

THE ROLE OF REGENERATIVE MEDICINE IN PAIN MANAGEMENT

INTRODUCTION

Regenerative Medicine offers an entirely new approach for repairing, replacing, maintaining, or enhancing organ or tissue function that has been lost due to disease, injury, or aging. By combining biomedical, biochemical, and biomechanical technologies, regenerative medicine strives to improve cellular migration, replication, and remodeling.¹ Treatment approaches include cell therapies, tissue engineering, gene therapy, immunomodulation therapy, and biomedical engineering.

Presently, clinical practice within the United States focuses mainly on approved autologous therapies utilizing platelet rich plasma (PRP) or bone marrow aspirate (BMAC). Here, we will review various stem cell therapies for pain management based on *in vitro* and clinical studies.

Mesenchymal Stem Cells (MSCs)

Adult stem cells are found throughout the body. The primary role of these cells is to maintain and repair the tissue in which they are found. Adult stem cells possess two very important properties- self-renewal and differentiation. Adult stem cells are multipotent, meaning they can differentiate into a subset of cell types. Stem cells have been used for many different disease states as they also have the capacity to change the local environment via their paracrine effect, which may render them capable of disease modification.

Mesenchymal stem cells (MSCs) arise from pericytes released from broken and inflamed blood vessels. Once released, the MSCs exhibit an immunomodulatory as well as a regeneration zone of influence. Additionally, they have an angiogenic and antibiotic effect. Caplan et al. describe the immunomodulatory zone as a curtain of bioactive molecules that prevent the body from mounting a chronic autoimmune reaction.^{3,4} MSCs address the regeneration zone, or the damaged tissue, by secreting a different set of bioactive molecules that have trophic functions. This results in four basic actions: apoptosis inhibition, inhibition of scar formation, angiogenesis, and mitogenesis. Lastly MSCs possess antimicrobial properties that can sense and address infection.^{5,6}

Sources of MSCs include bone marrow, adipose, peripheral blood, amniotic, umbilical, and placental tissue. Most clinical studies involve the use of autologous sources of MSCs due to their accessibility and low risk of infection. In terms of clinical practice, the most popular types are derived from bone marrow aspirate or lipoaspirate.

The International Society for Cellular Therapy (ISCT) set forth minimum criteria to define human MSCs: 1) plastic adherence in standard culture; 2) positive for endoglin, CD73 and CD90, and negative for CD45, CD34, CD14 or CD11, CD97-alpha or CD19, and HLADR surface markers; 3) *in vitro* differentiation into osteoblasts, adipocytes, and chondroblasts.⁷

BONE MARROW-DERIVED STEM CELLS

Bone marrow-derived stem cells are the most commonly used source and are permissible by the FDA and other governing bodies. Bone marrow aspirate has been approved and used safely for many years for the treatment of blood cancers and other hematological disorders. Bone marrow is the primary site of new blood cell production. Two main cell types are produced in bone marrow: hematopoietic cells (myelopoietic, erythropoietic, lymphocytes, plasma, reticular, monocytes, megakaryocytes, hematopoietic stem cells) and stromal cells (fibroblasts, macrophages, adipocytes, osteoblasts, osteoclasts, endothelial cells, MSCs). The frequency of stem cell production decreases proportionately with age.

Bone marrow MSCs are obtained via bone marrow aspiration of the iliac crest (most commonly) under sterile conditions. The aspirate is then typically washed and centrifuged to remove the plasma and buffy coat until the final “cell pellet” is obtained; in some instances, a separation medium may be utilized. This pellet is commonly referred to as Bone Marrow Aspirate Concentrate or BMAC. A milliliter of bone marrow aspirate yields approximately 6×10^6 nucleated cells; only 0.01% are bone marrow stem cells.⁸

Bone marrow MSCs are a population of cells found in bone marrow that are different from blood cells in a variety of ways. They are multipotent stem cells, giving them the ability to differentiate into bone, cartilage and fat cells. Additionally, they can support the formation of new blood cells. Two types of stem cells originate in bone marrow- hematopoietic stem cells and MSCs. There are also endothelial progenitor cells that have the capacity to form new vasculature. Hence, they have similar capabilities to stem cells.

Bone marrow-derived MSCs regenerate injured or damaged tissue via the following pathways- fibrinogenesis, osteogenesis, adipogenesis, and chondrogenesis.⁹ Bone marrow-derived MSCs home to injury sites via migratory properties in bone marrow, bone, and cartilage.¹⁰ Additionally, BMAC contains the following growth factors- basic fibroblast growth factor (b-FGF), platelet-derived growth factor (PDGF-BB), vascular endothelial growth factor (VEGF), transforming growth factor-beta (TGF- β), and bone morphogenetic protein-2 (BMP-2), to name a few.¹¹

While the number of stem cells found in BMAC is relatively low compared to those found in lipoaspirate, the growth factors and platelets contained in BMAC are believed to enhance their therapeutic ability. A study by Schafer et al. found that BMAC increases the concentration of PDGF-BB and VEGF in mononuclear cells.¹² Both PDGF-BB and VEGF contribute to angiogenesis.¹³⁻¹⁴

ADIPOSE-DERIVED STEM CELLS

Adipose-derived stem cells are a population of cells found in adipose tissue that have adipogenic, myogenic, neurogenic, chondrogenic, and osteogenic differentiation potential. Additionally, they possess angiogenic and vasculogenic tendencies.¹⁰ Human adipose stem cells were first characterized by Zuk et al., in 2001.¹⁵ In adipose tissue, MSCs originate in perivascular locations and can be found in the stromal vascular fraction (SVF). The regenerative components of SVF include preadipocytes, endothelial progenitor cells, macrophages, monocytes, pericytes, and MSCs.³

Rodbell and Jones pioneered the initial methods to isolate stem cells from adipose tissue in the 1960's.¹⁶ Current methods of adipose stem cell isolation involve tumescent liposuction which produces the minced adipose tissue fragments, depending on the size of the harvesting cannula used. This is followed by cell washing to remove some red blood cells, enzymatic digestion, centrifugation, and further cell wash steps to isolate the SVF.

