Narrative Review

Biologics in Interventional Spinal Procedure: The Past, the Present, and the Vision

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Disclaimer: There was no external funding in the preparation of this manuscript.

Conflict of interest: Each author certifies that he or she, or a member of his or her immediate family, has no commercial association (i.e., consultancies, stock ownership, equity interest, patent/licensing arrangements, etc.) that might pose a conflict of interest in connection with the submitted manuscript.

Manuscript received: 05-11-2023
Revised manuscript received: 05-25-2023
Accepted for publication: 07-19-2023

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Background: Orthobiologics have shown promise in repair, restoration and regeneration of damaged and degenerated spine, joint and musculoskeletal tissues. The role of MSCs is to reduce inflammation, gliosis, and oxidative stress, while encouraging angiogenesis, neuronal proliferation, cell survival, and differentiation. While autologous MSCs have homologous advantages, they present with challenges related to donor predisposition, harvesting skills, and processing times. In this regard, allogenic MSCs show promise, but face ethical challenges, contamination, and survival risks. Ongoing efforts to overcome challenges and enhance performance include bioprinting, tissue engineering, artificial intelligence, nanotechnology, and microenvironmental alteration, among many others. Genetically programmed MSCs are being explored and tissue regeneration is now considered a real possibility. In this article, we discuss some of the leading-edge technologies in the process of being developed and perfected for widespread clinical application.

Objectives: The aim of this narrative review is both to update on orthobiologics, especially MSCs and provide a vision for their future potential in interventional spine medicine.

Study Design: Narrative review.

Methods: The PubMed database of the National Institute of Medicine and Google Scholar were searched for keywords "mesenchymal stem cell," "mesenchymal stem cell + regenerative medicine," and "mesenchymal stem cell + spine." The bibliographies of these articles and authoritative Web sites were also consulted.

Results: There are hundreds of ongoing clinical trials exploring the role of MSCs in regenerative medicine for treating a wide range of diverse conditions, including spine conditions, neurodegenerative disorders, and cardiovascular disease.

Limitations: This visionary narrative review has several limitations. It is a narrative, rather than a systematic review. Many of the ideas and treatments presented here are not perfected and are still in development.

Conclusions: The role of MSCs in regenerative medicine is still emerging, but their promise for spinal cord injury and other disorders of the spine is clear. Using allogenic or autologous MSCs can help stimulate healing and neural regeneration remains a tantalizing possibility.

Key words: Mesenchymal stem cell, regenerative medicine, spine, spinal cord injury, nanotechnology, bioprinting, neural regeneration, degenerative disc disease, low back pain

Pain Physician 2023: 26:E775-E785

The first detailed description of the isolation of mesenchymal stem cells (MSCs) from human bone marrow dates back 30 years, with infusion of MSCs into humans reported as early as 1995 (1,2). The ability of MSCs to differentiate into cells with endoderm and neuroectoderm characteristics, that is, into neurons and their progenitors, has opened important new avenues in interventional spine techniques (3,4). MSCs derived from human bone marrow are the most-used type of stem cell in regenerative medicine because of their homing ability to navigate to the site of injury (5). While it can be easy to herald the potential of MSC treatments as a major

breakthrough in treating spinal cord injury, there still remain unanswered questions, therapeutic limitations, and technological gaps and limitations.

The Mesenchymal and Tissue Stem Cell Committee of the International Society for Cellular Therapy has set forth minimal criteria to define human MSC: plastic adherence in standard culture; expression of certain cell surface proteins (CD105, CD73, and CD90) and lack of expression of others (CD45, CD34, CD14, CD11b, CD79α, or CD19, and HLA-DR surface molecules); and that these MSCs differentiate into osteoblasts, adipocytes, and chondroblasts in vitro (6). MSCs have been extracted from bone marrow, adipose tissue, dental tissue, the endometrium, peripheral blood, salivary glands, skin, and synovial fluid, leading to a great deal of heterogeneity among depots. Despite these issues, MSCs offer numerous advantages over embryonic stem cells because MSCs are genomically stable, easy to obtain, self-renewing, and their harvesting poses minimal ethical challenges (7). But while basic science has accelerated our understanding of the molecular structure of mesenchymal cells and their promise to regenerative medicine, clinical science lags behind due to regulatory challenges, lack of high-quality data, and the rigors and time requirements needed for long-term clinical studies.

MSCs have 3 important therapeutic characteristics: they can differentiate into specific cell types; they secrete exosomes and cytokines that stimulate cell growth and proliferation; and they act as anti-inflammatory, antibacterial, and immunomodulatory vehicles. MSCs must come into direct contact with host tissue in order to be effective (8). Numerous things can affect the effective use of MSCs in regenerative medicine, including MSC potency (which can be variable), their pharmacological function, how the cells are harvested and handled, dose, and route of delivery (9). There are hundreds of ongoing clinical trials exploring the role of MSCs in regenerative medicine for treating a wide range of diverse conditions (8). The purpose of this narrative review is both to update on MSCs and provide a vision for their future potential in interventional spine medicine.

Spinal Cord Injuries

Globally, 250,000 to 500,000 people suffer a spinal cord injury each year and 90% of such cases are caused by trauma (10). In the United States, there are approximately 20,000 survivors of spinal cord injury every year (11). About 12% of traumatic spinal cord injury patients die on the scene or within the first 3 hours, while 48% die within 7 days (12). Survivors face lifelong functional deficits, disability, and painful symptoms. Men are about twice as likely to experience a spinal cord injury as women with the greatest risk occurring between the ages of 20 to 29 and over 70 years (10). By contrast, in the United States, 78% of all new cases of spinal cord injury occur in men at age 43, up from age 29 in the 1970s (13). Spinal cord injury is associated with numerous comorbidities, such as deep vein thrombosis, muscle spasms, osteoporosis, pressure ulcers, and respiratory illnesses (10).

