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Painful Intervertebral Disc: Cell Therapies

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ABSTRACT

Intervertebral disc-related low back pain is a common helath issue, responsible for disability for numerous patients world-wide. Disc degeneration is a process with an almost universal development at advancing age and is connected not only with low back pain, but also with disc herniation and neurological deficits. Conservative treatment for discogenic low back pain is mainly symptomatic, often has short effect and/or is inadequate for a subgroup of patients. Surgical treatment is does not address the biology of disc degeneration, is connected with morbidity and may hasten adjacent level disc degeneration. Among the biological treatments being investigated, aiming to hault or even reverse the degeneration process, cell therapy has attracted rising interest recently, including the administration of both autologous and allogenic stem cells and chondrocytes. In this study, it is attempted to review the recent literature concerning application of cell treatment to patients suffering from discogenic low-back pain and highlight certain promising results, as well as future obstacles for further clinical trials and possible clinical application of cell therapy. Twelve clinical trials and case reports have been included, all published since 2006.

KEY WORDS: Low Back Pain, Intervertebral Disc Degeneration, Cell- and Tissue-Based Therapy, Stem Cells, Chondrocytes

Introduction

Low back pain is an important health issue worldwide. It is a common cause of visit to the doctor, affects a measurable portion of the population and is often connected with disability. Its direct and indirect annual cost at the US economy is estimated up to 500 billion[1]. The degeneration of the intervertebral discs is a common cause of low back pain and disability. Disc degeneration, although in many cases asymptomatic, is associated with sciatica and disc herniation or prolapse. It alters disc height and the mechanics of the spinal column, possibly adversely affecting the behavior of other spinal structures such as muscles and ligaments. In the long term, it can lead to spinal stenosis, a major cause of pain and disability in the elderly [2]. Its incidence is rising exponentially with current demographic changes and an increased aged population [3,4].

The intervertebral discs lying between the vertebral bodies, are the main joints of the spinal column and occupy one-third of its total height [5]. Their major role is mechanical, as they constantly transmit loads arising from body weight and muscle activity through the spinal column. They provide flexibility, allowing bending, flexion, and torsion. They are approximately 7 to 10 mm thick and 4 cm in diameter (anterior–posterior plane) in the lumbar region of the spine. The intervertebral discs are complex structures that consisting of a thick outer ring of fibrous cartilage (the annulus fibrosus),

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and a gelatinous core (the nucleous pulposus). The fibrous ring is made up of a series of 15 to 25 concentric lamellae of alternating oblique collagen fibres, lying parallel within each lamella. The central nucleus pulposus contains collagen fibers, organized randomly and elastin fibers (sometimes up to 150 mm in length), arranged radially, and embedded in a highly hydrated aggrecan-containing gel. Between the disc and the vertebrae (cranially and caudially) lies a thin horizontal (usually under 1mm) layer of hyaline cartilage, the cartilaginous endplate [5].

The intervertebral disc is a highly hydrated structure, especially the nucleus pulposus, as in a healthy state, over 80% of its weight is water. The IVD contains a rich collagen network, formed mostly of type I and type II collagen fibrils,-making up approximately 70% and 20% of the dry weight of the annulus and nucleus, respectively. It provides tensile strength to the disc and anchors the tissue to the bone. Aggrecan, the major proteoglycan of the disc, is responsible for maintaining tissue hydration through the osmotic pressure provided by its constituent chondroitin and keratan sulfate chains. The proteoglycan and water content of the nucleus (around 15% and 80% of the wet weight, respectively) is greater than in the annulus (approximately 5% and 70% of the wet weight, respectively) [5,6]. Notochordal cells are present from the early embryonic formation of the intervertebral disc and undergo a gradual transition towards chondrocyte-like cells during the first decade of life[7]. These mature nuclear chondrocytes produce collagen type I, but reduced amounts of water-attracting proteoglycans and collagen type II.

Disc degeneration in the lumbar spine is almost universal over the age of 50 years[8]. This observation appears related to humans' recent evolution to an upright posture and S-shaped spinal column. However, the aetiology of intervertebral disc degeneration remains obscure and the current consensus is that it is "multi-factorial[7]. The process of degeneration consists of several changes at a cellular, biochemical, structural and biomechanical level. Among them is the increase of acidity, the decrease of the water content of the IVD and the intradiscal hydrostatic pressure. This is connected to the increase of the catabolic activity of the. There is a progressive increase in the expression of inflammatory cytokines like IL-1 and TNFa[9,10,11] , a reduction in the expression of proteoglycans and collagen type II genes and an increase of colagen type I expression with increasing degeneration, resulting in a limited tissue water-binding potential[7]. Loss of intradiscal pressure reduces disc height; increases stress concentrations within the disc; and increases shear forces in the nucleus. Additionally, MMP-3 production is reduced, and tissue inhibitor of metalloproteins-1 (TIMP) production is increased reducing remodelling of the extracellular matrix. In the annulus, loss of intradiscal pressure will reduce tension in annulus fibers and increase in- and out-ward bulging. This bulging can increase shear forces between laminae, leading to delamination of the translamellar bridges, and consecutive risk of tears. In the endplates, the loss of annulus tension and the reduced stress distribution by the nucleus will alter the biomechanical stresses on the endplates which may be the cause of endplate sclerosis, fractures, or Schmorl's nodes[12]. The increased vascular and neural ingrowth seen in degenerate discs and associated with chronic back pain is probably associated with proteoglycan loss, since disc aggrecan has been shown to inhibit neural ingrowth. Changes at a cellular level procede the visible structural changes of disc degeneration, such as loss of disc height, disc bulging and protrusion, sclerosis of the subchondral bone, development of osteophytes. The model of the "degenerative cycle " presented by Vergroesen et al[7], attempts to connect the separate procedures of disc degeneration in the "degenerative cycle", a positive feedback loop involving cells, extracellular matrix, and biomechanics.

