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Review Article

Current Progress in Mesenchymal Stromal Cell Therapies

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Abstract

Mesenchymal Stromal/Stem Cells (MSCs); which can be isolated from several tissues and body fluids including bone marrow, adipose tissue and placenta; are characterized by self-renewal, differentiation into various cell types, plastic adherence and specific surface markers on flow cytometry. Their immunomodulatory, immunosuppressive, anti-inflammatory, antimicrobial, regenerative and other properties enable them to have several clinical applications including the treatment of various injuries and tissue regeneration; several autoimmune disorders; graft versus host disease in hematopoietic stem cell transplantation; skin, pulmonary and cardiovascular disorders; neurological and eye diseases as well as several infectious diseases and their complications.

Recently, there has been plenty of advancements in the technical and therapeutic aspects of MSCs such as automated production and genetic engineering of MSCs in addition to the use of MSC secretome and these advancements have led to therapeutic approval of fifteen MSC products worldwide. However, the remarkable progress achieved in MSC research and studies on animal models has not translated into equivalent success on the clinical side. Also, there are several challenges that need to be overcome before having the widespread clinical utilization of MSCs and their products as novel therapeutics for several intractable medical diseases.

Keywords: Mesenchymal Stromal Cells; Extracellular Vesicles; Mitochondrial Transfer; Genetic Engineering; Human Clinical Trials; Regenerative Medicine; Therapeutic Drug Approvals

Introduction

Multipotent Mesenchymal Stromal/Stem Cells (MSCs) are a group of heterogeneous, non- hematopoietic, undifferentiated, fibroblast-like cells with the ability of self-renewal and differentiation into multiple cell lineages including adipocytes, osteocytes, myoblasts and chondrocytes [1-5]. They are characterized by the expression of CD73, CD90 and CD105 surface markers and the lack expression of hematopoietic markers such as CD34 and CD45 as well as CD14, CD11b, CD79a or CD19 or class II Human Leukocyte Antigens (HLA) [1,3,4]. MSCs can be found in nearly all body tissues including: Bone Marrow (BM), Adipose Tissue (AT), umbilical cord blood (UCB), placenta and amniotic membranes and they play a significant role in tissue repair and regeneration [5]. The biological functions of MSCs that are essential in their therapeutic effects include: (1) proliferation, (2) multipotency, (3) trophic ability, (4) homing/migration ability and (5) immunosuppression [6]. Different factors including donor age, biological source, route of administration and signaling pathways have an impact on the biological functions and consequently the clinical applications of MSCs [7].

MSCs have inherent immunomodulatory characteristics, trophic activity, high *in-vitro* self-renewal ability and they can be readily engineered to enhance their immunomodulatory functions [8]. Also, MSCs affect the functions of most immune effector cells via direct contact with immune cells and local microenvironmental factors [8]. They interact with immune cells both in the innate and adaptive immune systems, modulating immune responses and enabling immunosuppression and induction of tolerance [5].

Production, *Ex-vivo* Expansion and Cryopreservation of MSCs

MSCs are commonly derived from BM, AT, placenta and other sources. Alternative approaches include differentiation of induced Pluripotent Stem Cells (iPSCs) into MSCs or direct reprogramming of blood cells into MSCs [9]. Stem cells, including embryonic stem cells and iPSCs, are considered potent sources for the derivation of MSCs. However, PSC- derived MSCs may be superior to tissue-derived MSCs in: proliferation capacity, large-scale generation of MSCs, immunomodulatory activity and *in-vivo* therapeutic applications [10]. Clinical grade production of MSCs should comply with Good Manufacturing Practice (GMP) regulations and guidelines because GMP will maintain the highest levels of MSC quality and safety [11]. Efficient, cost-effective and reproducible protocols need to be created for large-scale production of MSCs with unique therapeutic potentials that far exceed tissue-derived MSCs, which could be a breakthrough in regenerative medicine [10]. Bioactive, micrometre-sized porous three-dimensional scaffolds adapted for stem cell expansion have been developed. These functionalized scaffolds significantly promote MSC proliferation while retaining stemness, resulting in higher expansion efficacies at a reduced cost [12]. *Ex-vivo* expansion of MSCs at high confluency alters the metabolic and transcriptomic states of these cells and this process maximizes the production of MSCs with enhanced immunomodulatory functions without priming, thus providing a non-invasive and generalizable strategy for improving the clinical utilization of MSCs [13].

The origins of low-temperature tissue storage research date back to the late 1800s and since then, numerous advancements related to cryopreservation have been developed. Cryopreservation allows the banking of a large number of cells and tissues that can be utilized for scientific research and medical applications [14]. It is crucial to cryopreserve MSCs in liquid nitrogen prior to clinical application while preserving their functionality as efficient cryopreservation greatly enhances the potential of MSCs in a range of biological domains [15]. The addition of cryoprotective agents such as Dimethyl Sulfoxide (DMSO), although toxic to cells at high concentrations, is a necessary step to protect against cell death during cryopreservation [14]. Usually, clinical studies are conducted by using cultured MSCs after short-term cryopreservation then thawing of MSCs prior to administration for the treatment of a wide range of medical disorders [16]. For MSCs, like hematopoietic stem cells, slow cooling is recommended for cryopreservation [14]. Recently, banking of cryopreserved perinatal MSCs for potential personalized medicine for later use in lifetime has raised growing interest in many countries around the globe [16]. There are several limitations to the current methods of MSC cryopreservation, necessitating thorough biosafety assessments before utilizing cryopreserved MSCs. Hence, new techniques must be developed to improve the effectiveness of cryopreserved MSCs in clinical stem cell treatment procedures and to protect the functional properties of MSCs such as their ability to differentiate and survive [15]. Viability and functional assays are critical steps in determining the quality of the cells post-thaw and improving the efficiency of the current cryopreservation methods [14]. In the future, it may be possible to create a high-throughput, completely automated and sterile system for MSC cryopreservation tailored specifically for clinical use and this will significantly promote the utilization of cryopreserved MSCs in clinical settings and enable clinicians to more efficiently prepare MSCs for their patients [15].

