. In one of the largest clinical trial to date, autologous chondrocytes were obtained from the patients after their discectomies, expanded in culture, and given back to them after cell expansion [42]. Despite the success seen in the trial, it was limited to only patients who required surgery for their disc herniation and to apply this harvesting process in patients who did not have surgery would necessitate a separate interventional procedure prior to cell expansion and reinjection. Allogenic cells donated from health donors and tissue banks would overcome this limitation, and as mentioned, the MSC cells had a significantly better outcome in reducing pain than did the chondrocyte cells [22].

Mesenchymal stem cells, also called medicinal signaling cells (MSCs), can be isolated from a number of different tissues and show promise for repairing tissues in the intervertebral disc [22]. The MSCs can renew themselves while maintaining an undifferentiated phenotype but when exposed to certain stimuli they undergo

differentiation into certain types of cells such as NP cells or chondrocytes. Bone marrow-derived MSCs are the most commonly used autologous stem cell but only represent a small percentage of the total number of cells present in the bone marrow. This low number further decreases with increasing donor age normally. Recent work with MSCs has shown that if cocultured with NP cells, the MSCs could be differentiated into NP cells [44], so MSCs taken from multiple sources could be induced into the type of cell needed for augmenting the intervertebral disc.

The cell number is also something that may be important but remains controversial as to what is the right cell number to inject into the disc. Pettine et al. used unexpanded bone marrow concentrate in their study with an average CD34 cell concentration of 1.66×10^6 per mL, and they used 2–3 mL for the injection [37]. The as of yet unpublished phase II study sponsored by Mesoblast (Mesoblast Ltd., Melbourne, Australia), and evaluating Rexlemestrocel-L, reportedly compared the injection of 6 and 18 million MSCs per disc and found that the six million injection worked better for reducing pain. Therefore, it is thought that identifying the optimal number to inject into each disc is important.

In addition to cell number, some authors have advocated for a carrier to keep the MCSs within the intervertebral disc and to assist and support the MSCs until the cells can graft on to the native anatomy [45, 46]. A carrier or scaffold can be important to protect the cells from the harsh environment of the degenerated disc and can restore some of the support and mechanical function of the disc while the regeneration can take place [47].

13.3 Level I Data with Allogeneic MSCs

13.3.1 Rexlemestrocel-L (Mesoblast)

In addition to the meta-analysis of the completed trials using cellular therapy to treat painful DDD, there are ongoing clinical trials currently being conducted at the time of the writing of this chapter including three open-label single-arm trials and two phase I/II randomized control trials. There have also been two large blinded placebo-controlled RCTs that have been completed. One of these completed trials is coming on the heels of some very positive but as of yet unpublished on the phase II Rexlemestrocel-L trial data and the other large placebo-control RCT is a triple-armed trial with 224 patients that has provisional results that have been reported at multiple conferences [48, 49]. This trial is evaluating a product that contains 6×10^6 combined with micronized disc material ground to a size of 300 μm that is used as an allograft carrier [49].

The phase I work with Rexlemestrocel-L was performed with an ovine model with STRO-1 and STRO-3 antibody-labelled allogeneic MCSs. The intervertebral discs were injected with chondroitinase at three levels to produce a DDD model, and the discs were treated differently (Fig. 13.2). One level was treated with MSCs plus hyaluronic acid (the carrier), one level was treated hyalgan alone, and the other level received no treatment (Fig. 13.2). An MR imaging exam obtained 9 months

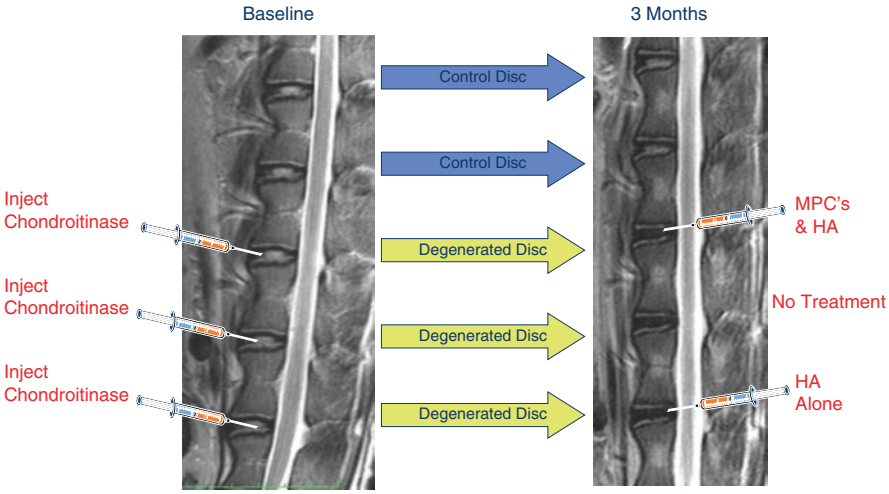


Fig. 13.2 Lateral STIR MR images of an ovine spine showing the baseline intervertebral discs being injected with chondroitinase (image on the left) and the status of the discs at 3 months (image on the right). The chondroitinase produced degeneration of the intervertebral discs at the three levels where it was injected. The three levels received different treatments with one level treated with MSCs and hyaluronic acid (the carrier), one level treated hyaluronic acid alone and the other level received no treatment

after the initial MRI and 3 months after the chondroitinase injection showed complete restoration of the fluid signal within the intervertebral disc treated with the MSCs and hyaluronic acid but no change in the degenerated absence of fluid signal in the other two discs that were not treated with the MSCs (Fig. 13.3).

The phase II trial studying the allogeneic MSCs produced by Mesoblast was a prospective, multicenter, double-blinded, controlled clinical study of two doses of allogeneic MSCs combined with hyaluronic acid in subjects with discogenic low back pain [24]. This study included patients had back pain for more than 6 months, a visual analog score (VAS) of >40, an Oswestry Disability Index (ODI) score of >30, and had failed at least 3 months of nonoperative care. The patient selection included intervertebral discs with a moderate amount of degeneration (modified Pfirrmann score 3–6) [50] without or with a protrusion that was less than 3 mm. Patients were excluded if they had clinically significant radiculopathy, sacroiliac pain, facet-mediated pain (diagnosed via relief from facet injection or medial branch block), severely degenerated discs, or full thickness tears of the annulus fibrosis.

There were 100 total patients that were randomized to one of four treatment arms receiving intradiscal injection: saline (control), hyaluronic acid (HA), HA and six million MSCs, or HA and 18 million MSCs. The pain (VAS) and disability (ODI) were evaluated at 1, 3, 6, 12, and 24 months [24].

At the 24-month follow-up 60.9% of patients receiving six million MPCs had ≥50% pain reduction ($p = 0.020$) and 47.8% ($p = 0.093$) of those receiving 18 million MPCs had more than a 50% reduction in pain (Fig. 13.4). This was compared

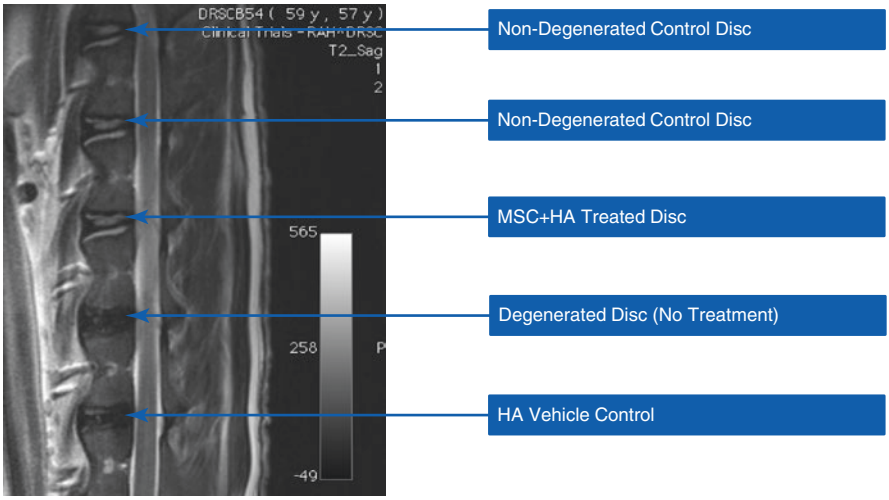


Fig. 13.3 Lateral STIR MR images of an ovine spine taken 6 months after injecting 0.5 million MSCs shows signal similar to the control discs in the disc treated with MSCs but no change in the appearance in the other discs from the MRI obtained 3 months after injecting chondroitinase

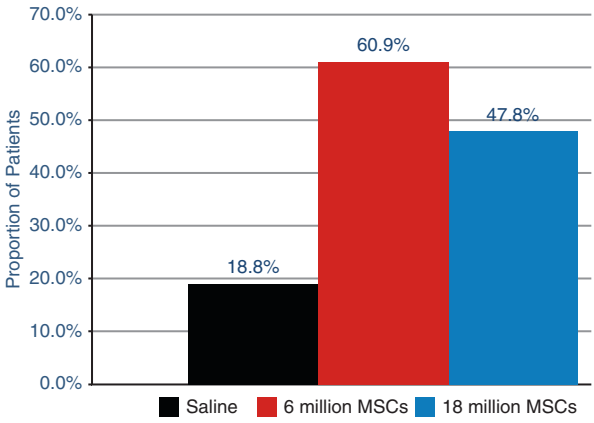


Fig. 13.4 Percent of patients with ≥50% back pain reduction at 24 months

to only 18.8% of controls that had this degree of pain relief. More than half of the patients in the six million MPC arm had complete or near-complete resolution of pain with VAS scores in the range from 0 to 10. The ODI disability scores improved ≥15 points in 56.5% ($p = 0.024$) of the patient injected with six million MSCs and in 60.9% ($p = 0.020$) of the patients in the 18 million MSC arm, compared to only 18.8% in the saline control group (Fig. 13.5) [24].