The regenerative capacity of the SVF is likely a result of the “sum of its parts”. This multi-component cellular result provides numerous pathways and mechanisms for regeneration to occur.³ Pre-adipocytes are the most plentiful component of SVF and share many characteristics and markers of MSCs.¹⁷ Endothelial progenitor cells release VEGF and insulin-like growth factor-1 (IGF-1), which activate angiogenesis.¹⁸ Macrophages contained in SVF have demonstrated immunomodulatory properties via cytokine expression, as well as plastic adherence, multilineage differentiation, and immunosuppression via T-cell modulation.¹⁹ Pericytes produce extracellular matrix components capable of improving cellular adhesion, migration, and cell interaction.²⁰

Cell surface markers, including clusters of differentiation (CD) are critical for cell-cell and cell-environment interactions and are used to define mixed cell populations. Despite an enormous amount of research, there is little consensus with regards to CD characterization of the SVF. Table 1 shows a comparison of SVF defined cell surface markers.^{17,21-27} Table 2 provides a comparison of cell surface markers between bone marrow and adipose-derived stem cells.²²

It is important to note that as of this publication, the FDA currently views the standard method of adipose-derived stem cell isolation as manufacturing a drug, therefore this is not permissible in clinical practice in the United States. Bone marrow aspirate concentration (BMAC) using density centrifugation methods is permissible so long as the procedure is carried out the same day.

Table 1: SVF cell surface markers

	MSCs (ADSCs)	Pericytes	Endothelial Progenitor Cells	Pre- Adipocytes	Transitional Cells	Macrophage/ Monocyte
Bourin et al., 2013	CD45- CD31- CD13- CD73- CD90+ CD105+ CD34+(unstable) CD45+(<50%) CD31-(<20%)	CD13+ CD29+ CD44+ CD73+ CD90+(>40%)	CD34+(<40%)			
Zimmerlin et al., 2013		CD45- CD31- CD146+	CD45- CD31+ CD34+	CD45- CD31- CD146- CD34+	CD146+ CD31- CD34+	
Hager et al., 2013			CD133+ CD146+ CD34+ CD31+			
Bianchi et al., 2013	CD90+ CD29+ CD34-	CD146+ CD34- CD45- CD56-			CD146+ CD34+	
Corselli et al., 2013		CD146+ CD34- CD45- CD56-	CD34+ CD146+ CD31+	CD34+ CD31- CD146-		
Trakutev et al., 2008	CD34+ CD31- CD45- CD144-			CD34+ CD45- CD31-		
Eto et al., 2013						CD45+ CD14+ CD34+ CD206-

Table 2: Differences between bone marrow and adipose tissue.

	SVF	BM-MC	ASC	MSC
CD34	>30-70%	>2-30%	>2-30%	<2%
CD45	>30-70%	>70%	<2%	<2%
CD13	>2-30%	>70%	<2%	<2%
CD73	>2-30%	>2-30%	>70%	>70%
CD90	>2-30%	>2-30%	>70%	>70%
CD105	>2-30%	>2-30%	>70%	>70%
CD10			>70%	>30-70%
CD36			>30-70%	<2%
CD106			>2-30%	>30-70%
CFU-U	>1%	>0.001%	>5%	>5%

SVF= Stromal Vascular Fraction

BM-MC= Bone Marrow Mononucleated Cells

ASC= Adipose Stem Cells

MSC= Mesenchymal Stem Cells

MSCs IN PAIN MANAGEMENT

It is well established that the effectiveness of MSCs are mainly due to their paracrine effects to promote a regenerative localized environment, rather than their direct effect or replacing tissue. For chronic pain and neuroinflammation, data suggests MSCs secrete the following noteworthy factors: transforming growth factor beta, interleukin-10, tumor necrosis factor-stimulated gene-6, hepatocyte growth factor 1, and matrix metalloproteinases.

Transforming growth factor beta (TGF- β) controls many functions in the cellular environment, including growth, propagation, differentiation, and apoptosis. TGF- β possesses both powerful immunosuppressive and neuromodulatory properties. It is thought that TGF- β may reduce chronic pain by inhibiting the activation and propagation of nerve-injury induced microglia and astrocytes.²⁸ TGF- β also decreases secretion of pro-inflammatory cytokines. However, the

mechanisms of TGF- β inhibition of neuropathic as well as chronic pain need further elucidation.²⁹⁻³¹

Interleukin-10 (IL-10) is known to be a powerful anti-inflammatory cytokine affecting inflammation and immunoregulation. The role of IL-10 remains elusive in pain management, with conflicting study results. A study by Li et al. demonstrated that the effects of MSCs were reversed by TGF- β and IL-10 antibody neutralization.³² This data suggests that IL-10 plays a critical role in reducing pain and inflammation.

Tumor necrosis factor stimulated gene-6 (TSG-6) is an immunomodulatory protein secreted by MSCs. Animal studies using various disease models suggest MSCs show dependence on TSG-6 for therapeutic effect. TSG-6 may shift cells such as macrophages from a proinflammatory state to an anti-inflammatory state. Injection of TSG-6 in ulcerative colitis mice reduces the severity of colitis and the number of inflammatory cells.³³

Hepatocyte Growth Factor-1 (HGF-1) is secreted in large amounts by MSCs. HGF-1 plays a role in angiogenesis and possesses the ability to promote cell growth, proliferation and resist apoptosis. Indeed it has a neuroprotective effect and *in vitro* studies illustrate that MSCs secrete HGF-1 which may have a protective effect on excitotoxicity-injured PC12 cells.³⁴ HGF-1 has demonstrated protective properties against autoimmune disease *in vivo* by activating regulatory T-cells, leading to the production of immunosuppressive cytokine IL-10.³⁵⁻³⁶

Matrix metalloproteinases (MMPs) are enzymes that are capable of degrading extracellular matrix proteins and can process a number of bioactive molecules. MMPs play a major role in neuroinflammation and pain through division and suppression of extracellular matrix proteins, cytokines, and chemokines.³⁷ Nerve injury-induced changes in the dorsal root ganglion are responsible for neuropathic pain.³⁸ MMP-9 produced by injured dorsal root ganglions provokes spinal microglia activation and neuropathic pain development due to MMP-9 pathophysiology, including IL-1 beta cleavage and microglia p38 activation in early phase neuropathic pain. MMP-2 activation leads to late-phase neuropathic pain via IL-1 beta cleavage and astrocyte ERK activation.³⁸ Tissue inhibitor of metalloproteinases (TIMP) suppress neuropathic pain. TIMP-1 diminishes early-phase neuropathic pain, while TIMP-2 diminishes late-phase neuropathic pain.³⁸

NEUROPATHIC PAIN

Neuropathic pain is one of the more difficult pain conditions to treat. Neuropathic pain is a progressive condition of the nervous system that is most often brought about by trauma, infection or ischemia. Neuropathic pain symptoms include hyperalgesia and allodynia.³⁹ Diabetic neuropathy is one of the most common and serious complications of diabetes mellitus and metabolic syndrome.⁴⁰ Common treatment for neuropathic pain involve pharmaceutical intervention to mask or diminish symptoms with little effectiveness and many side effects.