MSC Secretome: Exosomes and Extracellular Vesicles

The biological effector of MSCs is their secretome, which is composed of a heterogeneous pool of bioactive molecules, partially enclosed in Extracellular Vesicles (ECVs) [17]. The whole secretome, including stem cell exosomes obtained from AT derived from Stem Cells (AT-SCs), has been proven in many studies to have immunomodulatory, proangiogenic, neurotrophic and epithelization activity and can potentially be used for neurodegenerative, cardiovascular, respiratory, inflammatory and autoimmune diseases (AIDs) as well as wound healing treatment [18]. The secretome can be considered as a protein-based biotechnological product, it is probably safer compared with living/cycling cells, it presents virtually lower tumorigenic risk and it can be handled, stored and sterilized as an active principle ingredient [17]. The paracrine function of MSCs via the secretome and the unique stem cell properties of MSCs, such as proangiogenic, anti-inflammatory, immunomodulatory and antioxidative stress activities, represent the mechanisms of action underlying the use of MSCs for therapeutic purposes [19].

The MSC secretome, including ECVs, has recently been proposed as possible alternative to MSC therapy [17]. Due to limitations in the use of stem cells in cell-based therapy, their secretome with emphasis on exosomes seem to be a reasonable and safer alternative with increased effectiveness and fewer side effects. Moreover, the great advantage of cell-free therapy is the possibility of biobanking the secretome derived from AT-SCs [18]. For the clinical application of MSCs, the optimization of biological products (ECVs or exosomes) has to be performed based on their molecular properties and mechanisms of action [19]. The direct-to-consumer industry for secretome-based therapies appears to be primed for growth in the absence of appropriate regulatory frameworks and guidelines. However, such business activity requires tight regulations and monitoring by the respective national regulatory bodies to prevent patients from being conned and more importantly from being put at risk [20]. Although secretomes

possess therapeutic potential, their efficacy might be limited compared to cellular therapies [21].

The mechanisms through which administered MSCs can promote tissue repair include: (1) paracrine activity that involves secretion of proteins/peptides and hormones; (2) mitochondrial transfer (MT) by way of tunnelling nanotubes or microvesicles; and (3) transfer of exosomes or microvesicles containing RNA and other molecules [22]. The paracrine functions of MSCs are applied through secreting soluble factors and releasing ECVs like exosomes and microvesicles [23]. Exosomes can inherit similar therapeutic effects from their parental cells through vertical delivery of their pluripotency or multipotency [24]. Exosomes, diminutive ECVs, are integral to intercellular communication, harboring potential for applications in regenerative medicine and aesthetic interventions. However, the field struggles with the complexities of harmonizing exosome characterization protocols and safeguarding therapeutic integrity [25]. Compared to stem cells, stem cell-derived exosomes possess numerous advantages such as non- immunogenicity, non-infusion toxicity, easy access, effortless preservation and freedom from tumorigenic potential and ethical issues [24]. Stem cell-derived exosomes (SC-exos) exert comparable therapeutic effects to those of their parental stem cells without causing immunogenic, tumorigenic and ethical disadvantages [26]. Due to their content, exosomes promote tissue regeneration by different mechanisms, either by activating or inhibiting several signaling pathways involved in wound healing, extracellular matrix remodeling, immunomodulation, angiogenesis, anti-apoptotic activity and cell migration, proliferation and differentiation [27]. Exosomes sourced from various types of SCs can regulate major signaling pathways such as PTEN/PI3K/Akt/mTOR, NF-κB, TGF-β, HIF-1α, Wnt, MAPK, JAK-STAT, Hippo and Notch signaling cascades as well as minor pathways during the treatment of numerous diseases encountered in orthopedic surgery, neurosurgery, cardiothoracic surgery, plastic surgery, general surgery and other specialties [26]. Exosomes derived from SCs are capable of treating numerous diseases encountered in orthopedic surgery, neurosurgery, plastic surgery, general surgery, cardiothoracic surgery, urology, head and neck surgery, ophthalmology and obstetrics and gynecology. The diverse therapeutic effects of SC exosomes are a hierarchical translation through tissue-specific responses and cell-specific molecular signaling pathways [24]. The use of human exosomes derived from AT-SCs (AT-SC-exos) may provide an improved alternative to standard therapies used in regenerative medicine, as a cell-free new approach with multiple possibilities to be modulated according to the patient needs [27]. It is imperative to accentuate stringent methodological standardization within exosome research to fortify the validity and reproducibility of empirical findings. Amidst the burgeoning therapeutic optimism, the discipline must rectify methodological disparities and comply with regulatory mandates, ensuring the ethically sound and scientifically robust advancement of exosome-based therapeutic modalities [25].

MSCs can secrete ECVs including exosomes and microvesicles, which mediate their trophic effects on other cells [28]. ECVs are predominantly endosomal in origin and contain a cargo of microRNA (miRNA), messenger RNA (mRNA) and proteins that are transferred from their original cells to target cells [23]. ECVs carry a variety of intracellular molecules of MSCs including lipids, proteins, RNA (mRNA and noncoding RNA) and DNA and they deliver them into other cells to regulate tissue regeneration process [28]. ECVs secreted by MSCs preserve some of their parental cell features such as homing, immunomodulatory and regenerative potential [17]. Recently it has emerged that ECVs alone are responsible for the therapeutic effect of MSCs in several models of animal disease. Hence, MSC-derived ECVs may be used as an alternative MSC-based therapy in regenerative medicine [23]. The therapeutic effects of MSC-derived ECVs have been observed in a number of animal disease models [28]. ECVs retain some structural and technological analogies with synthetic drug delivery systems (DDS), even if their potential clinical application is also limited by the absence of reproducible/scalable isolation methods and GMP-compliant procedures [17].