In theory, MSC transplantation would promote recovery from spinal cord injury through reduction in inflammation, gliosis, oxidative stress, and stimulation of angiogenesis, cell survival, and the proliferation of remaining neurons (14). Unfortunately, there are few studies using MSCs in patients with spinal cord injuries and mixed results. A meta-analysis of 19 studies, including a total of 670 patients with spinal cord injuries, showed statistically significant improvements in the American Spine Injury Association (ASIA) impairment scale total grade, and among other sensory scores, but no significant improvements were found in motor or activities of daily living scores (15). In a 3-year prospective placebo-controlled study (n = 27) of the use of bone marrow MSCs in patients with acute complete spinal cord injury, no functional motor improvement occurred in any patient, although those treated with MSCs had improved bladder sensation, decreased spasticity, and better posture control (16). In an unblinded, single-center study of 13 spinal cord injury patients administered autologous MSCs in cultured autoserum in one infusion, 12/13 patients had neurological improvement at 6 months. Five patients were rated ASIA C at baseline and 100% progressed to ASIA D one day postinfusion (17).

Preclinical studies using animal models of spinal cord injury in mice, rats, and dogs (n = 34 studies) revealed a meta-analytic improvement in motor functional recovery with MSC treatment (18).

Disorders of the Spine

Damage to intervertebral discs is a leading cause of low back pain and disability. The intervertebral discs, located between cartilage endplates within the spinal column, are the largest nonvascularized tissue in the body (19). In studies, chondrogenic-differentiated MSCs provide best results, but progress has been limited because of lack of unifying definitions as to what distinguishes an annulus fibrosis vs a nucleus pulposus cell (20). To date, the use of MSCs to repair intervertebral discs has concentrated on the nucleus pulposus pheno-

type, although the annulus fibrosis phenotype may be of utility in herniated or "slipped" discs (21). In a study of 24 patients with chronic low back pain (cLBP), 12 patients were administered allogenic MSCs by intradiscal injection, and 40% exhibited rapid pain relief (22).

cLBP is a prevalent, potentially disabling, global health care crisis with about 20% of the population between the ages of 20 and 59 affected (23). A meta-analysis in cLBP patients revealed significant improvements in pain score and function based on the Oswestry Disability Index after 12 months following treatment with MSC injections (24). Mesenchymal precursor cells were administered via intradiscal injection in 100 cLBP patients in a randomized multicenter clinical trial and shown to provide safe, effective results in relieving pain durable to 36 months (25). In a study of 11 patients suffering monosegmental degenerative disc disease at L4-L5 or L5-S1, autologous MSCs in a tricalcium phosphate carrier were implanted in spinal surgery and patients were monitored in 4 visits over the next year. In all cases, autologous MSCs expanded and no adverse events were observed. Oswestry Disability Index scores, as well as pain relief, improved following surgery (26). Similarly, therapeutic potential of MSCs is also being explored in other spinal structures such as vertebral body, facet joint, capsule, ligaments, among other tissues that comprise the spine functional unit.

Therapeutic Potential of MSCs

MSCs are a class of multipotent stromal cells with the ability to self-renew and differentiate into terminally, specialized cells within the mesodermal lineage, such as adipocytes, fibroblasts, stromal cells, myoblasts, osteoblasts, and chondrocytes (27,28). Not limited to mesodermal lineages, MSCs have the potential to differentiate into cells with endoderm and neuroectoderm characteristics, namely neurons and their progenitors, making MSCs highly relevant to interventional spinal therapeutics (4,29). Natively, MSCs are distributed throughout perivascular regions of most tissues in the body and are thought to play a role in maintaining tissue remodeling, repair, and homeostasis by acting as a latent pool of stem/progenitor cells (29-31). MSCs have shown to have the ability to migrate to the local site of tissue injury, replace damaged and dying cells through differentiation, and modify the local microenvironment to promote tissue repair (32,33). The primary tissue repair mechanisms of MSCs reside in their secretory molecules and paracrine signaling (35). MSCs mediate immunosuppression in the local tissue environment through the inhibition of CD8+/CD4+ T lymphocytes, natural killer cells, dendritic cells, and the reduction of immunoglobulin production. They also stimulate local T regulatory cells to modulate immune activation of other immune cells (29,34). MSCs have low expression of HLA-I and do not express HLA-II, CD80, or CD86 on their cell surface, all of which are critical for T lymphocyte activation (29,35). For that reason, MSCs are unlikely to be targeted by T cells and have reduced immunogenicity when transplanted (36). However, this is not to say that they are immune privileged, as there is evidence for rejection for allogenic MSCs, albeit a slow and subdued one (36).

Sources of adult MSCs that have been extracted, cultured, and studied include bone marrow, adipose tissue, dental tissue, endometrium, peripheral blood, salivary gland, skin, and synovial fluid - all of which have shared and unique cell surface markers (7).

Current Innovations in MSC Therapy

The optimal transplantation method for MSCs is not known. With the injection method, only a portion of the total MSCs injected will actually engraft onto the injured tissue. A biomaterial "scaffold" can assure transport of as many MSCs as possible directly to the site of injury. A meta-analysis of 34 animal studies found that MSCs and scaffolds provided significantly greater motor function recovery than MSCs alone or placebo (18). Scaffolds as an adjunctive treatment to MSC transplantation in humans with spinal cord injury remain controversial. Collagen scaffolds were used with human umbilical cord MSCs in 2 patients with acute complete spinal cord injuries (one at T11 and one at C4) (37). At one year, there were no adverse reactions to the treatment and some degree of sensory and motor function recovery was observed in both patients (37). Further study is needed.

While 3-dimensional (3D) printing of the various structures of the central nervous system has not yet been achieved, a microscale continuous projection biomimetic printing method may help to reproduce certain complex structures needed for regenerative therapies of the spinal cord (38). A biomimetic hydrogel scaffold has already been tested in rats; it was successful in that the study found that cells adhered to the scaffold (38).

Nanotechnology

Nanotechnology encompasses both the creation of novel nanomaterials and their use in nanostructures; many nanotechnological applications relate to regen-

erative medicine. Nanoparticles can serve as carriers for targeted MSC delivery. Such nanostructured materials attempt to recapitulate the stem cell within a tissue and then guide the MSC toward creating the sort of environment that would permit further regeneration (39). Accurate targeting of the MSCs can be problematic, and a proof-of-concept study has evaluated the role of a magnetically activated "micro-robot" that can be deployed, guide the MSCs to proper placement, and then biodegrade after delivering the MSCs (40). Magnetic resonance imaging applications can be used to move these structures or to create a magnetic domain (41).