After a 3-year follow-up, 86% of the patients treated with the six million cell dose that successfully met the 24-month primary end point for pain reduction remained successful at meeting this end point at 36 months (Fig. 13.6) [24]. The

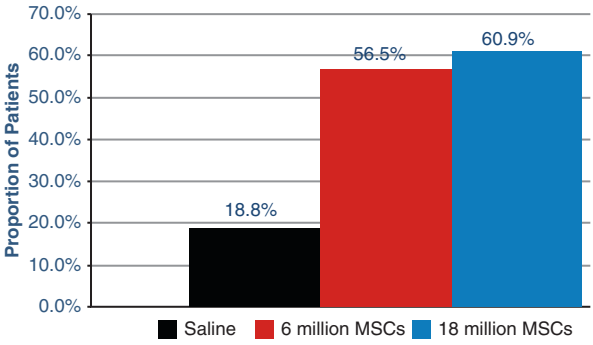


Fig. 13.5 Proportion of patients that had no subsequent intervention after the initial intradiscal injection with at least a 15 point improvement in the Oswestry Disability Index score at the 24 month follow-up

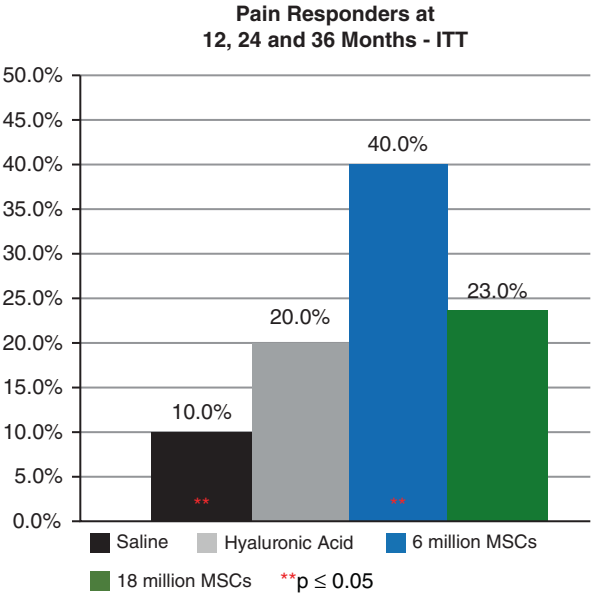


Fig. 13.6 Bar graph demonstrating that 86% of the patients treated with the six million cell dose that successfully met the 24 month primary endpoint for pain reduction remained successful at meeting this endpoint at 36 months. In this trial a pain responder is classified as having a greater than or equal to 40% reduction in pain and no additional intervention

3-year functional improvement showed that 92% of the patients treated with the six million cell dose that successfully met the 24-month primary end point for disability improvement remained successful at meeting this end point at 36 months (Fig. 13.7) [24].

Given this data from the phase II trial of 100 patients, it was concluded that MSCs cells injected into moderately degenerated discs causing discogenic back pain can demonstrate statistically significant improvement in pain and function at 3 years compared to normal saline controls [24]. The dose of six million MSCs demonstrated statistically significant better pain control but equivalent improvements in disability relative to 18 million MSCs. There were no SAEs reported in this trial.

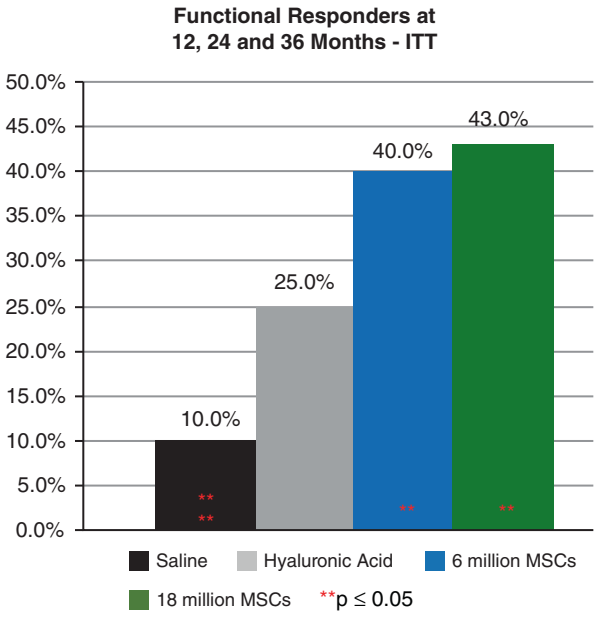


Fig. 13.7 Bar graph demonstrating that 92% of the patients treated with the six million cell dose that successfully met the 24 month primary endpoint for disability improvement remained successful at meeting this endpoint at 36 months. In this trial a functional responder is classified as having a greater than or equal to a 15 point reduction in ODI and no additional intervention

Table 13.3 Current trials involving cellular augmentation of the intervertebral disc in patients with internal disc disruption and discogenic back pain

Sponsor	N	Phase	Design	Cell type	Dosage	Outcomes
Red de Terapia	24	I–II	RCT, 2 arms	Autologous BMSC, cultured	25 M	VAS, ODI, SF-12, MRI, AEs
Bioheart	100	II	Open label, single arm	Autologous AMSC + PRP	Will vary	VAS, ODI
Biostar	8	I–II	Open label, single arm	Autologous AMSC	40 M	VAS, MRI, AEs
Inbo Han, CHA University	10	I	Open label, single arm	Autologous AMSC	20-40 M + HA	VAS, ODI, SF-36, MRI, DHI, AEs
DiscGenics	60	I	DBRCT (HA/Plac)	Allogeneic, cultured	3 M and 9 M + HA	VAS, ODI, EQ-5D, TUG

13.3.2 Ongoing Clinical Trials

At the time of writing this chapter, there were five ongoing trials evaluating the safety and effectiveness of MSCs for treating low back pain associated with DDD including three open-label single-arm trials and two RCTs (Table 13.3). In addition to the studies evaluated in the meta-analysis by Wu et al. [22] and the phase II trial by Mesoblast discussed above, there is a completed phase III Mesoblast trial and a nearly complete three-arm-blinded RCT evaluating a product called VIA Disc® made by Vivex Biomedical (Atlanta, Georgia, USA) (Table 13.4).

Table 13.4 Completed randomized control trials of cellular augmentation of the intervertebral disc in patients with internal disc disruption and discogenic back pain

Sponsor	*N*	Phase	Design	Cell type	Dosage	Outcomes
Mesoblast	404	III	RCT, three arms	Allogeneic MSCs	6MM 6MM + HA	VAS, ODI
Vivex	224	HCTP-361	RCT, three arms	Viable allograft	6 MM + micronized disc material	VAS, ODI

The largest trials are evaluating the use of allogeneic MSCs that are selected through a specific enzymatic reagent and mechanical process either by immunoselection and cell sorting or by isolating and growing intermediate cells into discogenic cells. These cells are already predisposed to in vitro or in vivo differentiation into cells of bone, fat, and cartilage lineages and are not immunogenic. It is the hope that allogeneic MSC therapy can offer "off-the-shelf" treatment with a defined product that has established potency assays and batch-to-batch consistency. In addition to the presence of growth factors, the cellular component is thought to have great promise due to its ability to respond to injury-specific microenvironmental cues by detecting injury, releasing a wide range of biomolecules, increasing proteoglycan synthesis, increasing migration and proliferation of nucleus cells, and by repairing the intervertebral disc [48].

13.3.3 Progenitor Stem Cells (VIA Disc® by Vivex)

The largest randomized control with provisional data at the time of writing this chapter is from the Viable Allograft for Intervertebral Disc Supplementation (VAST) Trial. This trial was performed to evaluate the safety and effectiveness of MSCs mixed with allograft disc material in treating patients with painful DDD.

There were 224 patients at five U.S. sites enrolled in the trial which was segmented into a treatment group, an NSM group, and a placebo-control group with a 3.5:1:1 randomization ratio. The first 24 participants were assessed at a 1-month posttreatment visit to assess for safety. There were two co-primary end points including back pain as measured by the visual analog scale (VAS) and function as measured by the Oswestry Disability Index (ODI). The primary end points were evaluated along with safety data and reported adverse events (AEs) and changes in clinical laboratory evaluations. The data was collected at baseline and at 3, 6, and 12 months. Structural evaluation was also performed, and imaging studies including X-rays and MR imaging were performed at 6 and 12 months.

