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Freeze-Dried Mesenchymal Stem Cells: From Bench to Bedside. Review

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This review describes the freeze-dried mesenchymal stem cells (MSCs) and their ability to restore damaged tissues and organs. An analysis of the literature shows that after the lyophilization MSCs retain >80% of paracrine factors and that the mechanism of their action on the restoration of damaged tissues and organs is similar to the mechanism of action of paracrine factors in fresh and cryopreserved mesenchymal stem cells. Based on the own materials, the use of paracrine factors of freeze-dried MSCs in vivo and in vitro for the treatment of various diseases of organs and tissues has shown to be effective. The study also discusses about the advantages and disadvantages of freeze-dried MSCs versus cryopreserved MSCs. However, for the effective use of freeze-dried MSCs in clinical practice, a more detailed study of the mechanism of interaction of paracrine factors of freeze-dried MSCs with target cells and tissues is required. It is also necessary to identify possible other specific paracrine factors of freeze-dried MSCs. In addition, develop new therapeutic strategies for the use of freeze-dried MSCs in regenerative medicine and tissue bioengineering.

1. Introduction

Stem cells are unique cells in the human body that are capable of both self-renewal and differentiation into different cell lines. The stem cell population is isolated from embryonic and postnatal tissues. Embryonic stem cells can be obtained both from existing human embryos and from embryos created by in vitro fertilization. However, obtaining embryonic stem cells through any of these methods is associated with ethical, religious, and political issues. In Georgia, as in many other countries, obtaining embryonic stem cells from embryos is prohibited.

This review will focus on mesenchymal stem cells (MSCs), which have been identified as a population of organized hierarchical postnatal stem cells capable of differentiating into osteoblasts, chondrocytes, adipocytes, cardiomyocytes, myoblasts, and nerve cells. Considering the fact that the mechanism of stem cell differentiation in vitro is more or less understood, the mechanism of stem cell differentiation into the desired phenotype in vivo is still the subject of discussion and requires more detailed studies. There is an opinion that the therapeutic effects of transplanted MSCs in vivo are not related to their regenerative activity and that paracrine factors of stem cells themselves can play a special role in the restoration of damaged organs and tissues.[1,2] An example is the conditioned medium in which stem cells are cultured. Studies have shown that secretomes, microvesicles, or exosomes secreted in the Conditioned medium by stem cells can restore damaged organs and tissues.[3–5] Numerous preclinical studies have been presented regarding the use of Conditioned medium for the treatment of myocardial infarction, liver diseases, focal cerebral ischemia, spinal cord injuries, and many others.[6–12] Conducted clinical studies have also shown the benefits of the conditioned medium of adipose tissue-derived stem cells on wound healing.[13] Other authors focus on the MSCs themselves, reporting that after their introduction into the systemic circulation; MSCs migrate to the damaged sites, attracted by chemotactic signals released by damaged tissues,[14] and actively participate in the restoration of damaged organs and tissues.

Another issue requiring more detailed study concerns the mechanism by which MSCs modulate and modify the immune response. Soluble factors, extracellular vesicles, and exosomes are reported to be behind their immunomodulatory action.[15]

There is also an ongoing discussion in the literature regarding the routes of MSC administration. For example, the introduction of MSCs through the systemic circulation leads to the dispersion of cells throughout the body, which significantly reduces their number and concentration in the damaged tissue. From this point of view, the direct introduction of MSCs directly into the lesion compares favorably with systemic administration. However, direct administration of MSCs in some cases may not be effective due to local inflammatory processes, which can create an unfavorable environment for the survival and functioning of transplanted stem cells.[3]

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2. MSCs and Their Application in Preclinical and Clinical Studies

The main sources of human MSCs are bone marrow (BM), peripheral blood, and adipose tissue, as well as the placenta, umbilical cord, cord blood, and others. Depending on the source and purpose of the study, various protocols for the isolation of MSCs have been proposed, including density gradient centrifugation method (Ficoll-paque, Percoll, and RosetteSep), pre-plate method, magnetically activated cell sorting (MACS), flow cytometry or immunomagnetic sorting methods,[16–20] and many others.