The purpose of this study is to review the current literature concerning clinical studies of cell therapies for the degeneration of the intervertebral disc during the last years. A search was conducted for relevant articles in the PubMed and Google Scholar internet databases, and a total of 7841 publications were found (including articles registered on both databases). After a screening of titles and "abstract" texts, 42 articles were chosen for a full text assessment, of which 13 articles refer to a total of 12 clinical studies, dating from 2006 (Table 1). The results of these studies are being presented and discussed further in this study.

Discussion

Current treatment options and the investigation of feasible cell treatment

Treatment options have been limited to conservative care, steroid injections, prescribed opiates, and surgery. The degenerated IVD surgery rate has shown recognizable growth in the last decades[13]. For herniated or bulging discs, with signs of compressed spinal nerves, (micro) discectomy will be considered. Alternatively, complete IVD replacement will

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be attempted, either by fusion surgery or total disc arthroplasty; however, these surgical procedures are highly controversial. The most common surgical approaches for discogenic back pain are spine fusions, with the clinical success rate ranging from 50% to 70%. Although existing surgical treatments provide better pain relief than

non-surgical interventions, they do not address the biology of disc degeneration, namely high pro-inflammatory cytokine levels or the inherent loss of nucleus pulposus cells[14,15,16,17,18]. Surgery, in most cases, can temporarily address changes from mechanical wear/stress and spinal instability, but there are many cases where the loss of motion from spinal fusion contributes to increased biomechanical stress due to alterations in spine kinematics and onset of degenerative cascade of adjacent segments. Genetic abnormalities are not addressed by surgery, leaving the patient susceptible to continued degenerative changes at other disc levels. In addition, surgical treatment can be applied after there has been a significant progress of the degeneration cascade connected with structural changes in the IVD and quite often, neurological symptoms. One also has to take the morbidity and the cost of a surgical intervention into account. Conservative therapy, on the other hand, is efficient for a short time and, although it may provide pain relief and improvement in disability, it does not slow or alter the degeneration cascade, and a subgroup of the treated patients proceeds to the chronic low back pain state.

As stated by Schol and Sakai[19], there is a "treatment gap" considering the option to treat intermediate low back pain, which cannot be alleviated by conservative treatment, but on the other hand is not characterized as disabling pain, which would make the patient a surgical candidate. Recent focus has been put on the development of novel regenerative therapies aimed at re-establishing a healthy IVD. Such treatments could involve protein, compound, biomaterial injections, and gene therapy, aiming to redirect or support endemic cells. In addition, investigators are exploring tissue engineering strategies to create biological IVD replacements.

There have been several clinical trials studying the transplantation of various cell populations, still leaving open questions regarding the ideal cell population for safe and effective IVD regeneration. The number of viable cells is already low in normal IVD (approximately $5x10^6$ /cm^3 in the NP and $9x10^6$ /cm^3 in the AF), and their functionality is further decreased during ageing and degeneration. In addition, increased cell senescence has been described in degenerative discs[20].

Cell populations researched for administration

For articular cartilage repair, autologous chondrocyte transplantation has advanced to an established procedure within the last two decades. Clinical application of autologous chondrocyte intradiscal transplantation is ongoing and trials are underway aiming to corroborate findings from initial studies[20]. In order to enhance their proliferative capacity and activity in terms of matrix production, cells isolated from disc tissue obtained during surgery can be stimulated by co-culture with autologous mesenchymal stem cells (MSCs). Short term co-culture with bone marrow derived MSCs under direct cell-to-cell contact significantly increased the growth and proteoglycan synthesis of human NP cells, while no indication of chromosome abnormalities and tumourigenesis was found[21]. The procedure appears safe and effective and is currently subject of a clinical study where such activated NP cells are injected in adjacent discs with underlying mild degeneration of patients undergoing fusion surgery. Recently, the feasibility of NP cell cryopreservation has been studied[22]. Results indicated no significant changes in cell proliferation and matrix production in cryopreserved compared to freshly isolated cells, opening the possibility of activating and transplanting the cells independent from the initial surgery and according to the patient's request. Moreover, the option for allogeneic cell transplantation can be considered. Indeed, allogeneic juvenile chondrocytes in combination with a protein-based carrier have been applied to patients with moderate lumbar disc degeneration and showed promising clinical and radiographic outcomes[23]. As an alternative to differentiated disc cells or chondrocytes, injection of MSCs has largely been investigated in animal models of disc degeneration and in certain clinical studies. Bone marrow remains the most common source for MSC harvest, although adipose tissue derived MSCs have shown regenerative potential in the disc as well[24]. In animal models of disc degeneration induced by annular puncture, nucleus aspiration or enzymatic means, implantation of MSCs has resulted in restoration of disc height, disc-like phenotype expression, discogenic extracellular matrix synthesis and improvement in MRI signals[25,26,27]. In vivo studies injecting MSCs in mouse[28], rabbit, and canine discs confirmed MSC differentiation, while also human cells implanted into rat or porcine discs were shown to adopt the chondrogenic or IVD-like phenotype[29-33].