The data on the first 24 patients out to the 1-year follow-up was available at the time of writing this chapter. At the 6- and 12-month time points, VAS back pain improved from 58.13, 60.0, and 59.75 in the allograft, placebo, and NSM subjects, respectively to 16.40, 28.60, and 16.0 at 6 months, and 9.85, 27.0, and 6.0 at 12 months (Fig. 13.8). At 3 months, the VAS of the NSM group was 55.0. There was an option for the NSM patients to crossover to the allograft treatment group at the 3-month time point and all subjects elected to crossover to allograft treatment. At

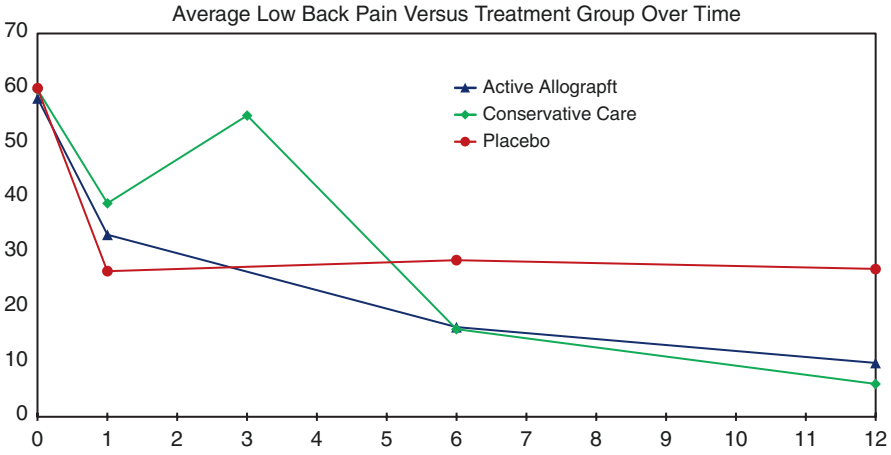


Fig. 13.8 Visual analog scores as measured from the baseline and at 1, 3, 6, and 12 months. All treated patients experienced pain relief with the allograft patients (blue line) and the conservative care (green line) experiencing the greatest amount of relief and the placebo patients (red line) experiencing the least pain relief. It should be noted that all conservative care patients crossed over to allograft treatment at 3 months

the 6- and 12-month time points, the ODI improved from 54.67, 50.40, and 51.75 in the allograft, placebo, and NSM subjects, respectively to 19.73, 15.25, and 22.50 at 6 months and 12.85, 17.5, and 20.0 at 12 months (Fig. 13.9). At 3 months, the ODI of the NSM group was 65.5 and all subjects crossed over to allograft treatment. At 12 months, there were no AEs in the first 24 participants, and the MRI evaluation showed anatomic improvement of the disc and enhanced nucleus signal (Figs. 13.10 and 13.11).

The first 24 subjects had full data collected at 1 year as part of a safety assessment. This was included as part of this large triple-arm prospective randomized control trial. The safety data showed that a delivery of a viscous cellular allograft can be done safely with no AEs in these initial subjects followed up to 1 year. The patients receiving the allograft had a very high level of pain decrease and functional improvement compared to the placebo and NSM cohorts, and those NSM subjects crossing over to allograft supplementation attained similar pain and functional improvements to those initially randomized to receive the active treatment (Figs. 13.8 and 13.9). The safety data was not powered for statistical significance, but the prominent improvements in pain and function trend towards the possibility of statistically significant differences at the final analysis of the data that will be performed after completion of this chapter.

The cellular disc allograft VIA Disc was originally developed after a cellular bone allograft and was used for an interbody fusion which resulted in a pseudoarthrosis. The pseudoarthrosis was revised, and an incomplete discectomy was determined to be the primary cause of the non-union. The residual disc fragments were sent to pathology for further evaluation, and spheroids of disc regeneration were found in the residual disc tissue surrounding the interbody fusion cage (Fig. 13.12).

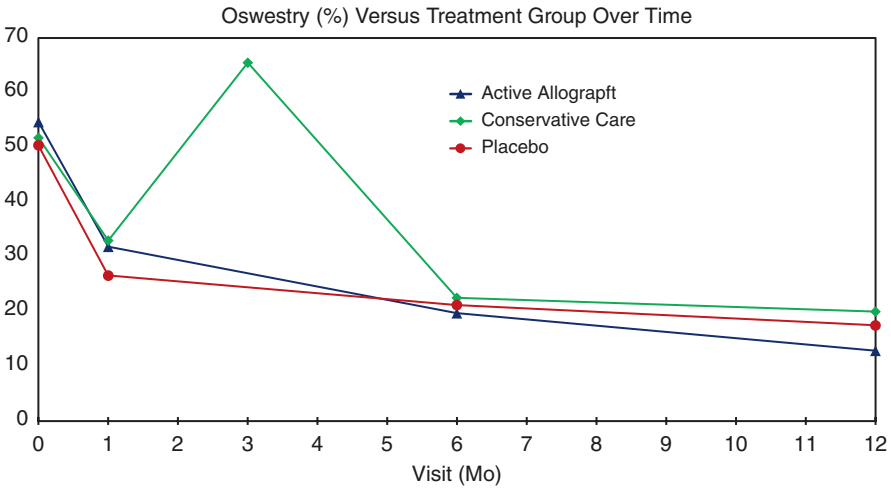


Fig. 13.9 Oswestry Disability Index scores as measured from the baseline and at 1, 3, 6, and 12 months. All treated patients experienced functional improvement with the allograft patients (blue line) experiencing the greatest degree of functional improvement and the conservative care (green line) and the placebo patients (red line) experiencing functional improvement to a lesser degree. It should be again noted that all conservative care patients crossed over to allograft treatment at 3 months

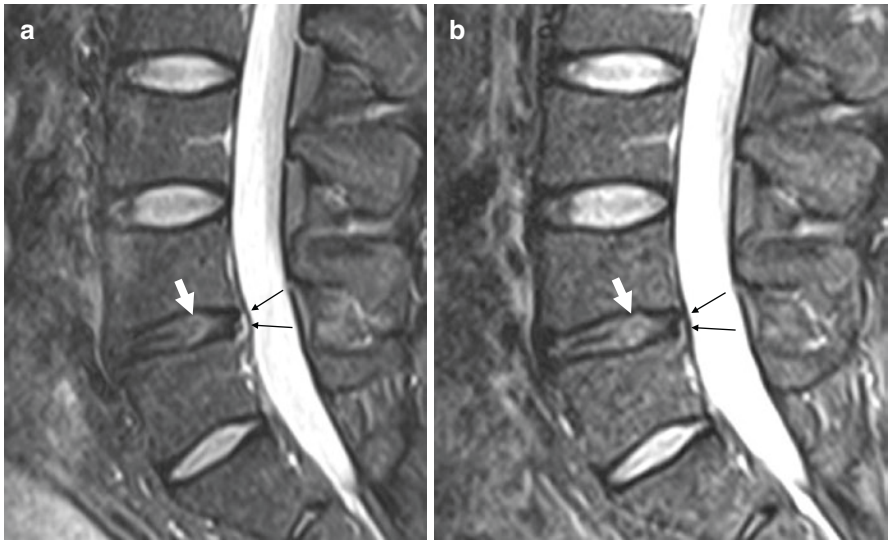


Fig. 13.10 Lateral STIR MR images of the lumbar spine from the same patient taken at baseline (**a**) and 6 months following injection of ViaDisc (**b**) showing less posterior prominence of the intervertebral disc at the 6 month time point (black arrows) and increased signal within the nucleus pulposis (white arrowhead). The subject's VAS and ODI decreased from 58 and 50 to 0 and 0 respectively at 6 months

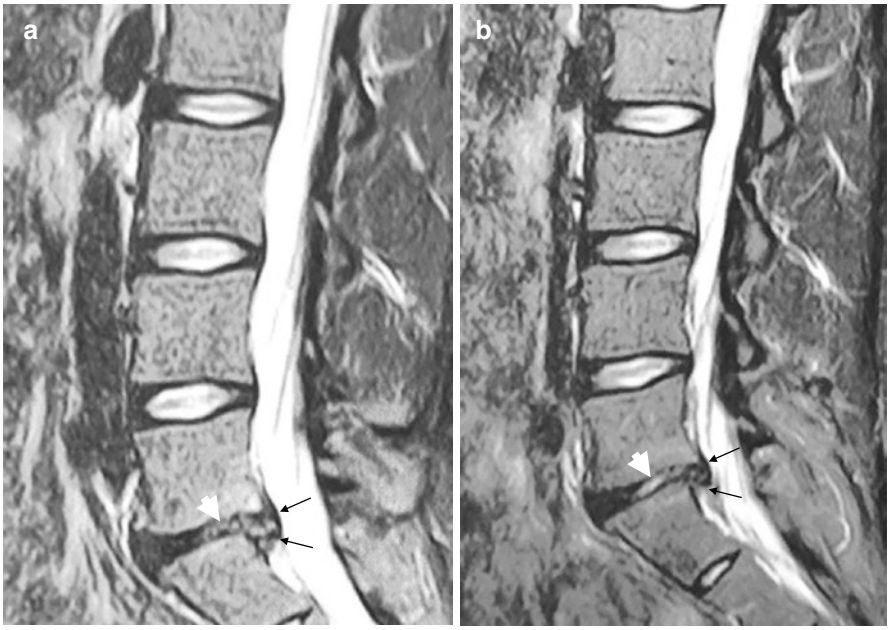


Fig. 13.11 Lateral STIR MR images of the lumbar spine from the same patient taken at baseline (**a**) and 1 year following injection of ViaDisc (**b**) demonstrates healing of a disc protrusion (black arrows) and increased signal within the nucleus pulposis (white arrowhead). The subject's VAS and ODI decreased from 70 and 58 to 15 and 22 respectively at 1 year

The cells, which are isolated by an enzymatic digestion process from the hypoxic region of the bone marrow immediately adjacent to the vertebral endplate, were then placed into a more concentrated solution, and micronized disc material was used as a carrier when injecting into the intervertebral discs (Fig. 13.13). The components of the liquid MSC solution and the micronized disc material comprise the VIA Disc product. The liquid component contains concentrated cells that are identifiable as MSCs, and the micronized disc material also contains various growth factors that are involved in cell survival and proliferation as well as in suppression of inflammation (Figs. 13.14, 13.15, 13.16, 13.17, and 13.18). The cells are stored in a dimethyl sulfoxide (DMSO)-free cryoprotectant at −75 °C and then thawed and reconstituted before use. The cell viability has shown to be consistently greater than 75% thereby ensuring that the target number of six million viable cells will be available for injection into the disc (Fig. 13.19).