The minimum criteria for determining human MSCs are positive expression of CD105 (SH2), CD73 (SH3), CD44, and CD90 markers and negative expression of CD45, CD34, CD14 or CD11b, CD79a markers or CD19 and HLA-DR surface molecules.[21]

The literature contains numerous preclinical and clinical studies on the use of MSCs for bone reconstruction, for the restoration of damaged cartilage, meniscus, tendons, ligaments, and intervertebral discs.[22–27] However, it should be noted that the choice of cell type for transplantation (autologous or allogeneic) is still a matter of debate. In this review, we will not describe the therapeutic benefits of autologous or allogeneic MSCs. They are well-described by many authors.[28,29] We only note that allogeneic and autologous MSCs have their own advantages and disadvantages in preclinical and clinical practice.

An analysis of the literature has shown that only in the last ten years (2012–2023) a large number of clinical studies have been conducted using autologous and allogeneic MSCs for the treatment of diseases such as liver cirrhosis,[30–35] cardiomyopathy,[36–44] spinal cord injury,[45–50] diabetes mellitus.[51–58] Almost all authors note that transplantation, especially of autologous MSCs, is safe and significantly improves the condition of patients. For example, a phase 2, multicenter, randomized, open-label study in 72 patients with baseline biopsy-proven alcoholic cirrhosis is presented.[59] Patients underwent both single and double injections of 5×10^7 BM MSCs into the hepatic artery 30 days after BM aspiration. The authors reported that Child-Pugh scores in both BM MSC groups improved significantly after BM MSC transplantation. They also noted that autologous BM MSC transplantation was safe and improved histological fibrosis and liver function in patients with alcoholic cirrhosis.

Another report demonstrated the therapeutic effect of cultured MSCs in patients with ischemic cardiomyopathy (ICM). Patients received MSCs via transendocardial injection of stem cells.[60]

A report on the treatment of twenty patients with spinal cord injury (SCI) is also presented.[61] According to the SCI classification of the American Spinal Injury Association (ASIA Impairment Scale), the authors reviewed 8 class A cases, 4 class B cases, and 8 class C cases. A total of 5 ml (1×10^8) of MSCs was administered to each SCI patient. The result analysis has shown a significant improvement in the function of sensory, motor, and autonomic nerves according to the American Spinal Injury Association Disorder Scale. The most common adverse events, fever, and headache, resolved within 24–48 h without treatment.

Our early clinical studies have shown that transplantation of autologous MSCs for the treatment of patients with spinal cord injury is a feasible and safe technique.[62] In total, the study included 18 patients with complete motor deficit and paraplegia caused by trauma to the thoracic and lumbar spine, without muscle atrophy and mental disorders. At least 750 million cells enriched in mononuclear cells suspended in 2 ml of saline were administered intrathecally. Studies have shown improvement in motor and sensory functions of varying degrees, observed in nine out of eighteen (50%) cases after bone marrow stem cell (BMSC) transplantation. According to the American Spinal Injury Association (ASIA) scale, seven (78%) of nine patients experienced a one-grade improvement, and two (22%) patients experienced a two-grade improvement.

3. Mechanism of Differentiation of MSCs

Here we briefly describe the main mechanisms of MSC differentiation into hepatocyte-like cells, cardiomyocytes, and insulin-producing cells in vitro and in vivo. For example, the differentiation of MSCs into hepatocyte-like cells is associated with the presence of hepatocyte growth factor (HGF), epidermal growth factor (EGF), and transforming growth factor-beta (TGF-β), fibroblast growth factor (FGF), insulin-like growth factor 1 (IGF-1), and others.[63,64] HGF has been reported to bind to its c-Met receptor and activate the PI3K/Akt and MAPK/ERK signaling pathways, leading to the activation of several transcription factors involved in hepatocyte differentiation. At the same time, EGF activates the MEK/ERK signaling pathway, which upregulates the expression of hepatocyte-specific genes such as HNF4α and CEBPα. As for TGF-β, it activates the Smad signaling pathway, which leads to an increase in hepatocyte-specific transcription factors such as HNF4α and HNF1α.[65–69]