Challenges for an effective cell therapy: disc microenviron-

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ment, possible adverse effects and administration issues

Cell therapies for disc degeneration,have to face certain challenges to emerge as viable treatment options. The intradiscal environment has characteristics that are not easily demonstrated in other tissues. It is elatively avascular, with low amounts of oxygen and nutrients available for any cell population. Furthermore, low pH and high hydrostatic pressure create a "hostile" environment[20]. Oxygen tension within the disc is significantly reduced towards the center of the nucleus pulposus (NP) and the disc cell metabolism is partly anaerobic, leading to high concentrations of lactic acid and low pH conditions. All those obstacles have to be overcome, to choose an effective cell population for the treatment[34]. The cells chosen (a) have to be able to adapt to the disc microenvironment, (b) should not "antagonize" the native cells for nutrients and oxygen (which could be an important factor to determine treatment dosage) and (c) in the case of not autologous cells a possible immune reaction has to be avoided. Concerning the latter, what leaves room for optimism is that the intradiscal microenvironment can be described as a relatively immunologically privileged environment, which can protect donor cells from a host reaction[23]. In addition, MSCs are immune privileged or immune evasive and inhibit immune responses in a manner not restricted by the HLA system. As a result, non-matched MSC are much better tolerated than other cell types. In fact, there are no reports of rejection in animal experiments and studies of transplanted MSC persistence in the host organism show the same values for autologous and allogeneic cells[35]. In humans, excellent tolerance to allogeneic MSCs has been reported in many clinical trials.

Other obstacles that have to be overcome are those related to the administration of the cells inside the disc. The integrity of the annular ring is of vital importance, and possible injury may provoke disc bulging and consequent hastening of the degeneration cascade. Interestingly, a recent animal study revealed that puncture with a 22G needle did not result in degenerative changes observed in radiography or histology[36]. The choice for an appropriate cell carrier is important as well, (a) to avoid cell leakage outside the disc (taking into account the high intradiscal pressure) and (b) to support the survival and proloferation of the administered cells. A study by Vadala et al, on animal intradiscal MSC injection, demonstrated (a) undesired migration (cell leakage) and (b) display of unwanted differentiation effects (osteophyte formation) at the treated vertebral levels[37]. A number of questions concerning the necessary storage, distribution and parameters concerning the possible cultivation of the cells to be transplanted arise as well. To transfer a feasible cell therapy from the experimental stage to clinical therapy, all those processes need to be clarified, taking into account patient safety and total treatment cost[38,39].

Clinical studies investigating cell therapies

In a case report by Yoshikawa et al[40], two cases were presented. Both patients had lumbago, leg pain and numbness. Myelography and magnetic resonance imaging showed lumbar spinal canal stenosis, and radiograph confirmed the vacuum phenomenon with instability. One patient had undergone an L4-L5 spinal fusion fifteen years prior due to left lower leg numbness and low back pain. At about 6 years following surgery, she began to experience low back pain, right lower leg numbness, intervertebral vacuum phenomenon, instability and lumbar spinal stenosis at L2–L3 and L3–L4. The other patient was operated at L4-L5. Marrow fluid was collected from the ilium and MSCs were cultured in an autogenous serum medium. In surgery, fenestration was performed on the stenosed spinal canal and then pieces of collagen sponge containing autologous MSCs were grafted percutaneously to the degenerated intervertebral discs. At the two-year follow-up, radiograph and computed tomography showed improvements in the vacuum phenomenon in both patients. On T2-weighted magnetic resonance imaging, signal intensity of intervertebral discs with cell grafts was high, thus indicating high moisture contents. Roentgenkymography showed improvement of lumbar disc instability. With intervertebral disc regeneration therapy, low back pain and neurologic symptoms improved. No adverse effects were reported.

In a study published by Orozco et al[41], ten patients with persistent low back pain, diagnosed with lumbar disc degeneration with intact annulus fibrosus, were treated with autologous expanded bone marrow MSC injected into the nucleus pulposus area. Clinical evolution was followed for 1 year and included evaluation of back pain, disability, and quality of life. The back pain was assessed via the Visual Analogue Scale, the disability via the Oswestry Disability Index and the quality of life via the short form-36 (SF-36) life quality questionnaire before the injections and at 3, 6 and 12 months after the injections. There was also an assessment of sciatic pain concerning six patients who presented such symptoms before cell transplantation. Magnetic resonance

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imaging measurements of disc height and fluid content were also performed, in T2-weighted sagittal images. There were positive results, as mean scores concerning pain (including sciatic pain), disability and quality of life were all improved. Pain and disability demonstrated statistically significant improvement in the first three months post-treatment with pain approaching 71% of optimal during the first year. The analgesic effect of the intervention was rapid, as most of the improvement in pain (85%) was attained by 3 months. The SF-36 questionnaire revealed, by the end of treatment, a significant improvement of the physical component with no change of the mental component. The treatment appeared to compare favorably with previous trials exploring physical treatments and spinal fusion with or without disc replacement or complemented with expanded disc material. Moreover, although there was no improvement in disc height, the fluid content of the affected disc segments was significantly elevated at 1 year following the intervention. No serious adverse effects or safety issues were reported.