Nanotechnology can be used to image MSCs, to deliver drugs within cells, to track MSCs, and others (42). Overall, nanotechnology enhances specificity and control of the MSC treatment, making it more versatile (43). Scaffolds for use with nanoparticles have been created in a variety of shapes and sizes, including some with specific surface topographies and others that allow for a controlled release of small molecules (44). The biomaterials can send signals, which MSCs can interpret for determination of their cell fate. To that end, chemical, physical, and topographical scaffolding features may play a role (44). Nanoparticles can also be used as carrier systems to promote targeted delivery of bioactive MSCs, which enhances regeneration (39). Nanotechnology monitoring systems that track the MSCs as they migrate within the body may be helpful in diagnostic procedures, as they are drawn toward injury and inflammation that diagnostic systems may not be able to detect (39).

Exosomes and Secretomes from MSCs

An exosome is a vesicle with a single membrane that is found in a variety of different types of cells and is present in MSCs (45). The MSC exosomes aid in cell-to-cell communication by secretion, protein, and RNA transport (45). First discovered in the early 1980s, exosomes were initially misinterpreted as waste products from cells, causing them to be downplayed until recently (46). While these extracellular vesicles are a type of paracrine molecule whose functions are not elucidated (47), exosomes likely support cellular regeneration and do not carry the same risks as transplanted cells (48). Exosomes specifically secreted by MSCs may aid in tissue repair (49).

Exosomes may be described as nanovesicles, that is, small organelles with a single membrane and a topology similar to their cell. Thus, they can remodel the extracellular matrix (ECM) and transmit signals (50). When properly deployed and directed, exosomes can transform the ECM, transmit signals, and trigger changes in pathophysiology (50,51). Stem cell products, including exosomes, are regulated by the US Food and Drug Administration (FDA), which is responsible to grant market clearance to all stem cell products (52). To date, no exosome-based product has yet been approved to market by the FDA. In specific, none of these products have been approved for orthopedic or neurological application (52).

First described in 2000 by Tjalsma et al (53), secretomes secreted by an organism, tissue, or a cell into the ECM. MSCs secrete secretomes that can regenerate nerves and relieve pain at inflammatory sites (54). Secretomes are of great interest to regenerative medicine because they may offer new avenues of treatment; it may be that secretomes have a beneficial synergistic effect when acting on host tissue (55). Secretomes are currently being studied for their potential role in regenerative medicine (56). In animal studies, MSC serotomes injected into lesion sites had neuroprotective features, reduced the cystic cavity, and preserved the spinal tract, all of which aided the recovery of locomotion (57,58).

Allogenic and Autologous MSCs

Allogenic stem cells from donors can be used immediately in allogenic treatments. Such cells can be specifically derived and excess cells banked for future use, so a series of allogenic treatments can be scheduled and deployed rapidly (59). The main drawback to allogenic MSCs is the potential for rejection (60). Immunosuppressive therapy may be required with allogenic MSCs (61). In this regard, autologous MSCs are considered (62,63). A drawback to autologous therapy is that the MSCs share the same comorbid burden as the patient. The heterogeneity of MSC populations has been a long-standing challenge to their effectiveness because these cells may come from different tissue depots, different donors, different cell cultures, and subjected to different expansion protocols. MSCs from certain tissue depots proliferate faster than others and some have more potent self-renewal capacity (64). Immunogenicity with autologous MSCs is more limited, but may still occur and has been observed in animal studies (65).

Risks associated with allogenic MSC therapy include the dangers of potential contamination, the potential for cytokine storm, tumor lysis, and even graft-vs-host disease (9,66). Over the long term, risks can include genetic instability, chromosomal mutations, and lack of or poor therapeutic response (66). Based on the limited clinical studies conducted and their adverse events, it may be that MSC therapies are relatively safe (66).

Current Challenges in MSC Therapy

A great challenge in regenerative medicine, in general, and MSC therapy, in particular, remains the low rates of cell retention and survival in clinical use (44). Cell therapy involves introducing stem cells into tissue for treatment of a disease or condition without the inclusion of gene therapy (67). Preclinical studies of MSC for treating spinal cord injury were promising, but subsequently, a few small human trials produced mixed results and modest benefits (68).

A major challenge in MSCs is the fact that population sources of MSCs are heterogeneous. For instance, the depot from which the MSCs were obtained may affect their proliferation rate or other attributes. Allogenic MSCs obtained from older individuals have a reduced differentiation potential, as well as a slower proliferation rate (69). Once in the body, MSCs can be washed away in circulatory activity or leak out of the body; low retention rates limit or preclude effectiveness. MSCs injected where they are needed, that is, into an inflammatory or ischemic environment, may not survive (70). When ECM-anchoring cells detach from the ECM, they may undergo apoptosis in a process known as anoikis (71). Stem cells normally resist anoikis (71), but therapeutic delivery of transplanted MSCs can trigger anoikis due to the loss of cell adhesion (71,72).

MSC grafts can facilitate bone healing, but attention must be paid to the microenvironment in which the grafts are to function (73). When MSCs are directed toward diseased microenvironments, they can struggle to survive. Improving their resistance to such hostile microenvironments can be a crucial component to their successful application (73). The solutions to bringing safe, effective MSC treatments to spinal patients reside in novel forward-looking technologies, such as bioengineering, microenvironment optimization, artificial intelligence (AI), and genetically enhanced therapies.

Bioprinting

Bioprinting utilizes additive manufacturing technology to combine living and nonliving materials into highly specific 3D structures (74). In contrast to other 3D printing techniques, bioprinting prints living cells within the medium ("bio-ink"), presenting both new medical opportunities and new engineering challenges (74). A great advantage of bioprinting technology is that it is highly customizable (75) and ideally suited for the body's microenvironments (76). In theory, at least, bioprinting technologies have the potential to revolutionize regenerative medicine (45). Bioprinting of organs, tissues, and even individual cells is possible, allowing for a native-similar replication of cellular architecture (77). A formidable challenge in bioprinting is "bio-ink," a name given to the living cells that are used in the processes; these bio-inks must be handled with extreme care and gentleness to preserve them (77). Bioprinting undifferentiated stem cells expands the potential of stem cell therapy, but it requires careful replication of the physiologic microenvironment (45).