MSCs have shown promise in the treatment of neuropathic pain in both animal and human studies. It is thought that this is due to the multipotency and paracrine effects of MSCs, in particular anti-inflammatory cytokines, proangiogenic factors, and neurotrophic factors.⁴¹ Pre-clinical animal studies for trigeminal neuropathic pain, diabetic neuropathy, and hind paw neuropathic pain all suggest that MSCs are an encouraging method to treat various neuropathic pains.⁴² For instance, a study conducted by Venturi et al.,⁴³ used transperineal injections of autologous adipose tissue with stem cells to treat fifteen female patients with pudendal neuralgia that were unresponsive to three months of medical treatments. Patients were examined clinically and evaluated using visual analog scale (VAS), validated SF-36 health survey, and pudendal nerve terminal motor latency (PNTML) before treatment, and twelve months following treatment. Twelve patients completed the protocol with no adverse events. Two patients reported no improvement in pain. The remainder of the patients showed significant improvement in VAS and SF-36 evaluation.

In another study, Vickers et al.,⁴⁴ demonstrated the effects of SVF in ten subjects with neuropathic trigeminal pain who were unresponsive to traditional pharmacotherapy. The female patients underwent liposuction and the SVF was obtained via enzymatic digestion, centrifugation, and resuspension. The SVF was injected locally into the appropriate pain areas. Patients were examined clinically and evaluated using a 10-point pain intensity scale as well as the evaluation of the use of anti-neuropathic medication before and at one week, one, three, and six months after treatment. There were no systemic or local adverse effects as a result of treatment. Outcome results at six months showed that five out of nine subjects had both reduced pain intensity scores and reduced need for anti-neuropathic medication.

In a study using autologous bone marrow mononuclear cells, 168 patients with refractory diabetic neuropathy were evaluated. These patients underwent bone marrow aspiration, the mononuclear stem cells were separated using Ficoll-Hypaque density-gradient centrifugation, then prepared for intramuscular injection. Patients were examined clinically and evaluated using the Toronto clinical scoring system (TCSS) and nerve conduction studies before and at one, three, six, twelve, eighteen, twenty-four, and thirty-six months following treatment. Results of the study confirmed decrease of the neuropathic signs and symptoms of neuropathy as evidenced by TCSS scores, motor nerve conduction velocity, sensory nerve conduction velocity, compound muscle action potential, and sensory nerve action potential. No adverse events or complications were observed.⁴⁵

Indeed, many of these studies are small in size yet the collective results support the premise that patients can expect to see improvements from stem cell therapy for neuropathic pain. Most importantly, it was safe. More studies are needed to further evaluate dosage and length of effect.

DISCOGENIC PAIN

Low back pain is one of the leading causes of disability with a large social and economic impact. Disc degeneration occurs via hydration loss in the nucleus pulposus. The hydration loss manifests in instability, culminating in disc herniation, spondylolisthesis, facet joint dysfunction, and ultimately spinal stenosis.

It has been well established that inflammatory cytokines secreted by the disc cells account for the pain experienced in disc degeneration.⁴⁶ This supports the notion that stem cell therapy maybe a viable option for discogenic pain. It is well understood that MSCs have a paracrine effect which may address inflammation in the nucleus pulposus, as well as promote recruitment of other substances leading to the development of proteoglycans and type II collagen.⁴⁷ The use of MSCs is appropriate due to chondrogenic differentiation and immunomodulatory tendencies. Preclinical studies utilizing MSCs have demonstrated safety and efficacy in discogenic pain.⁴⁸

Numerous human studies have shown favorable outcomes utilizing a variety of stem cell sources for discogenic pain. For instance, Mochida et al.,⁴⁹ evaluated the use of autologous cultured nucleus pulposus chondrocytes that were co-cultured with MSCs in nine subjects with Pfirrmann grade III disc degeneration and posterior lumbar intervertebral fusion. Patients were examined clinically and evaluated before and three years following treatment using the Japanese Orthopedic Association (JOA) scoring system for low back pain and MRI results. Clinical outcomes demonstrated significant improvement in JOA function and pain scores, as well as the safety of activated nucleus pulposus cell transplantation and the efficacy of the procedure to slow disc degeneration. Most importantly, there were no adverse events reported during the three-year follow-up period.

In another study conducted by Orozco et al.,⁵⁰ the efficacy of autologous expanded bone marrow-derived MSCs was evaluated in ten patients with degenerative disc disease and persistent low back pain. The patients received injections directly into the nucleus pulposus. Patients were examined clinically and evaluated before and one year after treatment using visual analog scoring (VAS), Oswestry disability index (ODI), and MRI. Clinical outcomes illustrated that 71% of the patients had a decrease in pain and disability.

Pettine et al.,⁵¹⁻⁵³ evaluated the use of nonexpanded autologous bone marrow concentration (BMAC) in twenty-six patients with moderate to severe discogenic low back pain. Patients were examined clinically before and at three, six, twelve, twenty-four, and thirty-six months following treatment utilizing ODI, VAS, and MRI. MRI results at one-year post procedure showed improvement of one modified Pfirrmann grade in 40% of the patients. After thirty-six months, six out of twenty-six patients moved forward to surgical intervention. The remaining subjects demonstrated evidence of safety and efficacy using bone marrow-derived MSCs as an intradiscal therapy.