In a study by Centeno et al[42], 33 patients with low back pain and degenerative disc disease presenting with a posterior disc bulge, diagnosed with MRI, underwent percutaneous, intradiscal, single-level injections of autologous cultured mesenchymal stem cells derived from bone marrow (posterior iliac crest), along with autologous platelet lysate. The results were promising after a six-year follow-up period. The improvement at the overall average for the last reported modified single assessment numeric evaluation (SANE) rating was 48.2%, at an average of 40.6 months post-treatment, with 50.4% reporting greater than or equal to 50% improvement. At 3-years post-treatment, 90% (30 out of 33) of patients reported > 0% improvement. In reference to the numeric pain score (NPS), they were found to be statistically significantly improved at 3 months, 4 years, and 5 years for the group of 25 patients who provided a baseline score. Functional Rating Index (FRI) change in scores was significantly different than baseline at 3 months and 5 years post- treatment. In addition to patient-reported outcomes, changes in IVD posterior projection or bulge beyond the vertebral body were also measured. A decrease in posterior disc bulge was detected in 85% of patients at an average of 6 months post-treatment. In determining how much of a decrease in bulge size measurement is clinically significant, patients with at least a ≥ 25% reduction in disc bulge reported significantly lower pain scores at 6 months compared to patients with a < 25% change in bulge size. All 3 of the reported adverse events were pain related and resolved, while one AE was reported, a large herniated nucleus pulposus, occurring months after the injection. This was either related to trauma from the needle procedure, or simply been a progression of the degenerative process.

In the study by Elabd et al[43], 5 patients with painful disc degenerative disease and pain, spasm, or functional disability in the low back, andfailed conservative treatments for at least 3 months, but no longer than 5 years received autologous, hypoxic cultured, bone marrow-derived mesenchymal stem cells. Four to six years after the cell transplant, they were re-examined to evaluate long-term safety and feasibility of this treatment. This follow-up consisted of a physical examination, completion of a quality of life questionnaire, and spine MRI. Four patients received injections in the intervertebral disc at the L5-S1 level and one at the L4-L5 level, and the amount of MSCs injected varied from 15.1 to 56.1 million. All five patients reported improvement of muscle strength, four patients improvement of mobility, while there was an overall improvement at the QoL questionnaire in the range of 10-90%. It is interesting to note that four out of five patients showed an improvement (reduction) of the protrusion size. Additionally all patients displayed maintenance or only mild worsening in disc height after long term follow up, and no adverse effects were reported.

In a randomized controlled trial by Noriega et al[35], 24 patients, diagnosed with Pfirrmann grade II-IV degenerative disc disease, unresponsive to conventional treatments (physical and medical) for at least 6 months and with 1 or 2 affected discs, with the lesion located at L1-L2 (n=1), L2-L3 (1), L3-L4 (3), L4-L5 (18), or L5-S1 (15) were divided into two groups at an allocation ratio of 1:1 (12 patients at each group). One group received MSCs ($25x10^6$ MSC in 2 ml of saline per disc) under local anesthesia and the other (control group) sham infiltration of paravertebral musculature close to the affected disc(s) with 2 ml of 1% mepivacaine. The MSCs were allogenic, received by five healthy donors. There was clinical evaluation and routine analyses, pain evaluation (VAS), Oswestry Disability Index (ODI) and short form-12 (SF-12) life quality questionnaire, at 8 days, and 3, 6, and 12 months after implantation. Quantitative MRI exploration was performed before the treatment and at 6 and 12 months after the injections. No major adverse events occurred. Eleven patients (8 controls /3 cell-treated) required brief treatments with NSAID-type analgesics for minor pains and 2 (1 control/1 cell-treated) required opioids. Both lumbar pain

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and disability were significantly reduced at 3 months after MSC transplantation, and the improvement was maintained at 6 and 12 months. Compared to the basal level of pain and disability, improvement was statistically significant at all time points except at 8 days, which could possibly be due to a placebo effect or the result from the anesthetic infiltration, although there is no indication of this extra-fast early improvement in the group of cell-treated patients. A fast decrease of pain was detected at the 8th day in the control group, but there was not any tendency to further improvement thereafter. The distribution of the cell-treated group is suggestive of a bimodal distribution in the Huskisson plot; a responders subgroup of 5 patients is close to the blue line that represents perfect treatment, whereas the other 7 (non-responders) resemble to controls, with no indication of effectiveness. The SF-12 life quality questionnaire did not reveal significant improvements of either the physical or the mental component scores. The height of the affected discs, as measured at the MRI imaging, had a bigger mean decrease in the controls than in the cell-treated patients, but the difference was not significant. Although the water content of the affected discs improved after treatment with the MSC, no statistical significance was observed. What is notable however is that the evolution of Pfirrmann staging was clearly different in the control and in the experimental group. In controls, there was a deterioration from (mean ± sem; n=20) Pfirrmann stage 3.15 ± 0.15 to stage 3.78 ± 0.16 ($p<0.001$), whereas in the cell-treated patients there was an improvement from stage 3.68 ± 0.13 to 3.18 ± 0.17 ($p<0.01$). The efficacy of allogeneic treatment found in the present trial (0.28) was smaller than the reported for autologous cells, 0.71[41]; yet, direct comparisons are difficult because the previous study by Orozco et al was uncontrolled. It would be most interesting to directly compare autologous with allogeneic cells in different arms of the same trial, in future studies.