At the time of writing this chapter, the data collection for the VAST trial was continuing. The 1-year data should be collected by the end of 2019 and complete results will be available shortly after that. Based on the previous data as discussed in the meta-analysis by Wu et al., the Rexlemestrocel phase II data and the

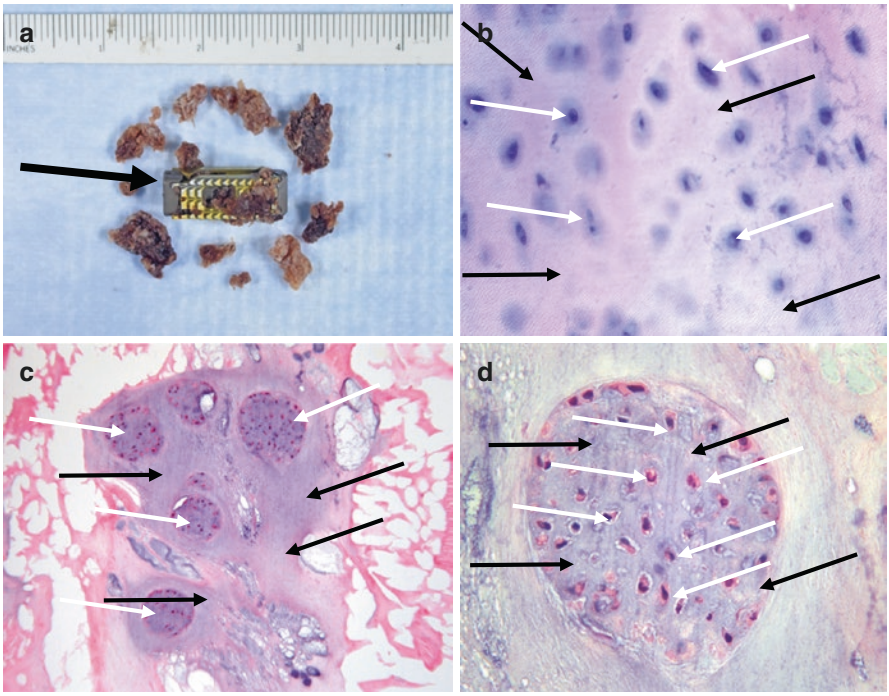


Fig. 13.12 (**a**) Residual fragments of the residual intervertebral disc removed from the pseudoarthrosis along the the interbody fusion cage (black arrow in **a**). Representative hematoxylin-eosin histophotomicrographs at 400× (**b**), 100× (**c**), and 200× (**d**) show fibrocartilate from the annulus and cloning of the chondrocytes (white arrows in **c** and **d**) surrounded by new matrix (black arrows in **c** and **d**). A high powered view (**b**) shows chondrocytes (white arrows in **b**) from the endplate of the vertebral body producing hyaline cartilage matrix (black arrows in **b**).

Fig. 13.13 Vial of allogeneic MSCs isolated from the hypoxic region of the bone marrow adjacent to the endplate containing six million cells (vial on left) is mixed with the micronized disc material (powder in small dish on the right) which forms a liquid with a thin paste consistency that can be injected into the intervertebral disc

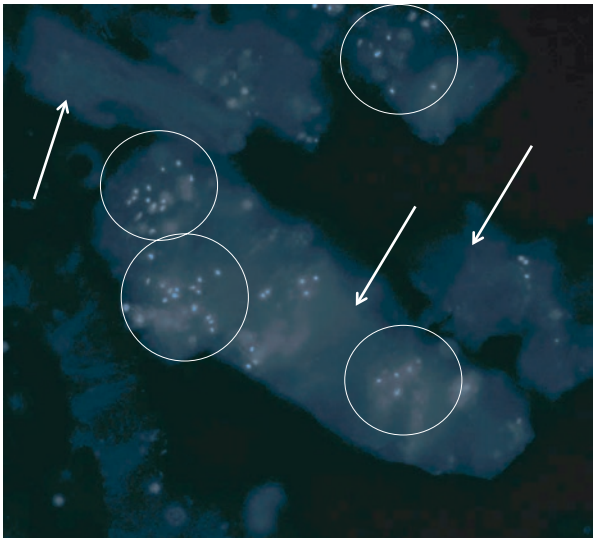


Fig. 13.14 Histophotomicrograph intervertebral disc material (white arrows) ground to 300 μm and having undergone DAPI staining showing DNA seen as light blue dots (within the white circles) by fluorescence microscopy

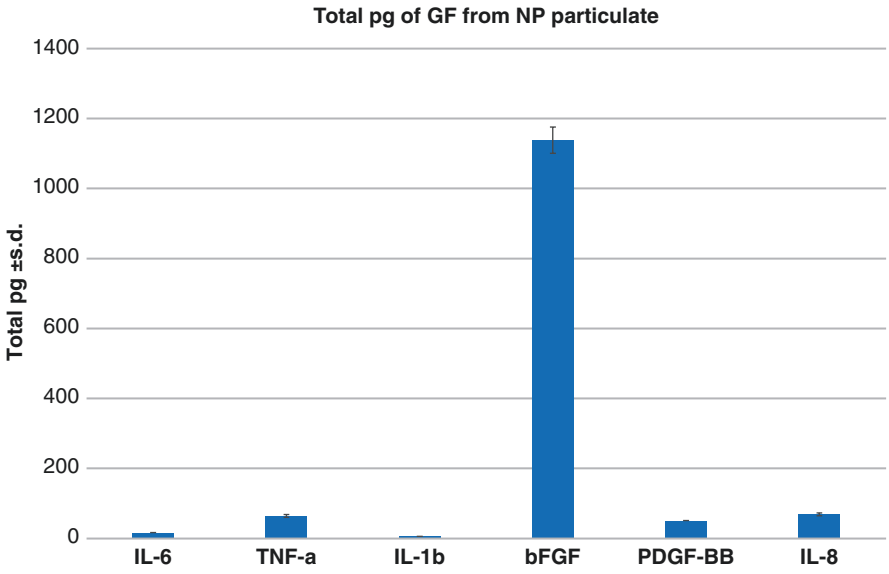


Fig. 13.15 A bar graph showing the measurements of the amount (in pg) and type of growth factors present in the micronized disc material that is mixed with the bone marrow derived MSCs to make ViaDisc. *IL* interleukin, *pg* pictograms, *TNF* tumor necrosis factor, *FGF* fibroblast growth factor, *PDGF* platelet-derived growth factor, *NP* nucleus pulposis

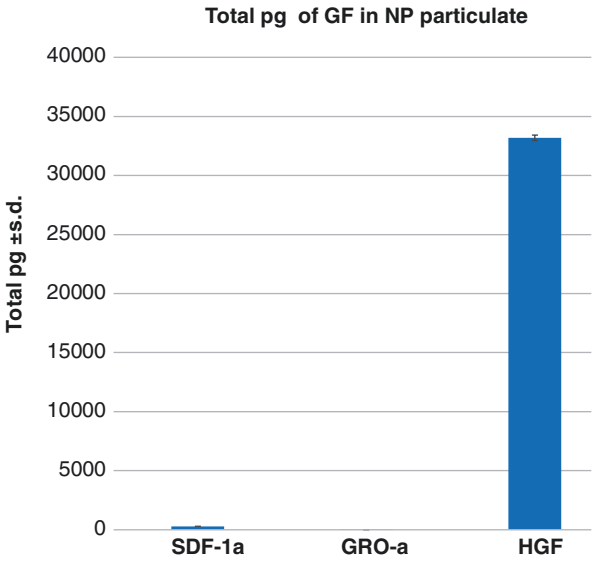


Fig. 13.16 Bar graph showing the measurements of the amount (in pg) and type of growth factors present in the micronized disc material that is mixed with the bone marrow derived MSCs to make ViaDisc. *SDF* stromal cell-derived factor, *GRO* gro alpha, *HGF* hepatocyte growth factor