A randomized study conducted by Noriega et al.,⁵⁴ evaluated twenty-four patients with chronic low back pain with lumbar disc degeneration that were unresponsive to conservative

treatments using allogeneic bone marrow MSCs via intradiscal injection. Subjects were examined clinically before treatment and after twelve months utilizing VAS, ODI, SF-12, and MRI. The MSC-treated patients showed significant reduction in lumbar pain and disability at three months. The reduction was maintained at six and twelve months. Overall average reduction in disability was 28%, while 40% of those treated reported 100% improvement.

Coric et al.,⁵⁵ evaluated the use of culture-expanded allogeneic juvenile chondrocyte cells in fifteen patients with lumbar degenerative disc disease and low back pain. Patients were examined clinically before treatment and at one, three, six, and twelve months following a single treatment utilizing MRI, ODI, Numerical Rating Scale (NRS), and the SF-36. Results from this study are very encouraging, with average ODI, NRS, and SF-36 all showing significant improvement from baseline. Ten patients (77%) showed improvement in MRI. Only three subjects underwent surgical intervention by twelve-month follow-up.

Centeno et al.,⁵⁶ explored the use of culture-expanded autologous bone marrow-derived MSCs in thirty-three patients with lower back pain and degenerative disc disease. Patients were clinically evaluated before and after treatment and up to seventy-two months utilizing MRI, numeric pain score (NPS), modified single assessment numeric evaluation (SANE) rating, functional rating index (FRI), measurement of intervertebral disc dimension, and adverse events. Study results were very encouraging. NPS scores were significant relative to baseline at all evaluation intervals. SANE ratings showed an average improvement of 60% at three years post treatment. Twenty patients underwent post treatment MRI and 85% demonstrated a reduction in disc bulge size of 23% post procedure.

A phase I study conducted by Kumar et al.⁵⁷ evaluated the safety and efficacy of combined treatment of autologous adipose-derived stem cells with hyaluronic acid derivative at two different doses in patients with chronic discogenic low back pain. Ten patients received either a dose of 2×10^7 cells/disc or 4×10^7 cells/disc. Safety and efficacy outcomes were determined utilizing VAS, ODI, SF-36, and MRI/X-ray at regular intervals for one year. Results demonstrated 60% significant improvement in VAS, ODI, and SF-36 in both dosage groups with no adverse events.

In summary, there are various studies suggesting MSCs may ameliorate symptoms of discogenic pain. Most importantly studies illustrate that the treatment is safe regardless of whether the cells were culture-expanded. These studies demonstrate that autologous MSCs from bone marrow would be a suitable in-office clinical application for discogenic pain with proper patient selection.

OSTEOARTHRITIS OR INFLAMMATORY PAIN

Osteoarthritis (OA) is considered to be the most common chronic joint disease in an increasingly growing elderly population. The Center for Disease Control (CDC) estimates that OA affects over 30 million adults in the United States, with an associated cost of \$185.5 billion

per year.⁵⁸ While most consider OA an age-associated chronic degenerative disease, further research has revealed that cellular imbalance between catabolic/pro-inflammatory and anabolic pathways are behind the debilitating effects of OA.⁵⁹

Conventional treatments for OA are designed to control symptoms rather than disease modification. Most are pharmaceutical in nature and carry unwanted side effects with only limited benefit. The standard care for joint pain for those unresponsive to conventional therapy is joint replacement surgery, which is accompanied by significant complication risk, including pain or loss of function.⁶⁰

OA treatments should have three objectives: relieve pain, control inflammation, and restore function and/or range of motion. MSCs are a suitable option for the treatment of OA due to their potential to regenerate cartilage tissue and control the inflammatory response. Cytokine and growth factor release convey immunomodulatory, anti-inflammatory, and analgesic effects.⁶¹ Collectively this configuration of cells can help suppress cartilage destruction and its associated pain and inflammation. Moreover, preclinical OA models confirm MSCs' ability to differentiate into chondrocytes and repair damaged cartilage, as well as regulate local inflammatory environments and support a favorable healing environment via paracrine activities.

OA has been the most intensively investigated pain condition treated with MSCs. The majority of all OA studies using MSCs involve an intra-articular injection into the affected area. It is well understood that intra-articular injections of MSCs are safe.⁶² Moreover, there is an abundance of evidence demonstrating varying degrees of efficacy with a direct intra-articular injection of MSCs, particularly in the knee.

Bone Marrow Derived Stem Cell Studies in OA

Centeno et al., conducted a safety study of 339 patients with various joint conditions utilizing culture-expanded autologous bone marrow-derived MSCs which showed a greater than 75% improvement in forty-one percent of patients treated, with greater than 50% improvement in 63% of those treated. Average reported pain relief improved throughout the two-year study period. In terms of adverse events, only two percent reported self-limited increased pain and swelling at the injection site.⁶³

In a smaller study of four patients, utilizing culture-expanded autologous bone marrow-derived MSCs showed improvement in VAS pain scores in all subjects from 24% to 50% at six months with no adverse events reported.⁶⁴ Although a small patient population, this study supports many other publications illustrating decrease in pain.

Emadedin et al., investigated the safety of cultured autologous bone marrow-derived MSCs in eighteen patients with OA of the hip, knee, or ankle. Each patient received one injection. Patients were clinically examined before treatments and at two, six, twelve, and thirty months after treatment using MRI, VAS, and Western Ontario and McMaster Universities Osteoarthritis

Index (WOMAC). All patients improved in terms of increased walking distance, decreased VAS, and WOMAC scores, which were supported by MRI. No adverse events were reported.⁶⁵

Orozco et al., conducted two prospective, observational studies to evaluate cartilage regeneration and clinical improvement in patients with OA. The first study involved an intra-articular injection of culture-expanded autologous bone marrow-derived MSCs in twelve patients with OA of the knee. Patients were evaluated clinically before and at twelve months after treatments using VAS, WOMAC scoring, and MRI. At twelve months, mean pain scores and WOMAC decreased significantly. MRI showed improvement in the quality of knee cartilage and no adverse events were reported.⁶⁶