Recent Technical and Therapeutic Advances

Among the major hurdles encountered in MSC therapy are: (1) inconsistent SC potency; (2) poor cell engraftment and survival; and (3) age or disease-related host tissue impairment [29]. The role of MSCs mainly depends on their paracrine components, namely the secretome, which are bioactive molecules affected by the local microenvironment and MSC culture conditions [30,31]. The components of MSC-derived secretome are not constant and are affected by the stimulation MSCs are exposed to. So, the components of the secretome can be regulated by the pretreatment of MSCs [30]. MSC paracrine mechanisms provide a promising framework for enhancing MSC therapeutic benefits, where the composition of secretome can be modulated by priming of the MSCs [31]. However, the recognition that MSCs primarily mediate therapeutic benefits through paracrine mechanisms independent of cell differentiation provides a promising framework for enhancing SC potency and therapeutic benefits [29]. Pretreatment of MSCs with hypoxia, inflammatory factors, three- dimensional (3D) culture, engineering methods and pharmaceutical stimuli or a combination of the above methods prior to application is a novel strategy to enhance the

immunomodulatory effects of MSCs and their secretome. However, these methods need to be optimized and new techniques need to be developed to better characterize and standardize the secretome [30]. To enhance the therapeutic capabilities of MSCs, the following strategies have been employed: (1) genetic manipulation; (2) preconditioning and optimization of cell culture conditions to preserve MSCs pluripotency and improve their therapeutic properties; (3) use of commercial drugs to enhance their self-renewal, differentiation and regenerative capacities; (4) use of biomolecules to improve the immunomodulatory or regenerative potential of MSCs; and (5) use of biomaterials to increase their viability, differentiation capacity and to produce extracellular matrix [32-34].

Mitochondrial Transfer from MSCs

MT is one way that has recently been found to be employed by MSCs to exert its biological effects [35]. Various studies have shown that MT from MSCs to other cells can affect vital processes such as proliferation, differentiation, cell metabolism, inflammatory responses, cell senescence, cell stress and cell migration [36]. MT from MSCs has been implicated in a spectrum of biological processes including regulation of: inflammatory response, cell death, proliferation, differentiation, migration, apoptosis, metabolism, stress responses, as well as promotion of tissue repair and regeneration [35]. Numerous studies have demonstrated that MT could lead to increased Adenosine Triphosphate (ATP) production, recovered mitochondrial bioenergetics and rescued injured cells from apoptosis [37]. Additionally, MT determines fates of both MSCs and recipient cells [35].

As MSC-mediated MT has emerged as a critical regulatory and restorative mechanism for cell and tissue regeneration and damage repair in recent years, its potential in SC therapy has received increasing attention because MSCs can enhance tissue repair after injury via MT and membrane vesicle release [38]. MT of MSC therapy has been shown to be a promising therapeutic strategy in mitochondrial dysfunction disease models due to the effectiveness of MSCs or isolated mitochondria transplantation in tissue repair [37]. Hence, understanding the molecular and cellular mechanisms of MT and demonstrating its efficacy could be an important milestone that lays the foundation for future Clinical Trials (CTs) [36].

MSC Membrane-Coated Nanoparticles

Recently, studies of nanomedicine have achieved dramatic progress. Hence, nanodrug delivery systems have been widely used in disease treatment [39,40]. Recently, SC membranes, owing to their immunosuppressive and regenerative properties have received attention as attractive therapeutic carriers for targeting specific tissues or organs [41]. MSC membrane has the characteristics of active targeting and immune escape of MSCs and has broad application potential as a new carrier in tissue regeneration and treatment of several diseases including inflammatory disorders and cancer [39]. MSC membrane-coated nanoparticles are an effective method with selective targeting properties, allowing entry into specific cells [40].

Single Cell Sequencing

Single Cell Sequencing (SCS) technology plays an important role in the field of MSC research [42]. SCS technology has been applied in the field of MSCs research and it has shown the following outcomes: (1) it enables more precise MSCs characterization and biomarker definition; (2) it uncovers the genetic structure and gene expression status of individual contained cells on a massive scale and reveals the heterogeneity among different subclusters within MSCs; (3) it provides insights into the dynamic transcriptional changes experienced by MSCs during differentiation; and (4) it links the molecular characteristics of MSCs with their clinical applications, contributing to the advancement of regenerative medicine [42,43].

Automated Production of MSCs

Obtaining MSCs for CTs manually is a difficult, time consuming and laborious process. Additionally, the prolonged period of *ex-vivo* expansion carries the risk of the cells becoming microbially infected [44]. Therapeutic application of MSCs requires large-scale production that should adopt stringent regulatory standards and GMP guidelines [45]. Automated, robotic and closed production systems, with built-in flexibility in relation to individual processing steps and complete protection of the cell product from operator-derived contamination, will provide the most efficient manufacturing strategy [46]. There are several automated MSCs production platforms that include: QuantumTM, CellQualia™, Cell XTM and Xuri™ cell expansion system W25 [44,45,47]. For example: the Cell XTM platform provides effective tools for automated assessment and processing of the heterogenous cells and colonies that serve as the starting materials for MSC manufacturing to reduce variation and reproducibility of manufacturing

processes [47]. Advancing automated manufacturing processes and platforms is essential for acquiring the full potential of MSCs-based regenerative medicine and accomplishing the increasing demand for cell-based therapies [45]. Fully controlled stirred tank bioreactor systems are an efficient production system for MSC-derived particles such as ECVs that may open and facilitate the path for clinical applications [48]. Collaboration between research institutions, MSC industry and regulatory bodies is essential to accelerate the translation of MSCs-based therapies into clinical practice [45].