There are currently several different ways to bioprint: multicellular bioprinting; microextrusion; laser-assisted bioprinting; stereolithography; cell aggregates;

Table 1. *Bioprinting options for MSC (45).*

Bioprinting Technique	Description	Advantages	Disadvantages
Cell Aggregates	Use of organoids rather than printing drops	Moderate printing speeds, high resolution, high throughput, good cell viability	Limited capability in terms of cell density, high costs
Droplet-Based Bioprinting	Single-cell printing	Allows for printing and depositing one cell at a time, ideal for academic or research applications, high cell viability	Delicate technique, high costs
Laser-Assisted Bioprinting	Cell-rich bio-inks are layered, one by one, onto a substrate	Moderate to fast printing, high resolution, limited shear stress on cells	Downside, may have difficult with viscous materials, high costs
Microextrusion	Creates continuous lines of cell-rich bio-ink	Allows for building stable 3D structures, can replicate ECM	Slower printing speeds, risk of shear stress
Multicellular Bioprinting	Cell-rich bio-inks are used in inkjet printers; delivery is in droplets	Moderate throughput speeds; relatively low cost	May result in some cell abnormalities
Stereolithography	Liquid photocurable polymer cured in UV light is used to construct layers	Allows for high resolution, good cell viability, photopolymerization can crosslink the constructions, allows for elaborate 3D constructs	Limited availability of resins

Abbreviations: MSC, mesenchymal stem cell; 3D, 3-dimensional; ECM, extracellular matrix.

and droplet-based bioprinting (45). Each method has its own particular advantages and drawbacks, as summarized in Table 1.

Repairing tissue in situ using MSCs utilizes bioprinting techniques, which, in turn, is dependent on the biomaterial(s) used (78). A variety of materials have been utilized: hydrogels; biopolymers; synthetic ECMs; and others (78). The 4 factors that must be addressed in this stem cell microenvironment are: cell migration; environmental remodeling; change in phenotype; and cell viability (78). Hydrogels can encapsulate the MSCs and since hydrogels are porous, biodegradable, and essentially made of water, they provide a suitable environment by permitting the encapsulated cells to maintain homeostasis as they adjust to their new microenvironment (79). However, hydrogels have limits depending on the ECM into which the MSCs are introduced (78).

For spinal applications, scaffolding rather than bioprinting may be a more effective approach. The MSCs attach themselves to the biocompatible scaffold which is then implanted, allowing the MSCs to slowly migrate beyond the scaffold and into the affected area. This method is particularly suitable for hard cartilage and bone (78).

Biological Device Combinations

In their native microenvironment, human MSCs are exposed to a continuous flow of chemical, physical, and mechanical cues that prompt them to differentiate (80). Early attempts to guide or influence this differentiation were ineffective for a variety of reasons, including an inability to provide precise technical controls along with a lack of scalability (80). Regenerative medicine requires tissue engineering to find ways under biomimetic conditions for seed cells to grow properly.

Functional nanoparticles can help allow scaffolds to produce cells with the appropriate biocompatibility, physical sensing, and other desired features (81). To this end, magnetized scaffolds have been used with an electrospinning process that assembles superparamagnetic iron oxide nanoparticles or gold nanoparticles, layer by layer (81). This results in a film-like structure of nanoparticles, improving hydrophilicity, and promoting scaffold elasticity, all of which contribute to osteogenesis (81). In other words, magnetized nanoparticles work as a biologically active interface between MSC and scaffolds (81).

The application of exogenous electrical stimulation to cells can affect cell proliferation, orientation, and bone remodeling (82). Functional assays performed in vitro have shown that the homogeneous delivery of electrical energy can cause human MSCs to elongate, differentiate, and orient themselves to the cytoskeleton without variations in pH levels or hydrogen production (82). Such stimulation usually involves conductive electrodes made of noncorrosive materials, such as titanium (83). In osteogenesis, both cell morphology and elongation can be crucial, making electrical stimulation an important technology (84). Using nanofiber scaffolding and a 2-layer microfluidic chip system, a 3D microenvironment can be created, allowing for seeding MSCs onto the scaffolds for optimization (84).

Biomechanics play an important role in bone marrow MSC differentiation (85). Protocols differentiate between MSC differentiation into cartilage cells, bone cells, or adipose cells. In an in vitro study, compression alone or shear force alone was not able to induce chondrogenic differentiation in MSCs, but when shear force was superimposed on dynamic compression, this combination allowed chondrogenic gene expression (85). Subsequent histology reports showed that the combination group (shear plus compression) was the only cell group to have sulfated glycosaminoglycan and collagen II (85).

Microenvironment Optimization

When MSCs are to be used in vivo, special attention must be paid to the creation of the most physiologically accurate microenvironment possible (45). Scaffolding and inclusion of biochemicals, such as peptides, exosomes, growth factors, and so on are crucial (45). The ideal microenvironment for MSCs seems to be a 3D spheroid (86). While there is general agreement on this premise, it is not clear how to best maximize the spheroid formation, which leads to greater cell survivability and better anti-inflammatory and proangiogenic potential (86). MSC spheroids, for example, secrete more beneficial trophic factors than individual dissociated MSCs (87). Transplanting MSCs in biomaterials increases their ability to aid in wound healing not just by localizing the MSCs at the desired site, but also by upregulating the secretion of trophic factors, for example, using a fibrin gel (87).

Notable recent advancements in biomaterials apply novel techniques using tissue-derived ECM, conjugated growth factors, and biochemically adjustable properties (44,88). Thermosensitive hydrogels using chitosan can provide an interesting microenvironment,

in that they change from a liquid state to a gelatinous state at room temperature; they are biocompatible and work well with 3D scaffold technologies (89).

Statistical Modeling and AI

The quantity, complexity, and sheer quantity of data generated in cell-based regenerative medicine have given rise to an interest in AI. A variety of AI algorithms are being explored to help semiautomate the challenging work of cell segmentation and assessments of the quality of a particular colony of cells (90). In fact, MSC therapy on a large scale is hardly feasible without AI due to the time-consuming process of evaluating colony morphology and the potential for human error in this tedious work (90).