At a phase-I clinical study by Kumar et al[44], 10 patients with chronic low back pain due to moderate IVD degeneration (Pfirrmann's grade III–IV at one or two levels based on T2- weighted MRI) and degenerative symptomatic discs confirmed by discography underwent a single intradiscal injection of combined HA derivative and autologous adipose tissue mesenchymal stell cells (AT-MSCs) at a dose of 2×10^7 cells/disc (n = 5) or 4×10^7 cells/disc (n = 5). The AT-MSCs were cultured for three weeks after isolation from subcutaneous abdominal adipose tissue, which was harvested via liposuction. Safety and treatment outcomes were evaluated by assessing VAS, ODI, SF-36, and imaging (lumbar spine X-ray imaging and MRI) at regular intervals over 1 year (1 week and 1, 3, 6, 9, and 12 months post transplantation). Based on discographic findings, AT-MSCs combined with HA derivative was implanted into the L4/5 disc in nine patients, whereas one patient received injections into the L4/L5 and L5/S1 disc. Seven of the 10 patients showed significant improvement ≥ 50% in the VAS and ODI at 6 months, whereas final treatment success (reduction ≥ 50% in the VAS and ODI compared with pretreatment VAS and ODI) was found in six subjects at the 12-month follow-up. No case of height loss at the lumbar X-ray or degeneration of the injected IVD was detected at the 12-month follow-up. Furthermore, the Pfirrmann grade of the transplanted disc increased from grade IV to grade III at the 6-month and final follow-ups in one case, who also achieved significant VAS improvement at 6 months. Among the six patients who achieved treatment success at the final follow-up, three cases showed increased water content based on the ADC map one year after the treatment. During the 12-month follow-up period, no adverse effects related to cell transplantation where observed. The treatment success rate was not different between the low-dose (2×10^7 cells/disc) and high-dose (4×10^7 cells/disc) groups. Out of the four patients classified as treatment failure, one reported significant pain relief for LBP (50% pain relief) at the 12-month follow-up, but the ODI improvement was < 30% and notable increases in Pfirrmann grade and in the ADC value were found at the 6-month follow-up. Two out of the "unsuccesfully treated" patients had other structural etiologies for chronic LBP: spondylolisthesis, spinal stenosis, facet joint arthritis, decreased disc height and disc herniation, while the other one had depressive symptoms, which might have resulted in treatment failure. Thus, careful patient selection is essential for achieving therapeutic success in stem cell therapy for chronic discogenic pain.

In a prospective analysis by Haufe et al[45], 10 patients underwent intradiscal injection of hematopoietic precursor stem cells (HSCs) obtained from pelvic bone marrow in an attempt to rejuvenate the disc. Patients were randomly offered the option of this study, and ten patients with confirmed disc pain via provocative discograms underwent intradiscal HSC injections. In the past, all patients were submitted to an endoscopic discectomy in an attempt to eliminate low back pain. Following intradiscal injection of HSCs, all patients underwent a 2-week course of hyperbaric oxygen therapy. These patients were followed up at 6- and

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12- month intervals to determine their degree of pain relief from this procedure. Of the 10 patients, none achieved any improvement of their discogenic low back pain after 1 year. Although animal studies suggest possible regeneration of disc via HSC injections, living human studies reveal that this effect does not correlate with reduced pain, and thus intradiscal HSC injection appears to be of little value.

In a prospective study by Coric et al[23], the safety and efficacy of allogenic juvenile chondrocytes delivered percutaneously for the treatment of lumbar spondylosis with mechanical low-back pain was evaluated. Fifteen patients were treated with a single delivery of juvenile chondrocytes (2 at L3–4 levels, 1 at L4–5 level, and 12 at L5–S1 levels; 12 levels at Pfirrmann Grade III and 3 levels at Grade IV). Each treatment consisted of a 1- to 2-ml injection (mean injection volume 1.3 ml) of juvenile chondrocytes (~10^7 cells/ml) combined with a fibrin carrier. Allogenic juvenile chondrocyte cells were harvested from the articular surface of cadaveric donor tissue and expanded in vitro. Patients were evaluated before the injection and at 1, 3, 6, and 12 months post treatment. The mean ODI, NRS, and SF-36 physical component summary scores all improved significantly from baseline, while the SF-36 mental component improved in a not statistically significant volume. At the 6-month follow-up, 13 patients underwent MRI, as one patient underwent CT imaging and another refused imaging. Ten (77%) of these 13 patients exhibited improvements on MRI. Three of the patients showed improvement in disc contour or height. High-intensity zones (HIZs), consistent with posterior annular tears, were present at baseline in 9 patients. Of these, the HIZ was either absent or improved in 8 patients (89%) at 6 months follow-up. The HIZ was improved in the ninth patient at 3 months, with no further MRI follow-up. Of the 10 patients exhibiting radiological improvement at 6 months, findings continued to improve or were sustained in 8 patients at the 12-month follow-up. In this study, no adverse effects were reported. Three patients (20%) underwent total disc replacement by the 12-month follow-up due to persistent, but not worse than baseline, LBP.