Genetic Engineering of MSCs

Insufficient engraftment and limited beneficial effects of MSCs indicate the need of approaches to enhance survival, migration and therapeutic potential of MSCs [49]. In recent years, genetic modification has been proposed to optimize MSC-based therapies, some of which are expected to enter CTs [50]. Genetic engineering methods can greatly amplify the paracrine actions of MSCs and broaden the therapeutic capabilities of MSCs, including transdifferentiation toward diverse cell lineages [51]. Also, targeting the delivery of therapeutics specifically to diseased tissue enhances their efficacy and decreases their side effects [52].

Distinct strategies have been applied to induce genetic modifications with the goal to enhance the potential of MSCs [49]. Non-viral gene delivery vectors are a promising and safe tool to engineer human MSCs *ex-vivo*. However, there is lack of standardized procedures to engineer human MSCs *ex-vivo*. Therefore, optimization of key transfection parameters is needed to effectively engineer human MSCs [53]. Enucleated MSCs; MSCs with their nuclei removed following genetic modification of cells; are effective vehicles for the targeted delivery of therapeutics because they retain most of the functionality of the cells, acquire the cargo-carrying characteristics of cell-free delivery systems and thus represent a versatile delivery vehicle and therapeutic system [52].

While the therapeutic potential of MSCs is exerted through the secretion of soluble mediators, genetic engineering can induce the expression of different proteins and soluble factors such as cytokines, enzymes, growth factors, chemokines, transcription factors and miRNAs which have a wide range of applications [49]. However, cell engineering can also affect safety and increase the cost of therapy based on MSCs. Therefore, the advantages and disadvantages of these procedures should be taken into consideration [51].

Clinical Applications of MSCs

The Current and Potential Clinical Applications of Mscs

Over the past decades, MSCs therapies have achieved tremendous advancements and MSCs have become promising candidates as effective treatment options for a variety of devastating medical conditions [54]. Recently, the clinical applications of MSCs have rapidly expanded to include: AIDs; Hematopoietic Stem Cell Transplantation (HSCT); several viral, bacterial, fungal and parasitic infections and their complications; skin, pulmonary and cardiovascular disorders; neurodegenerative, musculoskeletal and eye diseases; as well as regenerative medicine [23,55]. The safety and efficacy of MSCs have been well illustrated in several CTs, systematic reviews and meta-analyses [23,56]. The rapidly increasing number of CTs using MSCs will help to determine the promising therapeutic potential of these cellular therapies in various medical and surgical fields [56]. In order to build an effective cell therapy based on MSCs, it is essential to improve the clinical efficacy and decrease the side effects of MSCs such as infections and predisposition to cancer by adopting the following: (1) the use of specific products of MSCs such as ECVs and exosomes; (2) the use of MSCs derived from iPSCs; (3) genetic engineering; (4) using the most suitable cell source of MSCs; (5) selecting the most appropriate route of administration; (6) the use of certain stem cell doses; (7) standardization of production, purification, characterization, storage and administration protocols; (8) appropriate *in-vitro* manipulation strategies to promote their expansion and trigger specific therapeutic functions and (9) implementation of strict regulations, standards and quality control measures [23,55,57,58]. MSCs offer important therapeutic effects in the field of regenerative medicine depending on the production of paracrine factors and MSC-derived products such as ECVs, exosomes and conditioned media that modulate tissue environments and allow their repair following various injuries [57,58]. Recently, optimization of isolation, culture and differentiation procedures has permitted MSCs to improve closer to clinical uses in various AIDs and tissue regeneration [59,60]. MSCs have the following important characteristics that make them preferred candidates to use for regenerative medicine and AIDs: (1) high proliferation capacity to differentiate into various cells; (2) anti- inflammatory and immunomodulatory properties; (3) low tumorigenic effects along with poor immunogenicity; (4) migration to the sites of inflammation and (5) paracrine or autocrine roles that produce growth factors [55,59,60].

Studies on the use of MSCs in regenerative medicine and AIDs have shown the following results: (1) no significant relationship was found between the MSC therapy and incidence of cancer and infection; (2) although both autologous and allogeneic MSCs are valuable sources for tissue forming, autologous MSCs have been shown to be safe with minimal immunological threat; (3) the intravenous injection of MSCs is the most widely used form of administration; (4) the dosage commonly fluctuates between 1×10^6 cells/kg and 2×10^8 cells/kg; (5) repeated administration of MSCs is more beneficial than a single injection; and (6) safety and efficacy of MSC therapies in AIDS, bone diseases such as osteoarthritis and heart disorders has been broadly established [59,60].

In COVID-19 pneumonia, studies have shown that MSCs therapy can suppress aggressive inflammatory reactions and increase endogenous restoration by improving the pulmonary microenvironment; preserving alveolar epithelial cells and preventing lung fibrosis; treating pulmonary dysfunction caused by the lung infection; modulating immune responses; and promoting tissue regeneration [61,62]. In particular, intravenous administration of MSCs may radically reduce lung tissue damage in COVID-19 patients [61]. Studies have shown not only safety but also efficacy of MSCs in treating severe COVID-19 and acute respiratory distress syndrome (ARDS) [62,63]. The results of several CTs on the use of MSCs in the treatment of COVID-19 and its complications have shown the following beneficial effects: (1) MSCs can reduce the mortality rates and increase overall survival in patients with COVID-19 infection; (2) MSCs can optimize oxygen saturation levels and reduce the severity of COVID-19 pneumonia; (3) MSCs can improve the symptoms related to COVID-19 infection; (4) MSCs can reduce the duration of hospitalization and the need for invasive mechanical ventilation; (5) MSCs can improve lung function and radiological appearances in patients with COVID-19 pneumonia; and (6) MSCs can reduce the levels of cytokines and inflammatory markers such as C-reactive protein and interferon-gamma in the majority of patients with severe COVID-19 infection [62,63].