In health care, the main forms of AI currently in active use are machine learning (ML) and deep learning (DL). While a full discussion of AI is beyond the scope of this review, in short, ML can teach itself, while DL is more nuanced and based on layers of information. ML uses statistical tools to discern patterns in supervised or unsupervised learning situations, and uses statistical tools to help discern patterns (93). DL is a more complex, open-source system currently used in certain business applications, robotics, self-driving cars, and medical imaging interpretations (91). AI seems a valuable tool for regenerative medicine because working with large quantities of nonstable, heterogeneous MSCs necessitate such arduous testing methods that errors can occur even in the strictest settings (92). A long-term objective for AI in regenerative and other fields of medicine would be to accurately predict outcomes that could help guide clinical decision-making (93). The potential of AI, or rather a synergy of human clinical experience coupled with AI systems, may also help solve other MSC-related problems, such as length culture times, the laborious characterization of specific cells, faster identification of cells, fewer errors, truncated manufacturing processes, and eliminating production bottlenecks (94).

Genetically Programmed Stem Cells

MSC-based gene therapy introduces genes into the MSC using a viral or nonviral method to promote transgene expression. This is a very new strategy, but it has the potential to be useful in numerous and diverse applications, particularly in personalized medicine (95). Transduced human MSCs are able to maintain transgene expression in vitro and in vivo following stem cell differentiation (96). Existing gene delivery systems for MSCs are efficient, but they may induce permanent undesired genetic changes and promote tumors, leading to the investigation into more refined and other approaches (97).

Discussion

Regenerative medicine, in particular the use of MSCs, presents new options for the treatment of spinal disorders. This is particularly important for treatment of a spinal cord injury, which is characterized in large part by a cascade of multifactorial secondary effects that include diverse molecular processes: inflammation; neuronal death; ionic dysregulation; overwhelming presence of free radicals and lipid peroxidation; disruption of nerve pathways; dysfunction at the blood-brain barrier; and cellular apoptosis and necrosis, along with cavitation and retrograde degeneration (68). Left unchecked, this becomes a dangerous cascade of inflammatory responses, aberrant signaling, vascular damage, and cellular dysfunction (68). Two key processes are needed for relative recovery: removal of the accumulating cellular debris (macrophagy) and regeneration of neurons (68). Neuronal regeneration is a natural process for the axolotl (salamander), where the spinal cord injury triggers a glial response, causing these glia to migrate to the injured area, replace the missing neural tube, and stimulate axonal growth (68). Thus, the key to treating a spinal cord injury is to understand neural regeneration.

Stem cells divide and produce asymmetrical offspring; stem cells can differentiate into a variety of phenotypes. A totipotent stem cell is one that could develop into any potential cells, while multipotent cells have the potential for any number of cells (101). Important steps in advancing stem cell treatments are the modulation of phenotypic pathways, better transplant and imaging techniques, tissue engineering, and clinical trials. MSCs have specific appeal: they can be readily isolated; are available from multiple sources; can be easily preserved; have limited risk of causing tumors; and are free from technical and ethical controversies (101). MSCs are also known for rapid proliferation, another key advantage, and they have both autocrine and paracrine activity (102,103). MSC treatments have been used on patients with chronic spinal cord injury and while it was a safe treatment its neurological benefits were modest to none (104). This should not be viewed as a definitive failure, but rather as a need to find better techniques and improve our clinical under-

standing. The vision of this narrative review is that we have many - if not all - of the puzzle pieces needed to assemble a new way of treating spinal cord injury and other disorders of the spine.

This visionary narrative review has several limitations. It is a narrative, rather than a systematic review. Many of the ideas and treatments presented here are not perfected and are still in development.

Conclusions

The role of MSCs in regenerative medicine is still emerging, but their promise for spinal cord injury and other disorders of the spine is clear. Using allogenic or autologous MSCs can help stimulate healing and neural regeneration remains a tantalizing possibility. Breakthroughs in bioengineering, bioprinting, bio-inks, nanotechnology, and scaffolding may facilitate the use and effectiveness of MSCs. Regeneration depends, in part, on the ability of the MSCs to communicate back and forth with other cells by secretion and protein and RNA transport. To this end, exosomes and exosomes secreted from MSCs may play a crucial role in tissue repair. Numerous challenges remain, mainly involving the best way to transfer regenerative medical concepts into safe and effective clinical practice.