One of the first to conduct a randomized controlled trial involving fifty patients with OA of the knee utilizing peripheral blood stem cells was Saw et al.⁶⁹ Patients were randomized into two groups. One group received a series of intra-articular hyaluronic acid injections, while the other group received peripheral blood stem cells following knee arthroscopy and chondroplasty. Patients were evaluated at baseline and two years post treatment. Patients in both groups improved based on the International Knee Documentation Committee (IKDC) index score (reflecting pain, activity level) at twenty-four months. Patients receiving peripheral blood stem cells demonstrated increased cartilage quality at second-look arthroscopy.⁶⁹

Wong et al., analyzed the results of a study performing intra-articular cultured autologous bone marrow-derived MSC injections combined with hyaluronic acid or hyaluronic acid alone in fifty-six subjects with arthroscopic microfracture and medial opening sedge high tibial osteotomy. Patients were divided evenly into two groups- a cell treatment group and a control group. The primary outcome measure was the IKDC score at baseline, six months, one and two years post procedure. Secondary outcome measures included Tegner and Lysholm clinical scores as well as post procedural Magnetic Resonance Observation of Cartilage Repair Tissue (MOCART) scores at one year. While both treatment arms demonstrated improvement in Tegner, Lysholm, and IKDC scores, the cell recipient groups score showed statistically significant improvement.⁷⁰

Kim et al., evaluated the efficacy of intra-articular injection of autologous bone marrow aspirate concentrate (BMAC) with adipose tissue in forty-one patients with OA of the knee. Pain scores (VAS) and functional scales (SF-36, IKDC, Lysholm) were used for evaluation pre-procedure and three, six, and twelve months post procedure. Results showed significant improvement in both knee pain and knee function scores.⁷³

Soler et al., conducted a phase I-II prospective, open-label, single-dose, single-arm clinical trial assessing the feasibility, safety, and efficacy of ex-vivo expanded autologous MSCs infused intra-articular in patients with OA of the knee. Fifteen patients were clinically evaluated at baseline, six, and twelve months using VAS, SF-36 questionnaire, Lequesne functional index, WOMAC, and MRI. Results showed significant improvements in VAS, WOMAC, and Lequesne scores. The treatment was well tolerated with only a few reported mild adverse events (low back pain) while MRI showed signs of cartilage regeneration at twelve months.⁷⁴

Most recently, Chahal et al., conducted a phase II non-randomized, open-label dose escalation study in Canada to determine safety and efficacy of culture expanded autologous bone marrow-derived MSCs in twelve patients with late-stage knee OA. Patients were evaluated clinically at baseline and at one, two, and six weeks and three, six and twelve months using the following patient outcome measures: KOOS, WOMAC, Whole Organ MRI Scores (WORMS), collagen content (T₂ scores), synovitis, and inflammation and cartilage biomarkers. Significant improvement was noted in KOOS pain and symptoms, and WOMAC overall. Interestingly, the high-dose cell group showed significantly lower anti-inflammatory biomarkers.⁷⁶

A newly published study by Lamo-Espinosa et al., assessed the intra-articular injection of two doses of cultured autologous bone marrow-derived MSCs versus hyaluronic acid (control) in thirty patients with knee osteoarthritis. Patients were examined clinically at baseline, twelve, and forty-eight months utilizing VAS and WOMAC scoring. No adverse events were reported. The cell-dose groups improved clinically according to VAS and WOMAC scores.⁷⁷

Although all the studies are unique in their own way, they all demonstrate the safety of the use of bone marrow derived MSCs. Indeed, there was varying degrees of efficacy suggesting bone marrow derived MSCs may improve outcomes for patients suffering from OA.

Adipose Derived Stem Cell Studies in OA

Similarly, to the use of bone marrow derived MSCs studies, many clinical research studies have been completed demonstrating similar results. Here we will review several of them.

Jo et al., were the first to report results on dose-dependent direct intra-articular MSC injection for OA. This two-phase study consisted of eighteen patients with OA of the knee. Phase I patients received either a low-, mid-, or high-dose injection of culture-expanded autologous adipose-derived MSCs. Phase II subjects all received the high-dose treatment. While all groups demonstrated improvement in pain and function as evidenced by WOMAC, VAS, and KSS (Knee Society Score, an index that measures pain, stability, and range of motion), the results were statistically significant in the high-dose group at twenty-four months.⁶⁷⁻⁶⁸

Koh et al., conducted two studies utilizing autologous adipose-derived MSCs from the infrapatellar fat pad during arthroscopic debridement for the treatment of OA of the knee. The cells were injected intra-articular in a platelet-rich plasma suspension. Follow-up PRP treatments occurred on day seven and day fourteen post treatment. Study results demonstrated significant reduction of pain and improved quality of life via Lysholm score and KOOS two years post treatment.⁷¹⁻⁷²

An open-labeled phase I study conducted in France and Germany by Pers et al.⁷⁸ evaluated the safety of a dose escalation protocol of intra-articular injected cultured autologous adipose-derived MSCs in eighteen patients with OA of the knee. Three groups received either a low-,

medium-, or high-dose of MSCs. Outcome parameters included adverse event reporting and WOMAC scores at baseline, one week, three and six months post-treatment. At six months, treatment was determined to be safe with no serious adverse events reported. The low-dose cell therapy group demonstrated significant improvement via WOMAC score.

Bansal et al.,⁷⁹ evaluated the safety and preliminary effects of intra-articular injection of autologous adipose-derived SVF and platelet rich plasma in ten patients with OA of the knee. Patients were evaluated at baseline, three, six, twelve, eighteen, and twenty-four months utilizing WOMAC, six-minute walk distance, and MRI. Results displayed a significant decrease in WOMAC scoring and significant improvement in walking distance.

In a phase II randomized placebo-controlled study by Lee et al.,⁶² the safety and efficacy of a single intra-articular injection of autologous adipose derived MSCs in twelve patients with OA of the knee was assessed. Patients were evaluated over a six-month time frame. Outcome measures included WOMAC score, safety, and MRI. Results showed a significant improvement in WOMAC score at six months. There was no change in cartilage defect shown on MRI at six months. No serious adverse events were reported.