MSCs in Drug Delivery and In the Treatment of Cancer

MSCs and their ECVs have emerged as attractive options for the treatment of several cancers because they: (1) can modulate the immune response to neoplastic diseases; (2) home to tumor sites; and (3) can deliver antitumor drugs [64,65]. Unfortunately, MSCs have been reported to promote tumor growth and metastases [66]. Recently, several CTs have been registered to analyze the therapeutic values of tissue-derived MSCs, engineered MSCs and MSC-derived exosomes. However, the early results of these CTs have indicated not only safety but also and efficacy of MSCs and their ECVs [65].

MSCs have emerged as a promising platform for targeted drug delivery because of their unique properties particularly their innate ability to migrate and home to the sites of inflammation, including tumors, which can be exploited to deliver a variety of therapeutic agents directly to the target site [67]. Hence, MSCs are attractive cellular carriers of synthetic nanoparticles for drug delivery due to their inherent homing ability [68]. Under appropriate conditions, human MSCs can be efficiently loaded with synthesized microcapsules without damaging the integrity of cell structure [69].

MSCs can inhibit cancer cell proliferation, while being capable of potentially promoting tumor growth by supporting angiogenesis and increasing cancer SC invasiveness. Therefore, their use in cancer therapy is still controversial [70]. MSCs are promising candidates for developing genetic engineering and drug delivery strategies due to their inherent properties, including immune regulation, homing ability and tumor tropism [68]. The engineering techniques that are used to improve the therapeutic efficacy and targeting effectiveness while minimizing any loss of MSC function include: (1) viral or non-viral genetic engineering; (2) therapeutic agent incorporation; and (3) cell surface modification [71]. The strategy of using human MSCs as delivery vehicles for transferring microcapsules, containing bioactive material, across the tissue-blood or tumor-blood barriers to facilitate the treatment of stroke, cancer or inflammatory diseases may open a new therapeutic perspective [69]. To optimize their anti-cancer effects, diverse strategies have bioengineered MSCs to: (1) enhance their tumor targeting and therapeutic properties and (2) deliver anti- cancer drugs. Ongoing CTs are evaluating the therapeutic potential of bioengineered MSCs and setting the stage for future advances in MSC-based cancer treatment [70]. The therapeutic potential of MSCs is being investigated for cancer therapy, inflammatory and fibrotic diseases, in addition to other disorders [68]. So, it is essential to identify the potential applications of bioengineered MSCs in solid tumor targeting and anti-cancer agent delivery to position them as effective cancer therapeutics [70]. Hydrogels, acting as an ideal carrier, can enhance the therapeutic effects and application range of MSCs and exosomes derived from MSCs [72]. Clinical translation of MSC-based therapies is hindered by loss of MSC regenerative properties during large-scale expansion and low survival post-delivery, but these limitations might be overcome by designing hydrogel culture platforms to modulate the MSC microenvironment [73]. The clinical translation of hydrogel encapsulation into MSCs and their

secretome is a novel step to a very promising technology [74]. Hydrogel systems could be engineered to: (1) promote MSC proliferation and maintain regenerative properties; (2) improve MSC survival, retention and engraftment *in-vivo* and (3) direct the MSC secretory profile using tailored biochemical and biophysical cues [73]. The current and potential clinical applications of MSCs are shown in Table 1 [23,55,58-61,64-67].

The Remaining Challenges Facing the Clinical Utilization of MSCs

There are several challenges that face the clinical application of MSCs and their products and these include: (1) safety issues such as infection and predisposition to cancer; (2) clinical grade production as clinical application of MSC requires a large number of cells; (3) quality control measures covering all aspects from cell production to administration of MSCs; (4) ensuring successful translation of MSC therapies from animal models to human CTs; (5) performance of more randomized CTs and multicenter prospective studies that will ultimately determine the optimal conditions of MSC therapies in various acute and chronic diseases; (6) development of more robust pharmacodynamic, pharmacokinetic models which need to be applied in different clinical situations and to study failure of therapy and resistance to treatment; and (7) linking biologists, research teams, cell therapy laboratories, clinical teams and patient working groups in the context of different tissues and diseases [23,55,66].

Translation of Animal Studies on MSCs into Human Clinical Trials

Currently, MSCs are being widely investigated for numerous tissue engineering and regenerative medicine applications [75]. Generally, while MSC therapies have shown safety and efficacy in animal models, most human CTs have shown limited efficacy [76]. After nearly 3 decades of development and testing of MSCs in a broad spectrum of CTs, the outcomes of the majority of advanced and registered CTs in humans applying MSC therapy for diverse diseases have fallen short of the expectations that were raised by the encouraging pre-clinical animal data in a wide range of animal models [77-79]. An example is MSC transplantation in Chagas disease, where more success has been achieved in animal or preclinical studies than in human CTs [63,80]. Also, in inflammatory bowel diseases, the following major hurdles impede the direct translation of data derived from animal experiments to the clinical arena: (1) limitations of the currently available animal models that reflect humans; and (2) species specific differences in the functionality of MSCs derived from mice versus humans [81]. Recently, various SC types including MSCs have made significant progress in the treatment of several neurological disorders including Parkinson's disease, Alzheimer's disease, multiple sclerosis, stroke, spinal cord injury and traumatic brain injury with promising results from preclinical studies and early CTs and this progress is primarily attributed to their ability to replace lost or damaged neural cells, modulate the immune system and promote endogenous repair mechanisms [82].