In 2002 a prospective, controlled, randomized, multi-center study[46,47], comparing safety and efficacy of autologous disc chondrocyte transplant (chondrotransplant DISC) plus discectomy (ADCT), with discectomy alone was initiated. Interventional surgery for disc herniation is one of the most widely used and effective treatments for back pain that emerges within the broad scope of disc degeneration. Successful removal of herniated disc tissue offers the individual patient substantial relief for associated pain. However, the reduction of tissue involved in the surgical procedure anatomically compromises the function of the affected disc, and affects load transfer to adjacent discs. The goal of the clinical trial was to evaluate whether ex vivo expansion of autologous disc chondrocytes and subsequent percutaneous transplantation would positively affect the treated disc and potentially stabilize the spine in general. There was an aim to embrace a representative patient group, examining the traumatic, less degenerative disc, but also to include patients with persistent symptoms that had not responded to conservative treatment where an indication for surgical treatment was given. Patients having exclusively one level requiring surgical intervention were eligible for participation in the trial. Out of a total of twenty-eight patients, 12 received cell transplantation following discectomy and 16 were treated by discectomy alone. A single puncture with a minimal caliber cannula was used to achieve precise delivery and avoid significant trauma to the annulus. Chondortransplant DISC has been transplanted approximately 12 weeks following discectomy to assure healing of the annulus. Interim analysis was performed after 2 years; Oswestry (low back pain/disability), Quebec Back-Pain Disability Scale, as well as Prolo and VAS score were used for the evaluation, in 3-, 6-, 12-, and 24-month assessments. Differences in initial presentations between the control group and those receiving autologous cells were observed. Surgery, as expected, substantially reduced the patients' disability and pain. However, the trend in reduction of total sum score continued to decrease in patients whose treatment was supplemented by cell transplantation, while the control group did not sustain continual improvement. Descriptive analyses of the mean total sum score of the QBPD prior to sequestrectomy, prior to ADCT/control, and 3 months after ADCT/control demonstrated a decrease in mean and median sum scores in both groups. Although the mean and median values for both the ADCT and the control group decreased between first and second year, the assessments for the ADCT group were clearly lower. At 2 years follow-, both total sum score and disability index of the OPDQ were plainly lower in the ADCT group compared with the control, showing long-term therapeutic benefit in comparison to discectomy alone. Disc height was assessed by MRI. Comparison of the mean inter-vertebral disc heights and the vertebral heights revealed no differences between the groups. Concerning the hydration level of the IVDs, the

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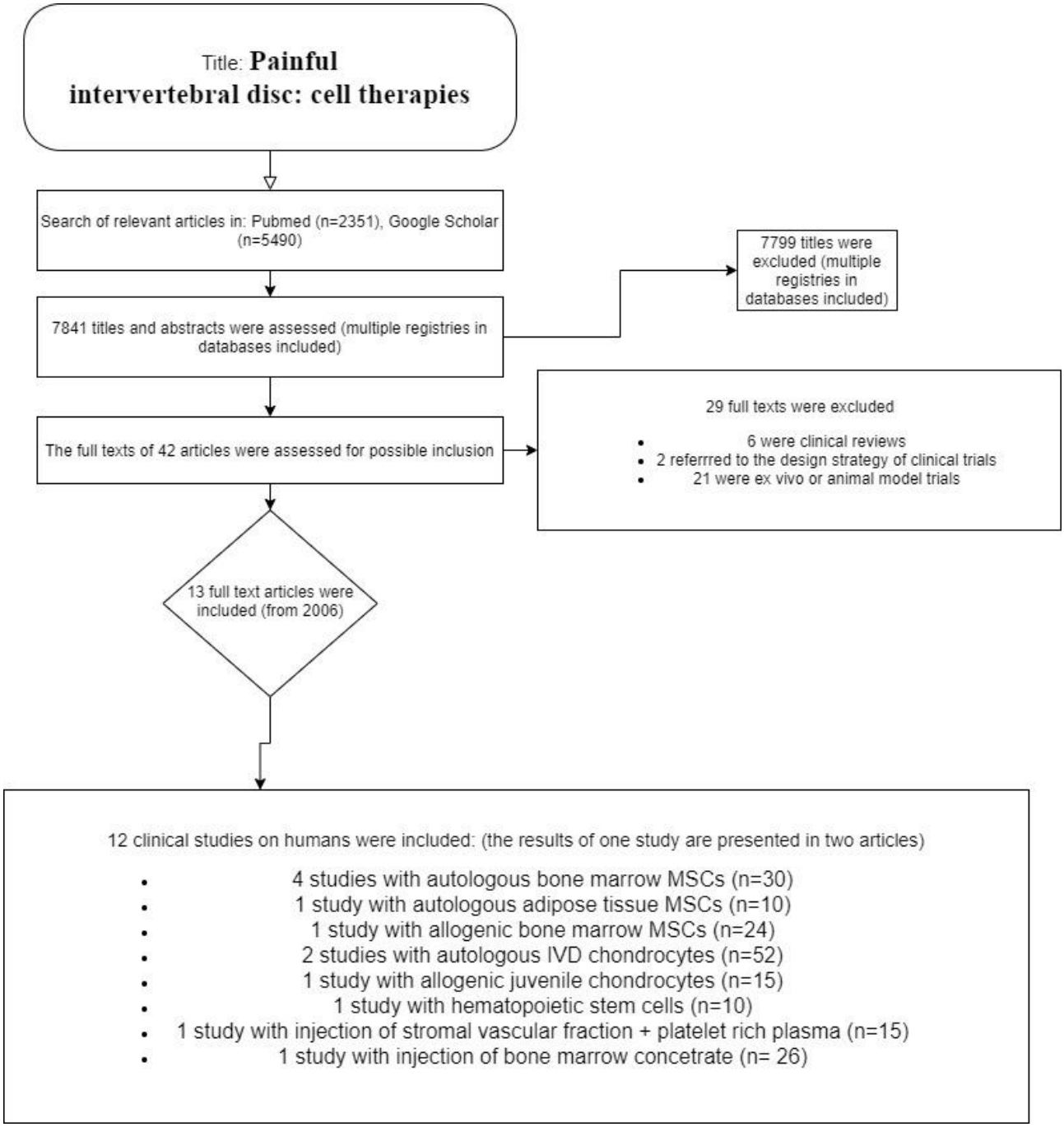
Table 1-Flowchart