A newly published Australian randomized controlled study by Frietag et al.⁸¹ evaluated the efficacy of autologous culture-expanded adipose-derived MSCs on pain, function, and disease modification in knee OA. Thirty patients were randomized into either a single injection, two injections (baseline and six months), or control group. Patients were evaluated at baseline, one, three, six, and twelve months utilizing the following outcome measures: Numeric Pain Rating Scale (NPRS), KOOS, WOMAC, and MRI Osteoarthritis Knee Score (MOAKS). Results demonstrated significant pain reduction and functional improvement. Moreover, MOAKS demonstrated modification of disease progression. No adverse events were reported.⁸¹ Frietag et al.⁸² also described the use of autologous culture-expanded adipose-derived MSCs for the treatment of acromioclavicular joint OA with positive findings. The patient received two injections, one at baseline and at five months. Outcomes were evaluated utilizing NPRS, QuickDASH (Quick Disability of Arm, Shoulder, Hand), and MRI. Results showed pain reduction and functional improvement as well as structural improvement via MRI. This is the first reported use of MSCs therapy in upper limb OA.

Mautner and Bowers conducted a comparison study of autologous bone marrow aspirate concentrate (BMAC) versus autologous micro-fragmented adipose tissue MSCs in seventy-six patients with knee OA. Outcome measures included VAS, KOOS, Emory Quality of Life (EQOL). Results demonstrated improved pain and function scores at one year for adipose and one and a half years for BMAC with no significant differences between the two modalities.⁶⁸ This is the first study to directly compare BMAC and adipose MSCs.⁸⁶

In summary, all though there are various different clinical study designs completed, they all illustrate that autologous adipose derived stem cells are safe with varying amounts of efficacy. They all do demonstrate improvement in quality of life for patients suffering from OA.

Allogeneic Derived Stem Cells

While the vast majority of clinical studies published utilize autologous stem cells for the treatment of OA, there is a growing body of evidence demonstrating that allogeneic sources are safe and effective. As the FDA compliance deadline for allogeneic product drug registration and approval approaches, more research will quickly emerge regarding safety and efficacy.

In a randomized study by Vega et al., the use of culture-expanded allogeneic bone marrow-derived MSCs from three healthy donors were used in thirty patients with OA of the knee. The patients were randomized into two groups- allogeneic cell group and hyaluronic acid group. Patients were evaluated at baseline and followed for one year utilizing the following outcomes: VAS, Lequesne, WOMAC, and MRI. Results showed significant improvement in VAS, WOMAC, and Lequesne compared with hyaluronic acid at six and twelve months. Patient satisfaction in the cell-treated group was 77%, with no serious adverse events.⁸³

Vangness et al., performed a randomized, double-blinded study to determine the safety of intra-articular injections of cultured allogeneic bone marrow-derived MSCs for knee OA, the ability of MSCs to promote meniscus regeneration, and the effects of MSCs on OA changes in the knee in fifty-five patients. The subjects underwent partial medial meniscectomy followed by an injection of either low-dose allogeneic MSC, high-dose allogeneic MSCs, or sodium hyaluronate vehicle control within seven to ten days post procedure. Patients were evaluated at baseline and through two years to determine safety, meniscus regeneration, and pain. Results showed significantly increased meniscal volume in both cell groups. Those receiving cell treatment also experienced significant reduction in pain according to VAS assessment at twelve months.⁷⁵

Matas et al., assessed the safety and efficacy of either a single or repeated intra-articular injections of umbilical cord-derived allogeneic MSCs in OA of the knee. Twenty-eight patients were randomized to receive hyaluronic acid at baseline and six months, single-dose umbilical cord MSCs at baseline, or repeated dose at baseline and six months. Clinical outcomes were evaluated at one, four, eight, twelve, twenty-four, thirty-six, and fifty-two weeks utilizing adverse event reporting, WOMAC, VAS, SF-36, and OARSI. Only patients in the cell-treated group experienced significant pain and function improvement from baseline. The umbilical MSCs group with repeated dose had the most improvement. No adverse events were reported and no differences in MRI were detected.⁸⁴

In summary, clinical studies utilizing MSCs for joint pain illustrate efficacy as well as safety, regardless of whether the cells were culture-expanded, or allogeneic. Patients quality of life was improved and they are satisfied with these treatments. However, correct dosing and standardized protocols have yet to be established. Most importantly, the overall results are very favorable compared to conventional treatment strategies. These studies suggest that allogeneic stem cells are a viable option for those suffering from inflammatory or OA pain.

CANCER PAIN

Pain resulting from cancer is a very common ailment. Cancer-related pain is experienced by 55% of patients undergoing treatments and by 66% of patients that have advanced, metastatic, or terminal disease.⁸⁶ Cancer pain is either a direct effect of the disease, such as nerve compression, tumor extension into the tissue or bone; or the pain is related to treatment side effects such as chemotherapy-induced peripheral neuropathy, nerve injury, or lymphedema. The gold standard for cancer pain treatment is pharmaceutical intervention, in the form of opioids, steroids, antidepressants, and anticonvulsants. Many of these of these medications are accompanied by undesirable side effects.

The use of MSCs in treating cancer pain stems from the premise that chronic inflammation leads to cancer development.⁸⁷ A large amount of research has been devoted to the role of cytokines and chemokines in cancer. While the anti-inflammatory effect of MSCs is well established, there is controversy surrounding the role of MSCs in tumor development. Numerous studies have shown that MSCs inhibit tumor growth; while just as many have suggested that MSCs possess protumor tendencies.⁷⁰ As a result of this controversy, there is little research regarding the use of MSCs for cancer pain. A pre-clinical study by Sun et al., evaluated the use of human bone marrow MSCs genetically engineered to express the human proenkephalin (hPPE) gene to treat bone cancer pain in a rat model.⁸⁹ Results demonstrated a decrease in pain threshold as well as a decrease in inflammatory cytokines interleukin-1 beta and interleukin-6. This demonstrates the possibility of using MSCs that may be genetically modified as a therapy for cancer pain, but more research is needed.

Author's Note: The author recommends that due to the even remote possibility of MSCs promoting tumor growth, that any patients evaluated for stem cell treatment should not have had any active cancer within five years of treatment and should have recent PAP smear, mammogram, CEA and PSA testing before treatment.