References

1. Haynesworth SE, Goshima J, Goldberg VM, et al. Characterization of cells with osteogenic potential from human marrow. *Bone* 1992; 13:81-88.
2. Lazarus HM, Haynesworth SE, Gerson SL, et al. Ex vivo expansion and subsequent infusion of human bone marrow-derived stromal progenitor cells (mesenchymal progenitor cells): Implications for therapeutic use. *Bone Marrow Transplant* 1995; 16:557-564.
3. Jiang Y, Jahagirdar BN, Reinhardt RL, et al. Pluripotency of mesenchymal stem cells derived from adult marrow. *Nature* 2002; 418:41-49.
4. Urrutia DN, Caviedes P, Mardones R, et al. Comparative study of the neural differentiation capacity of mesenchymal stromal cells from different tissue sources: An approach for their use in neural regeneration therapies. *PloS One* 2019; 14:e0213032.
5. Fu X, Liu G, Halim A, Ju Y, Luo Q, Song AG. Mesenchymal stem cell migration and tissue repair. *Cells* 2019; 8:784.
6. Dominici M, Le Blanc K, Mueller I, et al. Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. *Cytotherapy* 2006; 8:315-317.
7. Ullah I, Subbarao RB, Rho GJ. Human mesenchymal stem cells - current trends and future prospective. *Biosci Rep* 2015; 35:e00191.
8. Squillaro T, Peluso G, Galderisi U. Clinical trials with mesenchymal stem cells: An update. *Cell Transplant* 2016; 25:829-848.
9. Galipeau J. Mesenchymal stromal cells for graft-versus-host disease: A trilogy. *Biol Blood Marrow Transplant* 2020; 26:e89-e91.
10. World Health Organization. *Spinal cord injury*. Accessed 09/12/2022. www.who.int/news-room/fact-sheets/detail/spinal-cord-injury#
11. Jain NB, Ayers GD, Peterson EN, et al. Traumatic spinal cord injury in the United States, 1993-2012. *Jama* 2015; 313:2236-2243.
12. Lalwani S, Singh V, Trikha V, et al. Mortality profile of patients with traumatic spinal injuries at a level I trauma care centre in India. *Indian J Med Res* 2014; 140:40-45.
13. National Spinal Cord Injury Statistical Center. *Spinal cord injury facts and figures at a glance*. Accessed 09/12/2022. www.nscisc.uab.edu/Public/Facts%20and%20Figures%202019%20-%20Final.pdf
14. Liau LL, Looi QH, Chia WC, et al. Treatment of spinal cord injury with mesenchymal stem cells. *Cell Biosci* 2020; 10:1-17.
15. Muthu S, Jeyaraman M, Gulati A, et al. Current evidence on mesenchymal stem cell therapy for traumatic spinal cord injury: Systematic review and meta-analysis. *Cytotherapy* 2021; 23:186-197.
16. Saini R, Pahwa B, Agrawal D, et al. Efficacy and outcome of bone marrow derived stem cells transplanted via intramedullary route in acute complete spinal cord injury - a randomized placebo controlled trial. *J Clin Neurosci* 2022; 100:7-14.
17. Honmou O, Yamashita T, Morita T, et al. Intravenous infusion of auto serum-expanded autologous mesenchymal stem cells in spinal cord injury patients: 13 case series. *Clin Neurol Neurosurg* 2021; 203:106565.
18. Yousefifard M, Maleki SN, Askarian-Amiri S, et al. A combination of mesenchymal stem cells and scaffolds promotes motor functional recovery in spinal cord injury: A systematic review and meta-analysis. *J Neurosurg Spine* 2019; 32:269-284.
19. Kraus P, Lufkin T. Implications for a stem cell regenerative medicine based approach to human intervertebral disk degeneration. *Front Cell Dev Biol* 2017; 5:17.
20. Thorpe AA, Binch AL, Creemers LB, et al. Nucleus pulposus phenotypic markers to determine stem cell differentiation: Fact or fiction? *Oncotarget* 2016; 7:2189-2200.
21. Li X, Zhang Y, Song B, et al. Experimental application of bone marrow mesenchymal stem cells for the repair of intervertebral disc annulus fibrosus. *Med Sci Monit* 2016; 22:4426-4430.
22. Noriega DC, Ardura F, Hernández-Ramajo R, et al. Intervertebral disc repair by allogeneic mesenchymal bone marrow cells: A randomized controlled trial. *Transplantation* 2017; 101:1945-1951.
23. Meucci RD, Fassa AG, Faria NM. Prevalence of chronic low back pain:

Systematic review. *Rev Saude Publica* 2015; 49:1.