Differentiation of MSCs into cardiomyocytes requires bone morphogenetic protein 2 (BMP-2), which activates the Smad signaling pathway and increases the expression of heart-specific transcription factors such as Nkx2.5 and GATA4. Also, vascular endothelial growth factor (VEGF) which is able to activate the PI3K/Akt signaling pathway by increasing the expression of heart-specific transcription factors such as Nkx2.5 and GATA4. Insulin-like growth factor 1 (IGF-1) can activate the PI3K/Akt and MAPK/ERK signaling pathways, which upregulate the expression of heart-specific transcription factors such as Nkx2.5 and GATA4.[70–73] Differentiation of MSCs into insulin-producing cells requires factors such as fibroblast growth factor (FGF), insulin-like growth factor 1 (IGF-1), and activin-A, which is a dimeric glycoprotein belonging to the transforming family growth factors-β (TGF-β), a betacellulin protein encoded by the BTC gene on chromosome 4 at the 4q13-q21 locus in humans.[74–79]

4. Paracrine Factors of MSCs

MSCs secrete various paracrine factors such as cytokines and chemokines (interleukin-6 [IL-6], interleukin-8 [IL-8] and tumor necrosis factor-alpha [TNF-α]), growth factors (vascular endothelial growth factor [VEGF], transforming growth factor-beta [TGF-β] and platelet growth factor [PDGF], keratinocyte growth factor [KGF], angiopoietin-1 [Ang-1], factor 1 derived from stromal

cells [SDF-1], macrophage inflammatory protein-1 alpha [MIP-1α/CCL3] and macrophage inflammatory protein-1 beta [MIP-1 beta, CCL4, MIP-1ß] and erythropoietin [EPO]), extracellular vesicles (exosomes and microvesicles) containing various biologically active molecules, including proteins, lipids, and nucleic acids, enzymes (matrix metalloproteinases [MMPs]). Paracrine factors secreted by MSCs have a variety of therapeutic effects, including anti-inflammatory, immunomodulatory, and tissue regenerative effects.[80–82]

The molecular mechanism of MSC paracrine factors includes modulation of immune responses, stimulation of angiogenesis, stimulation of progenitor cell proliferation and differentiation, as well as regulation of production and remodeling of the extracellular matrix, which significantly enhances the repair and healing of damaged tissues.[83,84] Paracrine factor MSCs modulate the immune response by producing anti-inflammatory cytokines such as IL-10 and TGF-β, which in turn can suppress the activity of pro-inflammatory immune cells such as macrophages and T-cells.[85] In addition, MSC paracrine factors produce angiogenic factors such as VEGF and FGF-2, which promote endothelial cell proliferation and migration.[86]

However, in this process, in addition to paracrine factors of MSCs, the fate of MSCs themselves after their transplantation in vivo is of interest. We hypothesized that one part of MSCs that were previously subjected to the process of freezing/thawing after transplantation into the target tissue might die. This is primarily due to the fact that the freezing/thawing process can contribute to damage and decay of transplanted MSCs, which are manifested by cell shrinkage, cytoplasmic leakage, debris, the formation of apoptotic bodies, pores in the membrane cell, T-cell cytolysis and phagocytic absorption, which actively attack thawed MSCs.[87,88] Another part of transplanted MSCs can migrate from the injection site to nearby and distant tissues. The remaining part of MSCs, as we described above, enters into a complex process, including such stages as recruitment of MSCs to the site of tissue damage, interaction of MSCs with the tissue microenvironment, activation of signaling pathways, transcription factors, epigenetic modifications, and others. However, it is reported that nonviable cells, including apoptoic cells, have immunomodulatory property.[89] According to the authors, the treatment with dead MSCs (DMSCs) has the same therapeutic effect as the treatment with MSCs. Moreover, DMSCs per se in a therapeutic medicine of stem cells or apoptotic cells induced in vivo have the same immunomodulatory properties as fresh MSCs through the release of phosphatidylserine (PS). The latter was recognized by Mer Tyrosine Kinase (MerTK) and was involved in the modulation of immune cells. It has also been reported that apoptosis of mesenchymal stromal cells is required for their therapeutic function.[90]