MIGRAINE

Migraine is a complex episodic neurological disorder characterized by recurrent headaches, which may be accompanied by nausea, vomiting, and sensitivity to light and sound.⁹⁰ Visual aura is also experienced in approximately 30% of all migraine episodes, and migraines are three times more prevalent in females compared to males. Currently, there is no cure for migraine headaches. Conventional medical treatment consists of non-steroidal anti-inflammatory drugs (NSAIDs), triptans, anti-epileptics, beta-blockers, and calcium channel blockers, all of which vary in efficacy with significant side effects.⁹¹

The exact pathophysiology of migraine is not exactly known. There seems to be a hereditary component, as high as 50%. Most research points to the activation and sensitization of neurons in the trigeminal nerve as the trigger to migraine headaches. There are two possible theories as to how the nerve is aggravated- cortical spreading depression (CSD) and neurogenic inflammation. Cortical spreading depression is a wave of neural excitation activity followed by

depolarization of neurons. This may explain the aura some patients experience before the migraine episode. Neurogenic inflammation theory proposes that inflammatory neuropeptides lead to vasodilation and mast cell degranulation, followed by a release of various inflammatory compounds.⁹¹

Adult MSCs may help combat migraine due to their immunomodulatory effects. Studies using SVF have demonstrated to be effective for the treatment of trigeminal neuropathic pain. The activation of mast cells (which are derived from hematopoietic stem cells) triggers the release of inflammatory cytokines which result in many nervous system disorders, such as migraine.⁹⁵ Additionally, pericytes play a crucial role in neurological diseases. Used correctly, they may be used to increase blood flow, preserve blood-brain barrier function, and help repair glial scarring in areas of the central nervous system (CNS).⁹⁶ Moreover, MSCs studies have demonstrated that they are neuroprotective on dopaminergic neurons.⁹⁷

There are only two published studies utilizing cells for the treatment of migraine. Bright et al, treated four females with long histories of migraine or tension-type headache systemically with autologous stromal vascular fraction isolated from lipoaspirate. Two of the four women ceased having migraines after one month; this lasted for a period of twelve to eighteen months. A third patient had significant reduction in the severity and frequency of migraine over eighteen months. The fourth patient obtained a temporary decrease in symptoms for one month and was treated eighteen months later. Most importantly, the dosage and types of pain medication were reduced in all patients.⁹⁸

Mauksop and Rothaus investigated the intra-muscular injection of SVF in nine patients with severe migraine-related disability who failed botulinum toxin injections and three prophylactic drugs. The Migraine Disability Assessment Score (MIDAS) was used to assess the patients. Patients received injections in the pericranial, neck, and trapezius muscles and were evaluated at baseline and three months post treatment. The mean MIDAS score decreased thirty-four points. Seven out of nine patients reported a decreased MIDAS score but only two experienced meaningful improvement.⁹⁹

While these studies hold some promise for migraine treatment, the role of stem cells for migraines needs more research. Moreover, more studies with larger subject groups need to be completed to better evaluate the efficacy of stem cells for the treatment of migraine.

OPIOID TOLERANCE & OPIOID INDUCED HYPERPALGESIA

Two of the most troublesome side effects of opioid use/abuse is the occurrence of opioid tolerance and opioid-induced hyperalgesia. Opioid tolerance occurs when an individual requires greater amounts of opioids over time to obtain the original degree of therapeutic effect. This does not necessarily involve addiction. Increased dosages however, can lead to overdose and death. Opioid-induced hyperalgesia describes a situation in which individuals taking opioids to treat pain paradoxically develop an increased sensitivity to noxious stimuli.¹⁰⁰ Currently, there

are very few options in treating opioid tolerance or opioid-induced hyperalgesia other than medication rotation.

Molecular pathways and mechanisms tied to neuroinflammation appear to play similar, yet distinct roles in the development of both opioid tolerance and opioid-induced hyperalgesia. Opioids activate toll-like receptors on microglia, which leads to opioid tolerance. Opioid-induced hyperalgesia is mediated via μ -receptor dependent expression of P2X4 and the release of brain-derived neurotrophic factor (BDNF) from microglia.⁸² This pathway regulates microglia-to-neuron signaling, leading to the sensitization of spinal lamina I neurons and opioid-induced hyperalgesia. This suggests that mediating neuroinflammation would prove beneficial in treating opioid tolerance and opioid-induced hyperalgesia.

MSCs offer hope in the treatment of opioid tolerance and opioid-induced hyperalgesia, due mostly to their paracrine effects; mainly their immunomodulatory and anti-inflammatory effects. More specifically, MSCs impede opioid tolerance and opioid-induced hyperalgesia by hindering neuroinflammation. Repetitive opioid intake activates microglia and astrocytes in the spinal cord, as well as satellite cells in the dorsal root ganglion. Opioids also directly stimulate toll-like receptors (TLR-4), resulting in the manifestation of opioid tolerance. Additionally, opioids cause mu-opioid receptor (MOR)-mediated expression of purnergic receptors P2X4R and the release of BDNF from microglia. Opioid-induced hyperalgesia occurs due to the sensitization of lamina I neurons modulated by microglia-to-neuron signaling via the P2X4-BDNF-TrkB pathway.

MSCs may prevent neuroinflammation by paracrine release of a number of exosomes, microvesicles, and growth factors including indoleamine 2,3-dioxygenase (IDO), prostaglandin-2 (PGE-2), IL-10, TGF- β , leukemia inhibiting factor (LIF), human leukocyte antigen-G5 (HLA-G5), and TSG-6. MSCs communicate with microglia, astrocytes, macrophages, and other immune cells via various signaling pathways to discourage the manufacture and release of interleukin-1-beta, IL-6, TNF- α , BDNF, NO, and other pro-inflammatory bioactives that contribute to the development of opioid tolerance and opioid-induced hyperalgesia.¹⁰¹ Additionally, MSCs release growth factors that promote cell and tissue recovery via resident stem/progenitor cell stimulation, extracellular matrix remodeling, angiogenesis, and immune function modulation.¹⁰²