24. Sanapati J, Manchikanti L, Atluri S, et al. Do regenerative medicine therapies provide long-term relief in chronic low back pain: A systematic review and metaanalysis. *Pain Physician* 2018; 21:515-540.
25. Amirdelfan K, Bae H, McJunkin T, et al. Allogeneic mesenchymal precursor cells treatment for chronic low back pain associated with degenerative disc disease: A prospective randomized, placebo-controlled 36-month study of safety and efficacy. *Spine J* 2021; 21:212-230.
26. Blanco JF, Villarón EM, Pescador D, et al. Autologous mesenchymal stromal cells embedded in tricalcium phosphate for posterolateral spinal fusion: Results of a prospective phase I/II clinical trial with long-term follow-up. *Stem Cell Res Ther* 2019; 10:63.
27. Sarugaser R, Hanoun L, Keating A, et al. Human mesenchymal stem cells self-renew and differentiate according to a deterministic hierarchy. *PloS One* 2009; 4:e6498.
28. Ma S, Xie N, Li W, et al. Immunobiology of mesenchymal stem cells. *Cell Death Differ* 2014; 21:216-225.
29. Almeida-Porada G, Atala AJ, Porada CD. Therapeutic mesenchymal stromal cells for immunotherapy and for gene and drug delivery. *Mol Ther Methods Clin Dev* 2020; 16:204-224.
30. Bianco P. "Mesenchymal" stem cells. *Annu Rev Cell Dev Biol* 2014; 30:677-704.
31. Crisan M, Yap S, Casteilla L, et al. A perivascular origin for mesenchymal stem cells in multiple human organs. *Cell Stem Cell* 2008; 3:301-313.
32. Hofstetter C, Schwarz E, Hess D, et al. Marrow stromal cells form guiding strands in the injured spinal cord and promote recovery. *Proc Natl Acad Sci U S A* 2002; 99:2199-2204.
33. Kachgal S, Putnam AJ. Mesenchymal stem cells from adipose and bone marrow promote angiogenesis via distinct cytokine and protease expression mechanisms. *Angiogenesis* 2011; 14:47-59.
34. da Silva Meirelles L, Fontes AM, Covas DT, et al. Mechanisms involved in the therapeutic properties of mesenchymal stem cells. *Cytokine Growth Factor Rev* 2009; 20:419-427.
35. Rasmusson I, Ringdén O, Sundberg B, et al. Mesenchymal stem cells inhibit the formation of cytotoxic T lymphocytes, but not activated cytotoxic T lymphocytes or natural killer cells. *Transplantation* 2003; 76:1208-1213.
36. Ankrum JA, Ong JF, Karp JM. Mesenchymal stem cells: Immune evasive, not immune privileged. *Nat Biotechnol* 2014; 32:252-260.
37. Xiao Z, Tang F, Zhao Y, et al. Significant Improvement of acute complete spinal cord injury patients diagnosed by a combined criteria implanted with NeuroRegen scaffolds and mesenchymal stem cells. *Cell Transplant* 2018; 27:907-915.
38. Koffler J, Zhu W, Qu X, et al. Biomimetic 3D-printed scaffolds for spinal cord injury repair. *Nature Medicine* 2019; 25:263-269.
39. Corradetti B, Ferrari M. Nanotechnology for mesenchymal stem cell therapies. *J Control Release* 2016; 240:242-250.
40. Go G, Jeong SG, Yoo A, et al. Human adipose–derived mesenchymal stem cell–based medical microrobot system for knee cartilage regeneration in vivo. *Science Robotics* 2020; 5:eaay6626.
41. Engel E, Michiardi A, Navarro M, et al. Nanotechnology in regenerative medicine: The materials side. *Trends Biotechnol* 2008; 26:39-47.
42. Ferreira L. Nanoparticles as tools to study and control stem cells. *J Cell Biochem* 2009; 108:746-752.
43. Alexander A, Saraf S, Saraf S, et al. Amalgamation of stem cells with nanotechnology: A unique therapeutic approach. *Curr Stem Cell Res Ther* 2019; 14:83-92.
44. Zhao X, Li Q, Guo Z, Li Z. Constructing a cell microenvironment with biomaterial scaffolds for stem cell therapy. *Stem Cell Res Ther* 2021; 12:583.
45. West-Livingston LN, Park J, Lee SJ, et al. The role of the microenvironment in controlling the fate of bioprinted stem cells. *Chem Rev* 2020; 120:11056-11092.
46. Liang Y, Duan L, Lu J, et al. Engineering exosomes for targeted drug delivery. *Theranostics* 2021; 11:3183-3195.
47. Lu Y, Zhou Y, Zhang R, et al. Bone mesenchymal stem cell-derived extracellular vesicles promote recovery following spinal cord injury via improvement of the integrity of the blood-spinal cord barrier. *Front Neurosci* 2019; 13:209.
48. Dabrowska S, Andrzejewska A, Janowski M, et al. Immunomodulatory and regenerative effects of mesenchymal stem cells and extracellular vesicles: Therapeutic outlook for inflammatory and degenerative diseases. *Front Immunol* 2021; 11:591065.
49. Sun G, Li G, Li D, et al. hucMSC derived exosomes promote functional recovery in spinal cord injury mice via attenuating inflammation. *Mater Sci Eng C Mater Biol Appl* 2018; 89:194-204.
50. Pegtel DM, Gould SJ. Exosomes. *Annu Rev Biochem* 2019; 88:487-514.
51. Tang Z, Li D, Hou S, Zhu X. The cancer exosomes: Clinical implications, applications and challenges. *Int J Cancer* 2020; 146:2946-2959.
52. Food and Drug Administration. Consumer alert on regenerative medicine products, including stem cells and exosomes. Accessed 09/17/2022. www.fda.gov/vaccines-blood-biologics/consumers-biologics/consumer-alert-regenerative-medicine-products-including-stem-cells-and-exosomes
53. Tjalsma H, Bolhuis A, Jongbloed JD, et al. Signal peptide-dependent protein transport in Bacillus subtilis: A genome-based survey of the secretome. *Microbiol Mol Biol Rev* 2000; 64:515-547.
54. Kwon S, Yoo KH, Sym SJ, et al. Mesenchymal stem cell therapy assisted by nanotechnology: A possible combinational treatment for brain tumor and central nerve regeneration. *Int J Nanomedicine* 2019; 14:5925-5942.
55. Pajer K, Bellák T, Nógrádi A. Stem cell secretome for spinal cord repair: Is It more than just a random baseline set of factors? *Cells* 2021; 10:3214.
56. Pinho AG, Cibrão JR, Silva NA, et al. Cell secretome: Basic insights and therapeutic opportunities for CNS disorders. *Pharmaceuticals (Basel)* 2020; 13:31.
57. Tsai MJ, Liou DY, Lin YR, et al. Attenuating spinal cord injury by conditioned medium from bone marrow mesenchymal stem cells. *J Clin Med* 2018; 8:23.
58. Rosenzweig ES, Brock JH, Lu P, et al. Restorative effects of human neural stem cell grafts on the primate spinal cord. *Nat Med* 2018; 24:484-490.
59. Martín-Ibáñez R, Sareen D. Manufacturing of human iPSC-derived cell therapies: Road to the clinic. *Cell Gene Ther Insights* 2020; 6:177-191.
60. Miller LW. Cardiovascular toxicities of immunosuppressive agents. *Am J Transplant* 2002; 2:807-818.
61. Madrid M, Sumen C, Aivio S, et al. Autologous induced pluripotent stem cell-based cell therapies: Promise, progress, and challenges. *Current*

Protocols 2021; 1:e88.