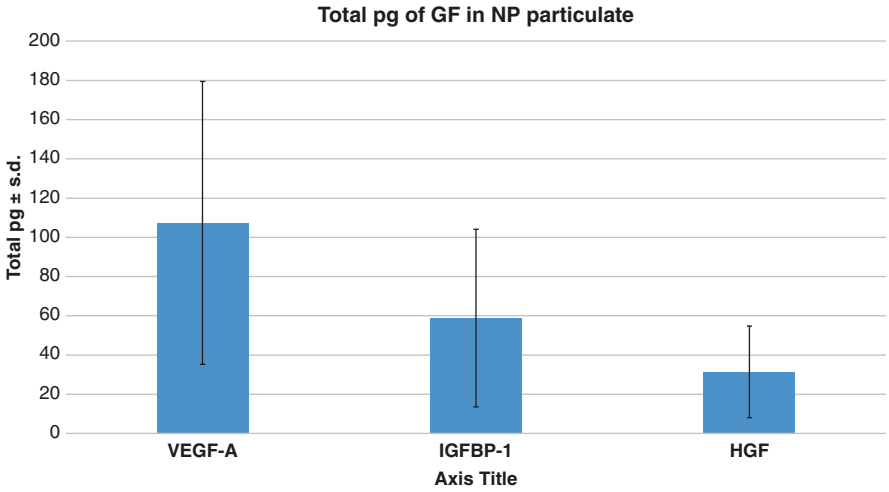
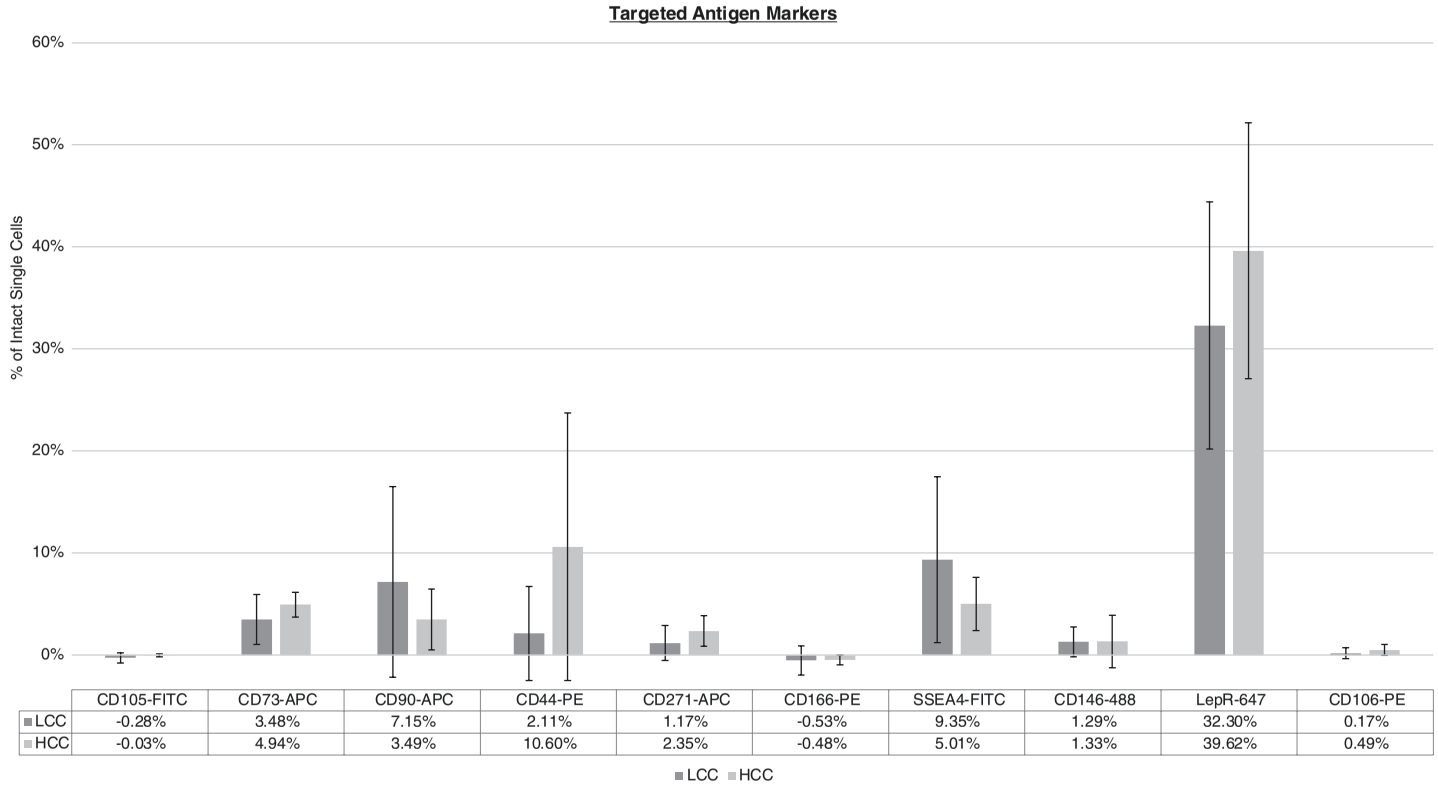


Fig. 13.17 A bar graph showing the measurements of the amount (in pg) and type of growth factors present in the micronized disc material that is mixed with the bone marrow derived MSCs to make ViaDisc. *VEGF* vascular endothelial growth factor, *IGFBP* insulin-like growth factor-binding protein, *HGF* hepatocyte growth factor



	CD105-FITC	CD73-APC	CD90-APC	CD44-PE	CD271-APC	CD166-PE	SSEA4-FITC	CD146-488	LepR-647	CD106-PE
LCC	-0.28%	3.48%	7.15%	2.11%	1.17%	-0.53%	9.35%	1.29%	32.30%	0.17%
HCC	-0.03%	4.94%	3.49%	10.60%	2.35%	-0.48%	5.01%	1.33%	39.62%	0.49%

Fig. 13.18 Bar graph showing the percentage of intact single cells with the antigen markers consistent with MCSs. *CD* cluster of differentiation, *FITC* fluorescein isothiocyanate, *APC* antigen presenting cell, *PE* phycoerythrin, *SSEA* stage-specific embryonic antigen, *LepR* leptin receptor

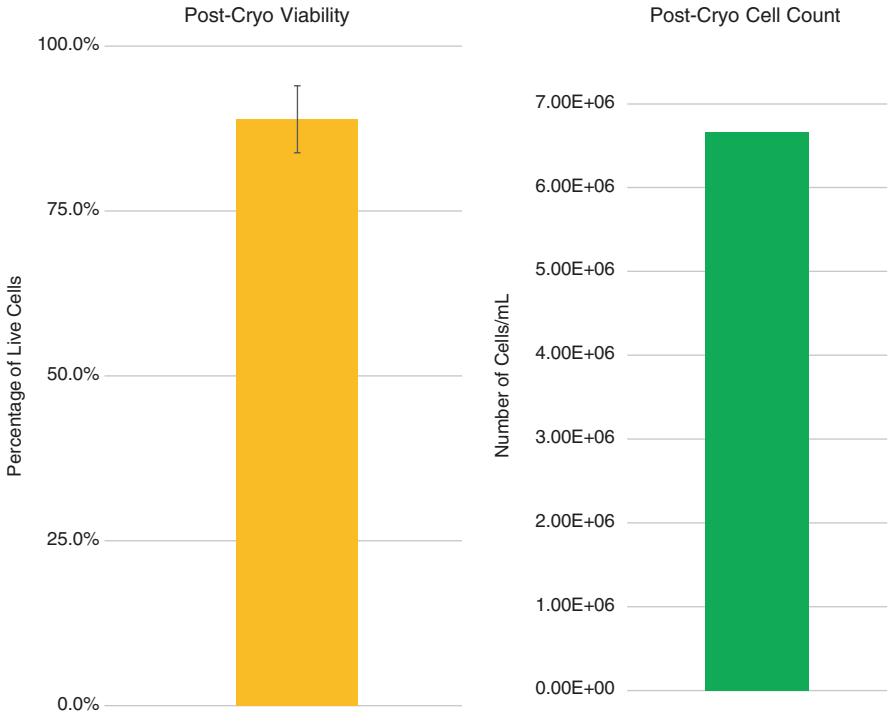


Fig. 13.19 A bar graph showing the percentage of viable cells (**a**) after the MSCs have been thawed. The cell number available after the thawing process is demonstrated in the bar graph on the right (**b**). The objective is to have consistently greater than 75% cell viability and to deliver at least six million viable cells to the intervertebral disc by injection

provisional data from the first 24 patients in the VAST trial, the final data should be optimal and could usher in an entirely new and highly effective treatment option for patients with stable discogenic back pain [22].

13.4 Intervertebral Disc Injection Protocol

13.4.1 Eligibility

Patients who are optimal candidates for injection of intradiscal biologics have painful discs that are moderately degenerated and tend to be typically younger and healthier than patients with severely degenerated discs. Most of the trials and use of intradiscal biologics have been in intervertebral discs that are moderately degenerated rather than discs that are severely degenerated due to the assumptions that moderately degenerated discs are capable of regeneration and should be treated at this point prior to when the degeneration can reach a point where the tissue cannot recover and/or be regenerated. There are certain eligibility criteria that are used to

Table 13.5 Patient eligibility criteria for intradiscal regenerative biologic treatment

1. Age 18 and 60 years inclusive
2. Male or female
3. Body mass index <35
4. Modified Pfirrmann grade (3–6)
5. Radiographic confirmation by MRI/X-ray of: (a) Translational instability defined as ≤5 mm or (b) Angular instability defined as ≤5
6. Back pain (with or without radicular leg pain) measured by: (a) ODI of at least 40% (b) VASPI of at least 40 mm
7. Pathologic level between L1 and S1
8. One or two vertebral level involvement that has been evaluated for at least 6 months and treated with conservative care
9. Psychosocially, mentally, and physically able to fully comply with physician treated with conservative care
10. No history of malignancy or chronic infectious disease (e.g., HIV, hepatitis)
11. Patients with mechanical instability and/or type III Modic changes should be excluded

determine whether a patient is an optimal candidate for an intradiscal regenerative biologic treatment. An example of a fairly restrictive set of criteria that has been used for intradiscal allogeneic MSC trials is shown in Table 13.5. Clinical application of these criteria can be less stringent than that used for an RCT and should be left to the discretion of the treating physician.

13.4.2 Standards for Pre-procedural, Intra-procedural, and Post-procedural Treatment

13.4.2.1 Pre-procedure

The procedure to inject the intervertebral disc should be described to the patient. An example of this is to discuss that the treatment is a human donor (or describe the product origin) product used to repair damaged discs resulting from DDD. Degenerative disc disease arises as the result in loss of hydration and ultimately tissue matrix within the intervertebral discs (IVD). The intradiscal biologic product is intended to replace or supplement the degenerated tissue in the disc in which it is injected. The disc injection procedure is a nonsurgical, minimally invasive procedure. The material is injected under fluoroscopic imaging into the nucleus pulposis of the disc and fills the voids in the damaged disc, providing hydration and supporting the disc to function as intended.