Pre-clinical studies show promising results using MSCs to reverse opioid tolerance and opioid-induced hyperalgesia. Researchers at the Cleveland Clinic¹⁰⁰ conducted pre-clinical studies in rodents using MSCs harvested from rat bone marrow. Opioid tolerance was induced in rats using daily morphine injections for three to four weeks. MSCs were delivered by either intravenous or intrathecal administration one or seven days before the administration of morphine to an opioid or control group. Outcome measures included paw withdrawal thresholds (PWT) to mechanical and thermal stimuli and tail flick tests. Intrathecal and intravenous MSCs did not cause any behavioral change in the control group. Both a single dose of intravenous or intrathecal administration in the opioid tolerance group significantly mitigated opioid tolerance for up to twenty-six days, regardless of the day of administration. The therapeutic effect took place as quickly as two days after treatment. In terms of opioid-

induced hyperalgesia, both intravenous and intrathecal administration of MSCs experienced similar degrees of anti-tolerance and anti-hyperalgesia effects. This is significant because intravenous injection is a much easier tolerated route of administration with less risk. Immunohistochemistry illustrated that the treatments significantly reduced microglia and astrocyte activation in the spinal cord. These findings are significant as both opioid tolerance and opioid-induced hyperalgesia are the leading causes of overdose and death when increasing doses are necessary for pain relief. Human studies are currently under development to substantiate the preclinical findings. If successful, this will radically change the treatment approach to pain management.

In summary, MSCs may be promising for opioid tolerance and opioid-induced hyperalgesia granted their main mechanisms of action are via paracrine effects. Indeed, preclinical data has illustrated a beneficial effect in animal models of addiction. More research and clinical work are needed to determine the efficacy of MSCs for opioid tolerance and opioid-induced hyperalgesia.

EXTRACELLULAR VESICLES

The use of extracellular vesicles are the next generation of possible therapies in the regenerative medicine sector. According to the International Society for Extracellular Vesicles (ISEV), extracellular vesicles (EV) refers to the generic term for particles naturally released from the cell that are delimited by a lipid bilayer and cannot replicate. EV subtypes should be defined by physical and biochemical characteristics and/or conditions/sources.¹⁰⁴ Another definition of EV is a collective term for heterogeneous, small, double-layered lipid membrane vesicles typically 30-2000 nm, which serve as carriers for biologically active signaling molecules such as mRNA, microRNA, proteins, enzymes, lipids, and DNA fragments.¹⁰⁵ EV are nanoparticles secreted by virtually all cell types in the body. Their main function is intercellular communication. Included in the EV category are exosomes, microvesicles, and apoptotic bodies. Due to the lack of specific markers, as well as overlapping characteristics of size, composition, and density, it is challenging to pinpoint various subtypes of EV. Exosomes are EV that are released from a cell following the merging of a multivesicular body (MVB, or intermediate endocytic compartment) with the plasma membrane. This process frees the intraluminal vesicles into the extracellular space, releasing the exosomes.¹⁰⁶ Exosomes tend to range in size from 40 – 100 nanometers. Microvesicles range in size from 50-1000 nanometers and originate via outward budding of the cell membrane. Apoptotic bodies or apobodies are released by cells experiencing apoptosis and range in size from 1- 5000 nanometers. EV can be isolated from most body fluids, as well as from the supernatant fluid of cell cultures. EV communication involves normal cellular processes as well as intercellular processes which are responsible for disease progression.¹⁰⁷

Exosomes may prove an important vehicle for the treatment of pain management. They provide intracellular communication and transport macromolecules between cells. They

contain a variety of bioactive materials including lipids (phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine, lysophosphatidic acid, ceramide, cholesterol, sphingomyelin), and nucleic acids (miRNA, mRNA). Exosomes from MSCs work in the same fashion as stem cells but are smaller in size, possess lower immunogenicity tendencies, and are not subject to the restrictions of MSCs in terms of cell survival and unknown behavior over time. Other advantages of exosomes over stem cell therapy center around issues with direct cell transplantation, including risk of emboli due to intravenous infusion, tumorigenesis, and immune activation from allogeneic sources.¹⁰⁸

Exosomes can be purified and stored for long time periods and they can be evaluated more accurately than their MSCs counterparts for safety, dosage, potency, etc.¹⁰⁹ This translates into a more potent, standardized dosing regimen as well as a faster approval pipeline with the FDA.

There are some advantages of using MSCs-derived exosomes for joint pain over MSCs themselves. While they both may possess the same properties, exosomes can be cultured and secured at a certain growth or differentiation point under controlled conditions. Also, the removal of environmental properties and tendencies allows for their use in a pathological environment (i.e., the exosomes will not yield to the inflammatory environment common to degenerative disease states).¹⁰⁹

MSCs-derived EV have been shown to confer a number of favorable effects in pre-clinical models of OA, joint inflammation, and cartilage injury. EV enhanced cartilage metabolism and reduced inflammation *in vitro*, and preserved cartilage and prevented the advancement of OA in mice after joint injury.¹¹⁰⁻¹¹¹ EV demonstrated immunomodulatory and chondro-protective effects *in vitro*, as well as reduced cartilage degradation and anti-inflammatory tendencies in *in vivo* mice models of OA.¹¹²⁻¹¹⁴ The use of EV in both rat and rabbit models of cartilage and osteochondral injury demonstrated significant defect repair as evidenced by the formation of hyaline-like collagen type II cartilage.¹¹⁵⁻¹¹⁷

Lui et al., studied the effects of exosome-mediated delivery of opioid receptor Mu small-interfering RNA (siRNA) for the treatment of morphine relapse *in vivo* and *in vitro* and morphine relapse in mice. Results demonstrated that the exosomes delivered the opioid receptor mu (MOR) siRNA into the brain and successfully inhibited morphine relapse via the down-regulation of opioid receptor mu expression levels. While more studies are needed, this could revolutionize the treatment of morphine, fentanyl, and methadone addiction.¹¹⁸

Exosomes hold promise for many degenerative conditions. Currently, human studies are underway using exosomes for conditions including cancer, diabetes, and wound healing. The current FDA position regarding exosomes is that due to their manipulation, they are considered a drug and not permissible in clinical practice at this time. However, due to their standardization abilities and acellular nature, they may be the gateway to many FDA-approved products in the regenerative medicine sector.

In summary, the regenerative medicine sector will soon have an impact on pain management. There is an abundance of evidence that demonstrates both safety and efficacy with the use of stem cells for pain. Indeed, controlled studies are still needed yet the evidence is relatively clear that stem cells help decrease pain and improve the quality of life for many. It remains to be seen if there will be large scaled controlled studies in the near future and the FDAs position on the use of cells for pain management.

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