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ADCT treated group showed a substantially higher normalization as a group; 41% normal fluid content compared with only 25% normal content in the control group at the 2-year follow-up. Perhaps most interesting of all the data to emerge from this study comes from inspecting adjacent discs either one, or two segments from the treated intervertebral disc. Fluid levels at both of these segments showed a substantially higher percentage of normal fluid content despite the fact that they were away from the surgical intervention site.

The effects of autologous chondrocyte disk transplantation were also studied at a prospective randomized multicenter phase I/II clinical trial, the safety results of phase I of which were presented by Tshugg et al[48]. The NDisc trial is a multi-center, randomized study with a sequential phase I study within the combined phase I/II trial with close monitoring of tolerability and safety. Twenty-four adult patients with a single-level lumbar herniated disk were randomized and treated with the investigational medicinal product NDisc plus or the carrier material only. The most commonly affected level of disk herniation was at L5/S1 in both groups. NDisc plus is an injectable, in situ polymerizing gel initially consisting of two separate components. Component A is a liquid matrix composed of modified albumin, hyaluronic acid and the cell culture medium containing autologous inter-vertebral disk cells dissolved in cell culture medium supplemented with human serum, chondroitin sulfate, insulin, BMP-2 and ascorbate. Component B is a solution containing bis- thiopolyethylene glycol. NDisc basic is used as control in the NDisc study, in which component A is modified and is a liquid matrix composed of cell culture medium, modified albumin, and hyaluronic acid, the cell suspension is replaced by an aliquot of cell culture medium without additives. Among the inclusion criteria were no previous lumbar spine surgery and no associated lumbar disease such as lumbar spinal stenosis, spondylolisthesis, or fracture. If patients showed an extensive damage of the annulus fibrosus intraoperatively that may subsequently pose a significant greater risk of recurrence or non-containment of the injected material, they were excluded from the trial. Transplantation was performed 90 days after sequestrectomy. In case of a degenerated intervertebral disc adjacent to the treated level, the same procedure was conducted additionally at the adjacent disk. Twenty of the 24 patients were treated, 12 patients with the IMP NDisc plus and eight patients with the control preparation NDisc basic. There were two cases, where adverse effects were assessed by the investigator as related to the medical intervention or to either of the study treatment. One patient of the NDisc basic group experienced spinal pain 21 days post implant (non serious adverse effect) assessed as related to both surgery and study treatment. One patient of the NDisc plus experienced an intevertebral disk protrusion assessed also as related to both surgery and study treatment. The patient underwent further surgery. Laboratory values such as interleukine-6 (IL-6) and C-reactive protein (CRP) as safety parameters were evaluated. These values in both treatment groups increased temporarily after 36 h of sequestrectomy and turned to normal thereafter. CRP did not change after implantation, whereas IL-6 showed minor changes with a peak at 42 h post implantation. There were no statistically significant differences in the results between the two groups. In the MRI, extradiscal fluid collection (EDFC) was observed in three patients (n = 2/12 in the NDplus group vs. n = 1/8 in the NDbasic group) after the implantation, but did not have any space-occupying effect. One of these patients demonstrated a recurrent disk herniation, which later also required surgery (7 months postoperative). Routine treatment (elective sequestrectomy) in the target patient population was considered to be associated with AEs such as recurrent disk herniation or ongoing or recurrent low back pain or sciatica in up to 25 % of patients within 2 years. Symptomatic reherniations occur in approximately 10% of patients with the highest risk within the first 6 months. Overall, the rates of radiological and clinical reherniations as well as of adverse effects are comparable with those expected in the early time course after elective disk surgery. No indications of harmful material extrusion or immunological consequences due to the treatment were observed.

In a study published by Comella et al[49] in 2017, the intradiscal injection of a mixture of stromal vascular fraction and platelet rich plasma was examined. A stromal vascular fraction (SVF) can easily be isolated from fat tissue in approximately 30–90 min in a clinic setting using a mini-lipoaspirate technique. The SVF contains a mixture of cells including ADSCs and growth factors and has been depleted of the adipocyte (fat cell) population. Platelet rich plasma is a mixture of growth factors and fibrin obtained from autologous peripheral blood. By combining PRP with SVF, there may be an increased number of growth factors and proteins which could translate to improved patient outcomes. Fifteen patients underwent a local tumescent liposuction procedure to remove approximately 60 ml of fat tissue. The fat was sep-