Risks, Benefits, and Alternatives

As with any spine injection procedure, the risks include infection, bleeding, nerve damage, localized increase in pain, anxiety, discitis, osteomyelitis, and immune reaction to the injected material. The risk of any of these occurring is quite low, and there were no adverse events recorded in the safety data obtained from the VAST trial, and Wu et al. reported no related adverse events in any of their included study

[22, 49]. There have also been no tumor formation observed in any clinical cases in stem cell transplantation during the observed follow-up period [22].

The potential benefits include improvements in pain, function, and quality of life. In the provisional data collected from the VAST Trial, the visual analog scale (VAS) improvement of 48.3 points and an Oswestry Disability Index (ODI) improvement of 41.8 were profoundly positive [49]. The meta-analysis by Wu et al. reported a mean VAS reduction of 44.2 points and a mean ODI improvement of 32.2 points [22]. Additionally, based on intradiscal stem cell data, the change and improvement appear to be a long-lasting or permanent benefit.

The alternatives to treatment in a patient with painful discogenic back pain include injections of anesthetic and anti-inflammatory agents into the disc, basivertebral nerve ablation, and fusion or disc replacement surgery. Intradiscal biologic treatment is recommended after injection therapies fail to provide durable relief of symptoms and in a disc that is mild to moderately degenerated with a Modified Pfirrmann Grade of 3–6. When there is severe degeneration with degenerative endplate change, either basivertebral nerve ablation or disc replacement surgery can be used if the lumbar segment is stable or surgical fusion if the segment is unstable.

13.4.2.2 Intra-procedure

Injection of the intradiscal biologics is performed with the same technique as any intradiscal injection. Intravenous access is obtained and the patient is given intravenous antibiotics just before the injection. The recommended antibiotics include 1–2 g of cefazolin (Ancef) and 80 to 160 mg of gentamicin. Patients with an allergy to penicillin should be given 600–900 mg of clindamycin in place of cefazolin with the higher dose for all antibiotics given to patients weighing 90 kg or more. Patients with impaired renal function should have the dosage adjusted accordingly. In addition to pre-procedure antibiotics, an injection of the anti-inflammatory medication ketorolac (Toradol) 30 mg intravenously given before and after the injection can help with procedural and post-procedural pain.

Moderate sedation is recommended in these patients as injection into a pain generating intervertebral disc can be painful. Typical agents given for moderate sedation include midazolam and fentanyl, and the dosage for this procedure can range from 1 to 5 mg of midazolam and from 25 to 100 μg of fentanyl. Other sedatives such as ketamine or propofol may be used in certain circumstances such as in an opioid-tolerant patient, if needed, but must be administered carefully by experienced personnel.

The injection into the intervertebral disc is performed the same as traditional lumbar discography with a 22-G spinal needle placed through Kambin's safe triangle into the disc from a posterolateral oblique approach using fluoroscopy or computed tomography (CT) guidance (Fig. 13.20). The needle is placed into the center of the disc (Fig. 13.20). An alternative approach is to use an 18-gauge needle that is placed just outside the intervertebral disc and a 22-G needle is placed through the 18-G needle into the nucleus pulposis of the target disc. The product is then injected through the 22-G needle into the disc using moderate consistent pressure. Following the injection, the needle(s) is/are removed, and the patient is taken

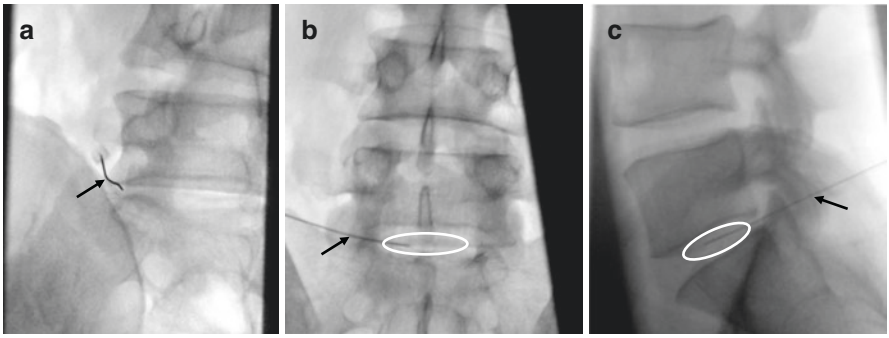


Fig. 13.20 Fluoroscopic views in the oblique (**a**), anteroposterior (**b**), and the lateral (**c**) views showing a 22 G needle (black arrows) places within the center of the intervertebral disc as confirmed on the anteroposterior and laterl fluoroscopic views (area within the oval in **b** and **c**)

to the recovery area for further observation and/or monitoring is done until they are ready to be released.

13.4.2.3 Post-procedure

The patient is instructed to limit physical activity to the normal activities of daily life and to limit strenuous activity for 72 h. After that they are instructed to resume all normal activities within reason keeping in mind that the regeneration time for the intervertebral disc can be up to 6 months.

Prescriptions are given to the patient for medications to take as needed including a steroid dose pack (i.e., Medrol Dosepak), a muscle relaxer (i.e., metaxalone 800 mg), and a narcotic (i.e., hydrocodone/acetaminophen or oxycodone/acetaminophen 10/325 mg). Although these medications are typically needed sparingly or not at all, there is a subgroup of patients that experience moderate-to-severe pain after injection of an intradiscal substance and oral medications may be needed promptly to treat this post-injection pain and discomfort. An icepack may be given to the patient to place over the injection site in the event of post-injection site discomfort.

A follow-up appointment is made 2–4 weeks after the injection to see how the patient is doing and to assess for significant post-injection discomfort. Additional follow-up appointments can be made at the discretion of treating physicians to assess the patient's treatment progress.

13.5 Noninfectious Reactions Seen with Intradiscal Biologics and Other Materials

Pain related to the intervertebral disc has been a known issue in healthcare for decades. The concept of discs that appear similar or just slightly different on imaging but that present with differing clinical presentations of pain is known but still a somewhat difficult concept to grasp. Over the years, there have been many attempts

to diagnose and treat discogenic-mediated pain. Discography has been and remains one of the standard diagnostic procedures used to evaluate the disc. Pressurization to create a provocative pain response is an important component of the study as is the assessment of contrast flow and the subsequent response to anesthetic infusion.

When the annulus fibrosis is breached during discography, this gives rise to the possibility of infectious discitis. The infection rate for discography had originally been reported to be 1–4% [25]. This unacceptably high infection rate has subsequently been profoundly reduced by sterile technique, different needle techniques, and pre-procedure IV antibiotics [26]. Modern techniques have resulted in negligible rate of discitis after discography [51].

Reactive changes that mimic infection have previously been reported including changes from seronegative spondyloarthropathies, neuropathic spine, acute cartilaginous nodes, and other conditions [52]. Recently, the authors of this chapter have noted noninfectious reactive changes of the disc associated with injections of many of the intradiscal biologics described above. Just as with some of the other noninfectious entities that can mimic discitis, these post-injection changes associated with intradiscal biologics can look identical to discitis on cross-sectional imaging (Figs. 13.21, 13.22, 13.23, and 13.24).

Anytime there has been a violation of the annulus, whether for discography or biologic treatment, discitis is possible. Differentiating between an infectious cause and a noninfectious cause or biologic immune reaction is of paramount importance. Discitis will most commonly present with an increase in axial back pain with or without radiating pain. There are similar clinical and radiological characteristics for both types of conditions. Timing of this increase in pain can be one of the important differentiating factors that separates infectious discitis from noninfectious inflammatory changes. Regardless of the type of process affecting the disc, a diagnostic workup must be done promptly to include MR imaging and laboratory testing with assessment of WBC (white blood cell count), CRP (C-reactive protein), and ESR (erythrocyte sedimentation rate).

Either form of reactive changed in and around the disc will appear similar on cross-sectional imaging. The T2-weighted images often reveal increased signal approximating the vertebral endplates adjacent to the suspected disc (Figs. 13.21, 13.22 and 13.24). This can be seen with or without endplate erosions or enlarged Schmorl's nodes (Figs. 13.21, 13.22, 13.23, and 13.24). The most common site for these changes will be at the center of the endplate where the endplate cartilage is thin and most nutrient transfer occurs. Laboratory values can be normal in both types of processes, especially if the inflammatory response is confined to the intervertebral disc. The probability of an infectious cause is higher when the laboratory values including the CRP, ESR, and the WBC become elevated, and the probability of a noninfectious process increases when these laboratory values remain either normal or very slightly elevated.