Additionally, despite being biologically appealing, the concept of tissue regeneration underlying first-generation and second-generation SC therapies has failed to translate into consistent results in CTs [83]. MSCs derived from mice and humans are not identical in their mechanisms of action in suppressing inflammation. Thus, preclinical animal studies with murine derived MSCs cannot be considered as an exact replica of human MSC-based CTs [81]. Several challenges exist that critically hinder successful clinical translation of MSC-based therapies including: (1) heterogeneity of their populations, (2) variability in their quality and quantity, (3) donor-related factors, (4) discrepancies in protocols for isolation, (5) *in-vitro* expansion and premodification and (5) variability in methods of cell delivery, dosing and cell homing [84].

Even though multiple preclinical as well as clinical studies have demonstrated remarkable properties for MSCs, the clinical applicability of MSC-based therapies is still questionable [84,85]. Developing further methods to understand and unlock MSC potential through intracellular and intercellular signaling, biomedical engineering, delivery methods and patient selection should all provide substantial advancements in the near future and greater clinical opportunities [78].

Appropriate animal models are crucial for the development and evaluation of regenerative medicine-based treatments and eventual treatments for debilitating diseases with the hope of application in upcoming human CTs [75]. Large animal models have been widely used to facilitate the translation of MSC from the laboratory to patient as they allow clinically relevant assessments of safety, efficacy and dosing prior to CTs and continue to provide a research platform that can be used to evaluate the value of cell-based therapies [86]. Cutaneous wound healing, bone regeneration, osteoarthritis and ischemic reperfusion injury represent examples of the types of disease states that may be investigated in large animal models such as pigs, cattle, sheep and goats using MSC-based therapy due to their overlapping similarities in structure and function that closely mimic the human

body [87]. Large animal models have been widely used to facilitate the translation of MSCs from the laboratory to patient as these SCs have been shown to have therapeutic benefits in a number of pathological conditions due to their multi-potent capacity [86]. With emerging fields such as exosome-based therapy, proper *in-vivo* models will be needed for testing efficacy and translational practice and livestock models should be strongly considered as candidates [87]. Also, the combination of SC biology with other cutting-edge technologies, such as gene editing, single-cell genomics, organoid models and advanced neuroimaging, holds great promise for developing safe and effective personalized and targeted SC-based therapies for certain diseases such as neurological disorders [82].

One systematic review and meta-analysis that included 122 articles, describing 54 distinct human diseases and 367 therapeutic interventions showed that: (1) the overall proportion of therapies progressing from animal studies was 50% to human studies, 40% to randomized CTs and 5% to regulatory approval; and (2) the median transition times from animal studies were 5, 7 and 10 years to reach any human study, randomized CT and regulatory approval respectively. Additionally, the meta-analysis showed 86% concordance between positive results in animal and CTs which was higher than what had been reported previously. Nonetheless, the low rate of final approval indicates potential deficiencies in the design of both animal studies and early CTs [88].

In another meta-analysis that included a total of 48 studies and 94 treatment arms, 42 studies and 79 treatment arms reported that MSC improved outcomes. This meta-analysis suggested that in preclinical studies, MSC have consistently exhibited therapeutic benefits. However, the findings demonstrate a need for considering variations in different animal models and treatment protocols in future studies using MSC to treat rheumatoid arthritis in humans to maximize the therapeutic gains in the era of precision medicine [89].

Clinical Trials on MSCs

MSCs offer remarkable potentials and this made them a favorable candidate for treating a large number of diseases. An overview on statistics obtained from the National Library of Medicine in the United States of America (USA) limited to 2016-2020 showed that 1240 out of 377,550 research studies were related to MSC-based therapy [90]. The recent advancements in the field of MSCs biology have: (1) facilitated the progress of particular guidelines and quality control approaches, which may eventually lead to clinical application of MSCs and (2) provided a new hope for the treatment of diseases and disorders that are yet to be treated. Hence, MSCs have been widely used not only in animal models but also in human CTs [59,91,92].

As of March 2023, more than 1476 CTs were listed in clinicaltrials.gov, the majority of which targeted disorders of the musculoskeletal, nervous, cardiovascular and immune- related disorders [93]. Several CTs have reported that both autologous and allogeneic MSCs are valuable sources for tissue formation. However, autologous MSCs have shown safety of administration with minimal immunological threat while allogeneic sources offer scalability and accessibility but pose potential immunogenicity risks [59,92]. Most of the CTs have focused on or tested 4 aspects of MSCs: (1) using different MSC doses, (2) comparing the effectiveness of MSCs from different sources, (3) using MSC's exosomes instead of the actual cells and (4) combining MSCs with other substances/cells [93]. In CTs, MSCs have consistently demonstrated safety. However, demonstration of efficacy has been inconsistent and many MSC trials have failed to meet their efficacy endpoint. Unfortunately, despite the large number of CTs using MSCs registered on clinicaltrials.gov., the number MSC therapies approved by regulatory agencies is very limited [94].

The first CT on MSCs was reported by Hillard Lazarus, et al., in the year 1995. In a phase I CT that included 23 patients with hematological malignancies, Lazarus, et al., evaluated the suitability of human BM derived Mesenchymal Progenitor Stromal Cells (MPCs) after *ex-vivo* culture expansion and intravenous infusion. However, only 15 patients completed the study and the trial showed safety of MPCs with no adverse reactions reported [95]. According to the ClinicalTrials.gov, 1014 CTs were registered till July 14th, 2021. In the first decade (from 1995 to 2005), there were only 9 registered CTs, then the number of CTs increased to 500 worldwide between 2006 and 2015 [96]. Between 2015 and 2021, 416 CTs based on the use of MSCs were implemented to treat various pathologies, according to the USA National Institute of Health-ClinicalTrials database (http://clinicaltrials.gov). The vast majority of these CTs were carried out in the USA and China and most of the published CTs evaluated effectiveness of MSCs in treating cardiovascular diseases, Graft Versus Host Disease (GVHD) and brain and neurological disorders, although some trials sought to treat immune system diseases and tissue regeneration [97].