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arated to isolate the SVF and the cells were delivered into the disc nucleus of patients with degenerative disc disease. The patients were diagnosed with degenerative disease of one, two or three lumbar discs and experienced predominant back pain after conservative treatment (physical and medical) for over 6 months. The annular ring had to be capable of holding the cell implantation as demonstrated by MRI image. Each injection included approximately 1cc of SVF/PRP suspension. If more than one disc was symptomatic, the SVF was divided and prepared with approximately 1cc of PRP. Clinical evaluations were scheduled at baseline, 2 and 6 months. The subjects were monitored for adverse events, range of motion, visual analog scale (VAS), present pain intensity (PPI), Oswestry Disability Index (ODI), Beck Depression Inventory (BDI), Dallas Pain Questionnaire and Short Form (SF)-12. Safety events were followed for 12 months. The patients demonstrated statistically significant improvements in several parameters including flexion, pain ratings, VAS, PPI, and short form questionnaires. Although ODI and BDI did not show statistically significant changes due to the low number of subjects in the trial, the data was trending positive. In addition, the majority of patients reported improvements in their Dallas Pain Questionnaire scores. Adverse effects other than soreness in the abdomen after the mini-liposuction procedure or soreness in the back after injections (which all resolved within 7-10 days) were not reported.

Another option concerning a potential cell treatment for disc degeneration could be the intradiscal injection of autologous bone marrow aspirate. In a prospective, open-label, non-randomized, single-arm study of Pettine et al[8], 26 patients received this treatment and had a three-year follow-up. All were surgical candidates seeking a consult from the author, had chronic low back pain persistent to conservative treatment and disc degeneration with an MRI confirmed modified Pfirrman grade of 4-7. They were injected with 2 ml autologous BMC into the nucleus pulposus of treated lumbar discs. Thirteen patients underwent an intradiscal injection of autologous BMC at a single symptomatic lumbar disc and 13 subjects had two adjacent symptomatic disc levels injected. A sample aliquot of BMC was characterized by flow cytometry and CFU-F (colony forming units-fibroblast, synonymous to bone marrow-derived MSCs) assay to determine progenitor cell content. There was an improvement of both the pain and the disability of the treated patients from the first post-treatment evaluation (3 months) and the improved VAS and ODI scores remained relatively stable during the 3-year follow-up period for the patients who did not undergo surgery during this period (20 out of 26 patients). There was a 71% improvement in ODI and 70% improvement in VAS in this BMC injection group after two-years and a slight decrease was observed in ODI and VAS scores from two to three years post procedure. Cellular analysis suggests patients with greater concentrations of progenitor cells (both CFU-F and CD34+/lineage– cell types) in their BMC experienced faster and greater pain reduction. MRI imaging showed eight out of 20 patients with imaging had at least one grade increase on the modified Pfirrmann grading scale for disc degeneration at one year. No patients presented worse MRI scores after one year. Patients with higher MSC concentrations, measured as CFU-F/ml, tended to have better outcomes than those with lower concentrations. Other than progression to surgery (six patients in total), there were no serious adverse events related to the study. The morbidity and cost of this percutaneous procedure are substantially less than a surgical option and the clinical results appear to be similar or superior to surgery for chronic discogenic low back pain.

The results of most of the clinical trials reviewed here are promising. Apart from the study evaluating the use of hematopoietic precursor stem cells by Haufe et al[45], all studies evaluating pain and disability showed positive results. In addition, the improvement in pain and disability scores was mostly not lost during the follow-up period, as was shown by the studies with a follow-up period of three to six years [8,42,43]. Although this tendency for long-term positive results has to be confirmed by studies with longer follow up-periods, it certainly leaves room for optimism. Radiological and MRI findings were promising as well. A common positive result was the fact, that the fluid content of the treated discs was higher a year post-treatment. In addition, treated discs tended to keep a steady height at the follow-up imaging. Although not as common, probably even more promising was the fact that in certain studies an improvement at the Pfirrman (or modified Pfirrman) score for disc degeneration was observed. The results of Noriega et al[35] and Pettine et al[8], are most notable. Furthermore, Centeno[42] and Elabd[43] et al both found that the majority of patients had a smaller posterior disc protrusion and disc bulge, respectively. Those findings indicate that the degeneration process of the disc may not only be slowed, but possibly reversed by cell treatment. Moreover, there were no

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serious adverse effects connected to the cell therapy reported (with the possible exception of one patient in the study by Centeno et al[42]) nor did cell therapy show to worsen the possibility of adverse effects when combined with surgery[46,47]. The combination of surgical and cell treatment is a possible therapy option that requires further investigation. These findings show that cell therapy can be a relatively safe treatment option. Further investigation with a prospective, randomized, blinded, placebo-controlled study design is necessary.

There are, however, issues that need to be addressed to safely apply cell therapy from clinical trials to "everyday" treatment. Firstly, the choice between cell populations, as well as between autologous and allogenic cell transplants requires further investigation, as clinical trials are still limited both in number and patient populations. Secondly, the appropriate therapeutic dosage and the cell carrier have to be defined, such as development of carriers that may imbue additional potential and scaffolds that enhance placement. While the study by Pettine et al[8] indicated a connection between viable cell population transplanted and clinical results, only one study[44] examined different dosage schemes without any statistically significant difference. Regenerative strategies targeting the repair of the annulus fibrosus and end-plates are also lacking[50]. In addition, the questions of cultivation, storage and adequate supply of the administered cells also have to be answered. In order to bolster and confirm the positive results and address the issues above, further investigation with bigger patient populations and comparison between different treatment options are required.

Conflicts of Interest

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