62. Eliopoulos N, Stagg J, Lejeune L, et al. Allogeneic marrow stromal cells are immune rejected by MHC class I- and class II-mismatched recipient mice. *Blood* 2005; 106:4057-4065.
63. Song B, Cha Y, Ko S, et al. Human autologous iPSC-derived dopaminergic progenitors restore motor function in Parkinson's disease models. *J Clin Invest* 2020; 130:904-920.
64. Almalki SG, Agrawal DK. Key transcription factors in the differentiation of mesenchymal stem cells. *Differentiation* 2016; 92:41-51.
65. Zhao T, Zhang ZN, Rong Z, et al. Immunogenicity of induced pluripotent stem cells. *Nature* 2011; 474:212-215.
66. Herberts CA, Kwa MSG, Hermsen HPH. Risk factors in the development of stem cell therapy. *Journal of Translational Medicine* 2011; 9:29.
67. Wei X, Yang X, Han ZP, et al. Mesenchymal stem cells: A new trend for cell therapy. *Acta Pharmacol Sin* 2013; 34:747-754.
68. Cofano F, Boido M, Monticelli M, et al. Mesenchymal stem cells for spinal cord injury: Current options, limitations, and future of cell therapy. *Int J Mol Sci* 2019; 20:2698.
69. Fehrer C, Lepperdinger G. Mesenchymal stem cell aging. *Exp Gerontol* 2005; 40:926-930.
70. Choumerianou DM, Dimitriou H, Kalmanti M. Stem cells: Promises versus limitations. *Tissue Eng Part B Rev* 2008; 14:53-60.
71. Rutherford TR, Elder AM, Lyons TR. Anoikis resistance in mammary epithelial cells is mediated by semaphorin 7a. *Cell Death Dis* 2021; 12:872.
72. Lee S, Choi E, Cha MJ, et al. Cell adhesion and long-term survival of transplanted mesenchymal stem cells: A prerequisite for cell therapy. *Oxid Med Cell Longev* 2015; 2015:632902.
73. Sui BD, Hu CH, Liu AQ, et al. Stem cell-based bone regeneration in diseased microenvironments: Challenges and solutions. *Biomaterials* 2019; 196:18-30.
74. Santoni S, Gugliandolo SG, Sponchioni M, et al. 3D bioprinting: Current status and trends—a guide to the literature and industrial practice. *Bio-Design and Manufacturing* 2022; 5:14-42.
75. Moore CA, Shah NN, Smith CP, et al. 3D bioprinting and stem cells. *Methods Mol Biol* 2018; 1842:93-103.
76. Dey M, Ozbolat IT. 3D bioprinting of cells, tissues and organs. *Sci Rep* 2020; 10:14023.
77. Shapira A, Dvir T. 3D tissue and organ printing-hope and reality. *Adv Sci (Weinh)* 2021; 8:2003751.
78. Gagan J, Fraze C, Stout DA. Three-Dimensional stem cell bioprinting. *Cell Stem Cells Regen Med* 2016; 2:10.
79. Gasperini L, Mano JF, Reis RL. Natural polymers for the microencapsulation of cells. *J R Soc Interface* 2014; 11:20140817.
80. Gupta K, Kim DH, Ellison D, et al. Lab-On-A-Chip devices as an emerging platform for stem cell biology. *Lab Chip* 2010; 10:2019-2031.
81. Chen H, Sun J, Wang Z, et al. Magnetic cell-scaffold interface constructed by superparamagnetic IONP enhanced osteogenesis of adipose-derived stem cells. *ACS Appl Mater Interfaces* 2018; 10:44279-44289.
82. Khaw JS, Xue R, Cassidy NJ, et al. Electrical stimulation of titanium to promote stem cell orientation, elongation and osteogenesis. *Acta Biomater* 2022; 139:204-217.
83. Gittens RA, Olivares-Navarrete R, Tannenbaum R, et al. Electrical implications of corrosion for osseointegration of titanium implants. *J Dent Res* 2011; 90:1389-1397.
84. Zhu S, Jing W, Hu X, et al. Time-Dependent effect of electrical stimulation on osteogenic differentiation of bone mesenchymal stromal cells cultured on conductive nanofibers. *J Biomed Mater Res A* 2017; 105:3369-3383.
85. Schätti O, Grad S, Goldhahn J, et al. A combination of shear and dynamic compression leads to mechanically induced chondrogenesis of human mesenchymal stem cells. *Eur Cell Mater* 2011; 22:214-225.
86. Murphy KC, Whitehead J, Falahee PC, et al. Multifactorial experimental design to optimize the anti-inflammatory and proangiogenic potential of mesenchymal stem cell spheroids. *Stem Cells* 2017; 35:1493-1504.
87. Murphy KC, Whitehead J, Zhou D, et al. Engineering fibrin hydrogels to promote the wound healing potential of mesenchymal stem cell spheroids. *Acta Biomater* 2017; 64:176-186.
88. Zhao X, Cui K, Li Z. The role of biomaterials in stem cell-based regenerative medicine. *Future Med Chem* 2019; 11:1777-1790.
89. Rao W, Huang H, Wang H, et al. Nanoparticle-Mediated intracellular delivery enables cryopreservation of human adipose-derived stem cells using trehalose as the sole cryoprotectant. *ACS Appl Mater Interfaces* 2015; 7:5017-5028.
90. Mukherjee S, Yadav G, Kumar R. Recent trends in stem cell-based therapies and applications of artificial intelligence in regenerative medicine. *World J Stem Cells* 2021; 13:521-541.
91. Saba L, Biswas M, Kuppili V, et al. The present and future of deep learning in radiology. *Eur J Radiol* 2019; 114:14-24.
92. Srinivasan M, Thangaraj SR, Ramasubramanian K, et al. Exploring the current trends of artificial intelligence in stem cell therapy: A systematic review. *Cureus* 2021; 13:e20083.
93. Liu YYF, Lu Y, Oh S, et al. Machine learning to predict mesenchymal stem cell efficacy for cartilage repair. *PLoS Comput Biol* 2020; 16:e1008275.
94. Coronnello C, Francipane MG. Moving towards induced pluripotent stem cell-based therapies with artificial intelligence and machine learning. *Stem Cell Rev Rep* 2022; 18:559-569.
95. Mohammadian M, Abasi E, Akbarzadeh A. Mesenchymal stem cell-based gene therapy: A promising therapeutic strategy. *Artif Cells Nanomed Biotechnol* 2016; 44:1206-1211.
96. Lee K, Majumdar MK, Buyaner D, et al. Human mesenchymal stem cells maintain transgene expression during expansion and differentiation. *Mol Ther* 2001; 3:857-866.
97. Haridhasapavalan KK, Borgohain MP, Dey C, et al. An insight into non-integrative gene delivery approaches to generate transgene-free induced pluripotent stem cells. *Gene* 2019; 686:146-159.
98. Wang X, So KF, Xu XM. Chapter 1 - Advances and challenges for neural regeneration research. In: So KF, Xu XM. (eds). *Neural Regeneration*. Academic Press, Oxford, 2015, pp 3-17.
99. Busuttil F, Rahim AA, Phillips JB. Combining gene and stem cell therapy for peripheral nerve tissue engineering. *Stem Cells Dev* 2017; 26:231-238.
100. Armaiz Flores A, Wang H. The use and delivery of stem cells in nerve regeneration: Preclinical evidence and regulatory considerations. *Ann Plast Surg* 2018; 80:448-456.
101. Dasari VR, Veeravalli KK, Dinh DH. Mesenchymal stem cells in the treatment of spinal cord injuries: A review. *World J Stem Cells* 2014;

6:120-133.

102. Sobacchi C, Palagano E, Villa A, et al. Soluble factors on stage to direct mesenchymal stem cells fate. *Front Bioeng Biotechnol* 2017; 5:32.
103. Baez-Jurado E, Hidalgo-Lanussa O, Barrera-Bailón B, et al. Secretome of mesenchymal stem cells and its potential protective effects on brain pathologies. *Mol Neurobiol* 2019; 56:6902-6927.
104. Liau LL, Looi QH, Chia WC, et al. Treatment of spinal cord injury with mesenchymal stem cells. *Cell Biosci* 2020; 10:112.