In the search for an effective therapy during the COVID-19 pandemic, the number of registered CTs increased dramatically [62,98-101]. As of November 16, 2020, at least 63 CTs on the treatment of COVID-19 using MSCs were registered [98,99]. In the years 2021 and 2022, more than 30 additional CTs using MSCs (predominately obtained from the UCB, BM, AT) to treat the complications of COVID-19 infection; such as sepsis, ARDS and the cytokine storm; were registered. In the published CTs, MSC therapy was found to be safe and effective in preclinical and clinical studies [62,100,101].

Global Approvals of Mesenchymal Stem Cell Therapies

Globally, fifteen MSC therapies have already been approved for various clinical indications by regulatory agencies around the world with ten MSCs products approved in Asian countries: five approved in South Korea, two approved in Japan, one in India, one in Iran and one in China [7,94,102-108].

In 2010, the first product that contained autologous MSCs was approved by the Korean Food and Drug Administration (KFDA) in South Korea. The product, named Queencell, was manufactured by Anterogen and it was approved for the treatment of subcutaneous tissue defects [94,102]. The 4 other MSC products that were approved in South Korea include: (1) Cellgram-AMI (autologous BM-MSCs) for acute myocardial infarction in 2011, (2) Cupistem (autologous AT-MSCs) for complicated fistulae of Crohn's disease in 2012, (3) Cartistem (allogeneic UCB-MSCs) for knee cartilage defects associated with osteoarthritis grade IV in 2012 and (4) NeuroNata-R (autologous BM-MSCs) for the treatment of progressive amyotrophic lateral sclerosis in 2014 [94,102,103,105].

The two MSC products approved in Japan were: (1) Temcell HS (allogeneic BM-MSCs) was approved in September 2015, as one of the first cellular and tissue products for the treatment of steroid refractory (SR) acute GVHD in children and adult patients undergoing HSCTs for hematologic malignancies following the promising results of 2 CTs (phase 1/2 and phase 2/3); and (2) Stemirac (autologous BM-MSCs) was approved for treatment of spinal cord injuries in the year 2018 [7,94,102,105]. In 2016, Stempeucel (allogeneic BM-derived MSCs) was approved for the treatment of critical limb ischemia due to Buerger's disease and peripheral arterial disease in India, while MesestroCell (BM-MSCs) was approved for osteoarthritis treatment in Iran in 2018 [7,102-106]. In January 2025, the National Medical Products Administration in China gave conditional approval to Platinum Life Excellence Biotech Co. Ltd. to market its product Amimestrocel injection (human UCB- MSCs; Ruibosheng) as the first human UCB-MSC therapy to treat SR acute GVHD in the country [108].

In Europe, the European Medicines Agency (EMA) had already approved 2 MSC products: (1) in the year 2018, the EMA authorized the first marketing of allogeneic AT-MSCs (Alofisel) for the treatment of complex perianal fistulas in Crohn's disease and this represented a breakthrough in the field of MSC therapy and (2) Holoclar was approved to replace damaged cells in the corneal epithelium in adults to treat moderate to severe limbal stem cell deficiency caused by burns [7,94,102,103,105,107]. In the USA, only one MSC product has been approved so far. On December 18, 2024, the FDA in the USA approved Remestemcel-L-rknd (Ryoncil, Mesoblast, Inc.), an allogeneic BM-MSC therapy, for SR-acute GVHD in pediatric patients 2 months of age and older [94,102]. In 2012, Prochyma (commercial allogeneic BM-MSCs) for pediatric SR-GVHD were licensed in Canada and New Zealand [7,94,103,105,107]. The MSC therapies that have been approved in several countries around the world are shown in Table 2 [7,94,102-108].

1. Regenerative medicine and tissue repair:

a. Myocardial ischemia.
b. Acute myocardial infarction.
c. Cardiac dysfunction.
d. Dilated cardiomyopathy.
e. Chronic non-healing wounds.
f. Critical limb ischemia
g. Peripheral vascular disease.
h. Ischemic stroke.
i. Traumatic brain injury.

j. Spinal cord injuries.
k. Liver injury.
l. Radiation-induced lung fibrosis.
m. Tissue repair: bone, cartilage, muscle, skin, myocardium, trachea, etc.

2. Treatment of autoimmune diseases:

a. Systemic lupus erythromatosus.
b. Rheumatoid arthritis.
c. Systemic sclerosis.
d. Ankylosing spondylitis.
e. Multiple sclerosis.
f. Type 1 diabetes mellitus.
g. Ulcerative colitis.
h. Crohn's disease.
i. Type II refractory celiac disease.
j. Myasthenia gravis.

3. Stem cell and solid organ transplantation:

a. Enhancement of engraftment in Hematopoietic Stem Cell Transplantation (HSCT).
b. Prevention and treatment of Graft Versus Host Disease (GVHD) following HSCT.
c. Improvement of outcome of solid organ transplantation by immunomodulation and induction of transplantation tolerance.

4. Treatment of various infections and their complications:

a. Bacterial infections including sepsis complicated by adult respiratory distress syndrome.
b. Viral infections such as human immunodeficiency virus, hepatitis B and C viruses, and COVID-19 infections.
c. Parasitic infections such as Chagas disease, schistosomiasis, and malaria.
d. Mycobacterial infections such as tuberculosis.

5. Drug delivery: different mechanisms are involved MSC-based drug delivery including: delivery of suicide genes; delivery of oncolytic viruses; and delivery of therapeutic proteins.

6. Treatment of cancer: examples include: leukemia; lymphoma; myeloma myeloma; myelodysplastic syndromes; lung cancer; prostate cancer; pancreatic carcinoma; ovarian cancer; head and neck tumors; and breast cancer.