There should be an increased suspicion of infectious discitis, if fluid signal is observed within the nucleus pulposis [53]. Extra annular or paraspinal inflammatory response or fluid signal also elevates suspicion for infection and should increase the urgency to treat [53]. In these cases, antibiotics should be started and a disc

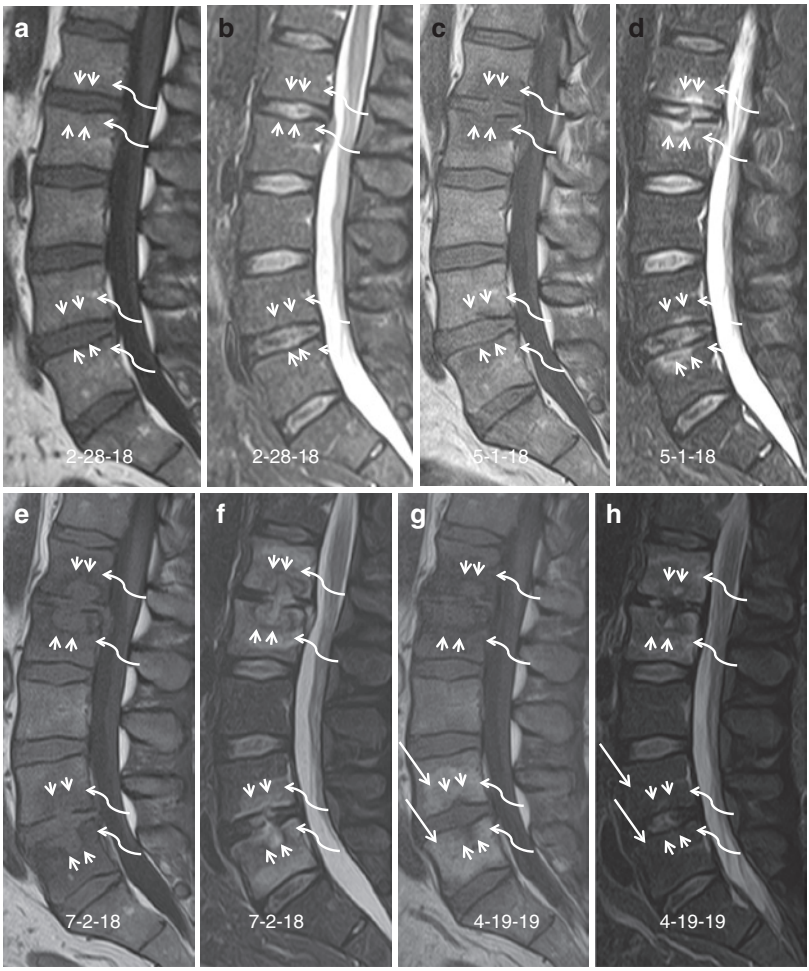


Fig. 13.21 (**a**–**f**) Lateral T1-weighted (**a**, **c**, **e**, and **g**) and STIR (**b**, **d**, **f** and **h**) MR images taken from a 44 year old male before (**a**, **b**) and after (**c**–**h**) injection of autologous stem cells. The dates are displayed at the bottom of each image. Signal and endplate changes were noted at the injected L1–2 and L4–5 levels progressing from the normal pre-procedure appearance of the endplates (white arrowheads in **a**) and the marrow (curved white arrows in **a**) to erosions of the endplates best seen on the T1-weighted images (white arrowheads **c**, **e**, and **g**) and prominent endplate edema characterized and best seen as increased signal on the STIR images (curved white arrows in **d**, **f**, and **h**). The edema was first noted when the patient initially presented with pain just over 2 months after injection (curved white arrows in **c** and **d**) and the edema progressed to its maximal amount approximately 4 months after injection (curved white arrows in **e** and **f**) and began to normalize approximately 13.5 months after injection (curved white arrows in **g** and **h**) with regions surrounding the L4–5 endplate resembling Modic type 1 endplate changes (straight white arrows in **g** and **h**) with fat signal on the T1-weighted images (straight white arrows in **g**) which is isointense to the surrounding marrow on the STIR images (straight white arrows in **h**). The patient's sed rate and C-reactive protein levels were never greater than 22 and 1 respectively and the patient was treated expectantly with medication for pain and no antibiotics due to a provisional diagnosis of non-infectious reactive changes rather than discitis primarily due to the time course of appearance of symptoms and the normal sed rate and C-reactive protein

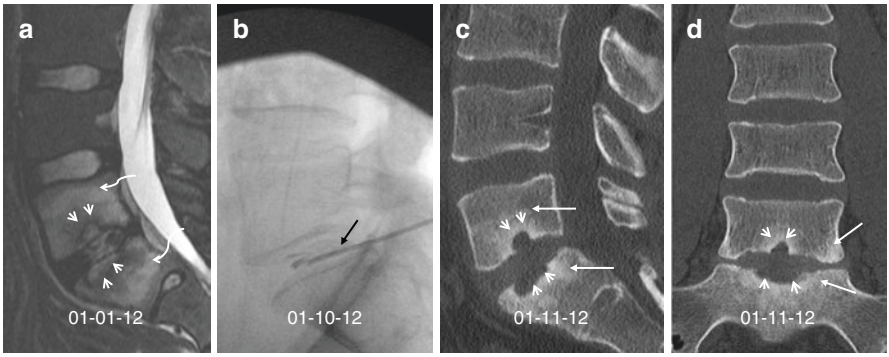


Fig. 13.22 (**a**–**d**) Lateral T2-weighted MR image (**a**), lateral fluoroscopic image (**b**) and sagittal and coronal CT reconstructed images (**c** and **d** respectively) from a 23 year old male taken after injection of fibrin sealant (Biostat) on 08-05-11 show edema in the L5 and S1 vertebral bodies (curved white arrows in **a**) along with endplate erosions (white arrowheads in **a**, **c** and **d**). The CT images also show reactive osseous sclerosis around the endplate erosions (straight white arrows in **c** and **d**). The lateral fluoroscopic image shows a needle and auger device (black arrow) sampling the disc tissue. The dates are displayed at the bottom of each image and the patient's symptoms began approximately 4 months after injection of the MSCs. Disc biopsy showed no evidence of discitis with a negative gram stain and culture and the patient's sed rate and C-reactive protein levels were never greater than 22 and 1 respectively. He was treated expectantly with medication for pain and no antibiotics due to a provisional diagnosis of non-infectious reactive changes rather than discitis primarily due to the time course of appearance of symptoms and the normal sed rate and C-reactive protein

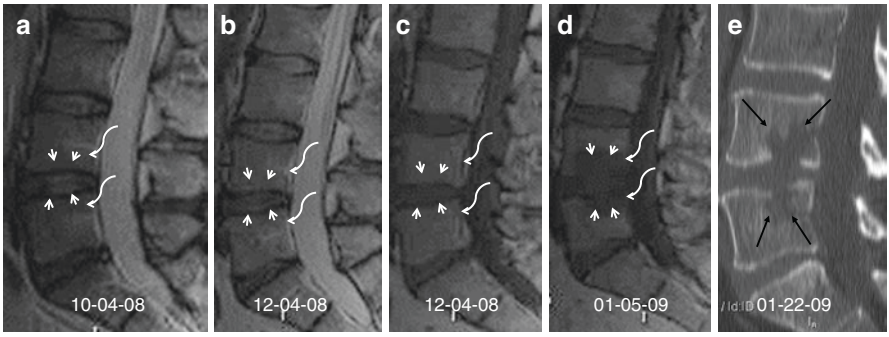


Fig. 13.23 (**a**–**e**) Sagittal T2-weighted (**a**) taken before injection of BMP-7 and sagittal T2-weighted (**b**) sagittal T1-weighted (**c** and **d**) MR images and sagittal CT reconstruction (**e**) images taken from a 44 year old male after injection of BMP-7 at the L4-5 level done on 10-22-08. The dates are displayed at the bottom of each image. Signal and endplate alterations are seen progressing from the normal pre-procedure and early post-injection appearance of the endplates (white arrowheads in **a**, **b** and **c**) and the marrow (curved white arrows in **a**, **b** and **c**) to erosions of the endplates best seen on the T1-weighted images (white arrowheads in **d**) and prominent endplate edema best seen as decreased signal on the T1-weighted MR images (curved white arrows in **d**). The CT images taken 3 months after the injection of BMP-7 show prominent endplate erosions at the L4–5 level (black arrows in **e**). The edema was first noted when the patient initially presented with pain at 10 weeks after injection (curved white arrows in **d**). Disc biopsy done 3 months after the injection showed no bacteria and the culture of this specimen was negative. The patient's sed rate and C-reactive protein levels were normal at 14 and 0.9 respectively and the patient improved with supportive treatment resulting in a provisional diagnosis of non-infectious reactive changes rather than discitis primarily due to the time course of appearance of symptoms and the negative inflammatory markers

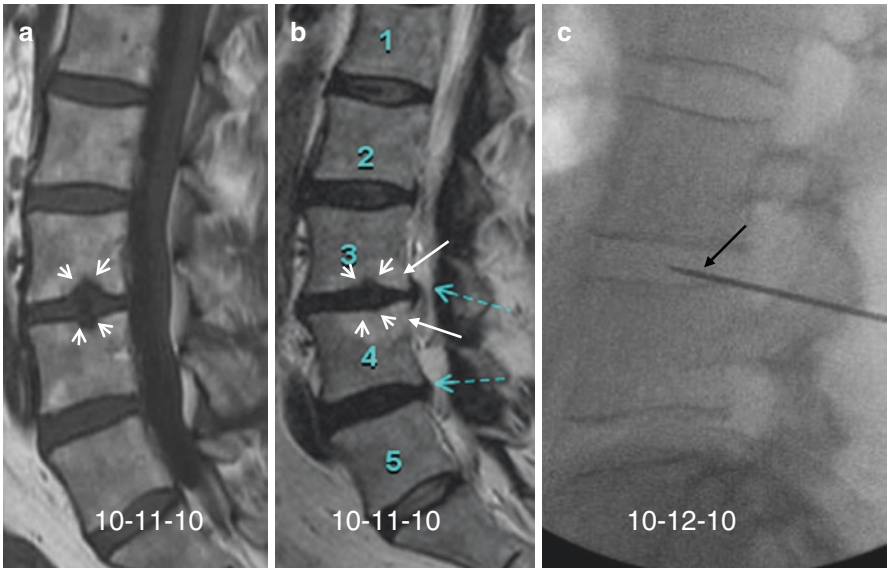


Fig. 13.24 (**a**–**c**) Sagittal T1-weighted (**a**), sagittal T2-weighted (**b**) and lateral fluoroscopic image from a 56 year old female after injection of a fibrin sealant (Tisseel) done on 08-30-10. The dates are displayed at the bottom of each image. Endplate erosions are seen on both the T1 and T2-weighted MR images (white arrowheads in **a** and **b**) and marrow edema is seen adjacent to these erosions on the sagittal T2 weighted image (white arrows in **b**). Disc bulging at both the L3–4 and L4–5 levels was also present and best seen on the sagittal T2-weighted images (blue dashed arrows in **b**). The edema was first noted when the patient initially presented with pain less than 6 weeks after injection (white arrows in **b**). Disc biopsy (black arrow in **c**) done 6 weeks after the injection showed numerous gram positive cocci. The diagnosis of discitis was made and the patient was treated with intravenous antibiotics

biopsy and aspiration should be considered. The most effective method of acquiring a sample of the nucleus is by using a mechanical biopsy device such as a core biopsy needle or an auger type device such as the Dekompressor (Stryker Corporation, Kalamazoo, MI). These techniques typically produce a large enough sample for gram stain and culture. During a biopsy, the disc can be flushed with antibiotics and, depending on the results of the biopsy, intravenous antibiotics can be started.