7. Other miscellaneous applications:

a. Macular degeneration, corneal reconstruction and corneal transplantation.
b. Liver fibrosis, liver cirrhosis, end-stage liver disease and hepatic failure.
c. Bones and joints: osteogenesis imperfecta, osteoarthritis, and osteoporosis.
d. Aging frailty.
e. Amyotrophic lateral sclerosis.
f. Parkinson’s Disease.
g. Idiopathic pulmonary fibrosis.
h. Chronic obstructive airway disease.
i. Renal disorders.

Table 1: Current and potential clinical applications of mesenchymal stem cells.

Name of MSC product	Source of MSCs	Indication of MSC Therapy	Country/Region of Approval	Producing Company and Year of Approval	Autologous or Allogeneic	Dose of Stem Cells and Route of Administration
Queen-cell	AT-MSCs and other cell types	Connective tissue disorders	South Korea	Anterogen 2010	Autologous	1×10^6 cells/mL
Cellgram	Human BM-MSCs	Acute myocardial infarction	South Korea	Pharmicell 2011	Autologous	Dose varies according to body weight: 10-18 mL [$5\text{-}9 \times 10^7$ MSCs]
Cupistem	Human AT-MSCs	Fistulae of Crohn's disease	South Korea	Anterogen 2012	Autologous	Dose varies according to fistula diameter: $3\text{-}6 \times 10^7$ MSCs in 1- 2 mL
Cartistem	Human UCB-MSCs	Advanced Osteoarthritis of Knee Joint	South Korea	Medipost 2012	Allogeneic	Dose depends on the size of the lesion: 7.5×10^6 cells/vial
Prochymal (Reme-stemcel-L)	Human BM-MSCs	Acute steroid-refractory GVHD in children	Canada New Zealand	Osiris Therapeutics and Mesoblast 2012	Allogeneic	100×10^6 MSCs/ 15 mL, 2.5×10^6 MSCs/ mL after reconstitution Intravenous infusion
NeuroNataR	Human BM MSCs	Amyotrophic lateral sclerosis	Republic of Korea	Corestem 2014	Autologous	1×10^6 MSCs/kg Once every 2 weeks
Holoclar	Limbal Stem Cells	Limbal stem cell deficiency due to ocular burns	EMA in Europe	Chiesi Farmaceutici 2015	Autologous	79,000–316,000 cells/cm²
Reme-stemcel-L	Human BM-MSCs	Acute and refractory GVHD in children	FDA in the United States of America (USA)	Mesoblast 2015	Allogeneic	2-8 million cells/kg Intravenous injection
Temcell HS	Human BM-MSCs	Acute and refractory GVHD	Japan	JCR Pharmaceuticals 2015	Allogeneic	2 million cells/kg twice weekly for 4 weeks, at rate of 4 ml/minute. Intravenous infusion
Stem-peucel	Human BM-MSCs	Critical limb ischemia	India	Stem-peutics 2016	Allogeneic	1 or 2 million cells/kg Intramuscular injection
Alofisel	Human AT-MSCs	Complex perianal fistulas in Crohn's disease	EMA in Europe	TiGenix and Takeda 2018	Allogeneic	4 Vials each containing 30 million MSCs/6 mL
Mesestro-Cell	BM-MSCs	Osteoarthritis	Iran	Cell Tech Pharmed 2018	Autologous	4×10^7 cells for both knees Intra-articular Injection
Stemirac	BM-MSCs	Spinal cord injuries	Japan	Nipro Corp 2018	Autologous	50 to 200 million cells Intravenous infusion

remestemcel-L-rknd	BM-MSCs	Acute steroid-refractory GVHD in children	FDA in the USA	Ryoncil, Mesoblast. 2024	Allogeneic	2 X 10^6 MSC/kg, twice weekly for 4 weeks Intravenous infusion
Amimestrocel	Human UCB-MSCs	Acute steroid-refractory GVHD	China	Platinum Life Excellence Biotech Co. Ltd. in Jan 2025	Allogeneic	Intravenous infusion
- MSCs: Mesenchymal Stem Cells - BM: Bone Marrow - UCB: Umbilical Cord Blood - AT: Adipose Tissue - GVHD: Graft Versus Host Disease - EMA: European Medicine Agency - FDA: Food and Drug Administration						

Table 2: List of approved MSC therapies.

Conclusion and Future Directions

After nearly three decades of development and research, MSCs and their products have achieved tremendous advancements that have translated into the approval of fifteen MSC- based therapies worldwide. Also, MSCs have shown great potential to become effective and possibly curative therapeutic options for several chronic and intractable diseases. In order to establish an effective MSC therapy, it is essential to adopt the following strategies: using specific products of MSCs such as ECVs and exosomes; using MSCs derived from iPSCs; using automated robotic production of MSCs to satisfy the increasing demand for MSC-based therapies; applying genetic engineering methods to amplify the paracrine actions of MSCs and broaden the therapeutic capabilities of MSCs; using MT of MSCs to lay the foundation for future CTs; using the most appropriate: cell source, route of administration and cell dose; standardization of production, storage and administration protocols; and implementation of strict regulations and unified standards. Also, in order to optimize MSC industry and therapeutics it is vital to: apply strict quality control measures to cover all stages of MSC therapy from manufacture till administration of stem cells or their products; perform more randomized CTs and multicenter prospective studies that will ultimately determine the optimal conditions of MSC therapies; and link various teams including: researchers, cell therapy laboratory scientists, clinical teams and patient working groups. Thus, MSCs may reshape the future of medical therapeutics and may eventually become curative for several chronic and intractable medical diseases provided the remaining challenges are overcome.

Conflict of Interest

The author declares no conflicts of interest.

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