Noninfectious discitis has been documented after numerous types of biologic injections into the intervertebral disc. These changes are usually seen between 8 and 16 weeks after the disc injection procedure which is a longer time interval than changes due to infection that usually occurs within 4 weeks post-procedure (Table 13.6). The incidence of noninfectious discitis after biologic intradiscal injection cannot be accurately defined at this time due to the uncommon occurrence of this entity and the inconsistency of the various biologic products. Each of the products tested have potentially different mechanisms of actions within the intervertebral disc but the biologic reactive response may appear similar on the follow-up imaging evaluations. The exact etiology of these types of inflammatory reactions is

Table 13.6 Potential complications associated with injection of biologics into the intervertebral disc and the post-injection timeframe in which these complications usually occur

Timeline of potential complications with intradiscal biologics	
Timeline, weeks	Complication
0–2	Pain with injection – Under 2 cc causes minimal irritation and pressure – Over 2 cc can begin to cause mechanical expansion of disc – Spasm (consider lumbar corset, muscle relaxant)
2–4	Infection
8–16	Biologic flare

still a point of debate but are probably different from product to product. Bone morphogenetic protein-7 (BMP-7), for example, is a protein that stimulates bone and cartilage growth. In a long bone fracture model, this protein initially causes osteolysis followed by osteogenesis. It has also been shown to promote cartilage growth, and the intradiscal study was based on an animal model that showed improvement of disc hydration. The inflammatory changes seen with BMP-7 (Fig. 13.23) was one of the first well-documented examples of noninfectious discitis with a biologic agent.

Early treatment of reactive and/or inflammatory changes in and around the intervertebral disc was initially very aggressive and consisted of a biopsy, intradiscal antibiotics, and subsequent intravenous antibiotics for at least 4–6 weeks or more. As we have seen the noninfectious inflammatory response more frequently, we have learned that the changes do not necessarily indicate and infect and our treatment has evolved. The current recommended treatment, once infection has been excluded, is palliative. Pain control and other supportive measures are applied with a watch-and-wait approach.

13.6 Intradiscal Exosomes

As previously discussed, cellular MSC-based regenerative treatments offer great potential in treating discogenic back pain. The cellular therapies are known to supplement the existing cell population by injecting cells either with or without a carrier. These treatments have been shown to promote nucleus pulposis cell proliferation, decrease inflammation, lessen apoptosis, and contribute to multipotent cell differentiation [54]. It has been shown that MSCs cocultured with nucleus pulposis cells can differentiate along the nucleus pulposis lineage either by direct or indirect contact methods and can cause the degenerated nucleus pulposis cells to regain a normal phenotype [55].

The cell-to-cell communication has been known to occur by secreted molecules and by cell surface molecules that contact other cells by specialized molecules in connected channels [56, 57]. The secretion occurs via extracellular microvesicles known as exosomes that are released by cells and are an essential component of the intercellular environment [58–60]. Exosomes are microvesicles that are produced in the endosomal compartment of most cells and, when they fuse with the cell surface, these microvesicles are released as exosomes.

The presence of microvesicles was originally reported by Chargaff and West in the 1940s and for many years were considered inert debris until De Broe et al. suggested these microvesicles may result from a specific process [61, 62]. It is now accepted that most cells release exosomes and recent studies indicate they have important roles in the function of stem cells [63].

Recently, attention has been turned to the function of exosomes which have been found in nearly all biological fluids including blood, urine, semen, and milk [64–66]. Exosomes are nanosized and range in diameter from 30 to 120 nm [67]. They contain multiple components including proteins, mRNAs, miRNAs, lipids, cytokines, noncoding RNAs (ncRNA), and ribosomal RNAs (Fig. 13.25) [67]. They also contain proteins involved in membrane fusion and transport including annexins, flotillin, and GTPases [67]. The two-layer membrane of the exosomes protects the integrity of their contents so that they are stable over long distances and during the interaction with target cells [64].

The exosomes can play unique roles in cell-to-cell communication based upon their cellular origin, and MSC-derived exosomes may provide an acellular alternative to traditional MSC treatment of the intervertebral discs [68]. This possibility has been investigated previously, and it has been shown that exosomes taken from nucleus pulposis cells can stimulate MSCs to differentiate into a nucleus pulposis cell phenotype and can stimulate the existing degenerated cells to regain a nondegenerate phenotype that produces and enhances matrix production [69]. This study suggests that exosomes can potentially stimulate repair of degenerated intervertebral discs and may play a role in the treatment of discogenic back pain. The variety of different functions of exosomes along with the potential to deliver a very concentrated dose of subcellular material taken from specific cell types offers great

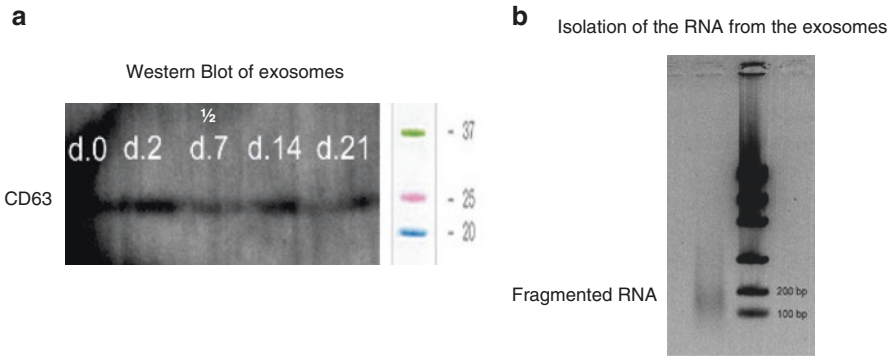


Fig. 13.25 (**a**) Western blot of fluid taken from a preparation of MSCs show the presence of a protein with a CD63 antigen. This antigen is mainly associated with membranes of intracellular vesicles or exosomes. (**b**) Agrarose gel electrophoresis using 1% agarose gel stained with ethidium bromide on an acellular fluid taken from a preparation of MSCs shows fragmented RNA observed around the 100 and 200 bp DNA standards

potential to regenerate the existing nucleus pulposis cells of degenerated intervertebral discs and could be used alone or in combination with MSCs. Additional investigation of the use of exosomes for this purpose will be essential to determine both the effectiveness of this therapy and the optimal use of these nano-biologics.

13.7 Conclusions

Back pain from DDD and IDD is exceedingly common, costly, and a very debilitating disorder. If this disorder can be accurately diagnosed and characterized, treatment may be accomplished with a combination of simple medications or biologically active treatments that are largely needle-based and can be delivered percutaneously. Stable discogenic back pain is not entirely adequately treated with conventional surgery or nonsurgical management but recently developed intradiscal needle-based therapies including the intradiscal biologic treatments are showing great promise.

While some biologic materials such as fibrin sealant and other isolated growth factors such as BMP-7 and GDF-5 have not been shown to be efficacious in treating discogenic back pain, other biologics such as platelet-rich plasma, alpha-2-macroglobulin, and MSCs have. Possibly the greatest promise for the treatment associated with the greatest safety and efficacy are the treatments that involve cellular augmentation of the intervertebral disc. The traditional lack of substantial data and large clinical trials is being remedied by recent RCTs that are producing high quality data and by the continued development of new technologies such as carriers for the cellular therapy.

Techniques for delivery of the intradiscal biologics will need to be reasonably standardized with good clinical practices followed to keep the treatment success high and the peri-procedural complications low. This is mostly refinement of existing techniques combined with a recognition of the nuances of the technical delivery of the new biologic materials.

The increase in the use of biologics will likely to continue to produce some unknown effects including the reactive changes to most of these biologics that have the characteristic of noninfectious immunogenic inflammatory changes. It is important to identify this as different from an infectious inflammatory condition as the treatments for these two conditions are entirely different.

Newer materials such as exosomes have great potential and promise as a biologic nanotechnology but its use in the intervertebral disc has not been studied to any significant degree. Further investigation will be necessary to determine the dose, method of delivery, and the optimal degenerative stage for optimally effective exosome treatment.

Overall intradiscal biological and cellular treatment of patients with discogenic low back pain holds great promise and potential. In patients who have not adequately been benefitted from conventional therapies, these treatments may be the therapeutic tool that produces the most optimal result.

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