The studies have shown that deletion of the apoptotic effectors Bcl-2 antagonist killer (BAK) and Bcl-2 associated x (BAX) prevented the death of MSCs and attenuated their immunosuppressive effects in disease models used for determining MSC activity. The authors note that MSC apoptosis and their efferocytosis induce changes in metabolic and inflammatory pathways in alveolar macrophages, causing immunosuppression and reducing the severity of the disease. Thus, they have concluded that the host response to dying MSCs is the key to their therapeutic effects.[90]

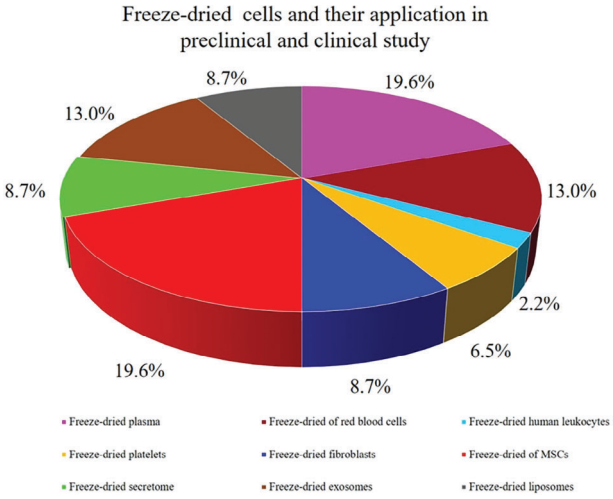

Figure 1. Freeze-dried cells and their use in preclinical and clinical studies.

5. Freeze-Dried Cells

The survival of cells in a dry and dehydrated state is one of nature's most fascinating phenomena.[91] For example, many organisms from a wide variety of taxa have the ability to survive extreme dehydration. Through a phenomenon called "anhydrobiosis", organisms use various protective proteins such as LEA (Late Embryogenesis Abandoned) and heat shock proteins (HSPs), antioxidant system proteins, or transport proteins,[92,93] which give them the ability to survive in extreme dehydration conditions. Based on the above mentioned, the increased interest of researchers in the lyophilization process is justified.

Freeze-dried, also known as lyophilization, is a combination of the processes of removing water from a biological substance by freezing it, followed by sublimation with ice in a vacuum, in which solid ice turns directly into vapor, bypassing the liquid state. Even till this date, there is still debate about who actually invented the lyophilization method. It is believed that in 1890 the German pathologist Richard Altmann first dried frozen tissue pieces for histological sections by placing them in a vacuum desiccator at a temperature of −20°. However, this method remained unnoticed for many years. Since the 1900s, Benedict and Manning, Jacques Arsène d'Arsonval, Leon Shackell, and others have reported sublimation. However, Tival H. and Elser W.J. were the first to patent freeze-drying systems with improved freezing and condenser stages.[94]

At present, significant advances have been made in freeze-dried technology and it is being used in such industries as pharmaceuticals, medicine, biotechnology, and others. It has to be noted that lyophilized cell material and its derivatives have been used in preclinical and clinical studies for many decades. For example, the freeze-dried plasma,[95–104] red blood cells,[105–110] freeze-dried leukocytes,[111] freeze-dried platelets,[112–114] freeze-dried fibroblasts,[115–118] freeze-dried MSC,[119–127] freeze-dried secretome,[128–131] freeze-dried exosomes,[132–137] freeze-dried liposomes[138–141] dried and others. **Figure 1** presents lyophilization data of various cells and their application in preclinical and clinical studies. As reported by the authors, the freeze-dried and

reconstituted platelets retain fundamental hemostatic properties necessary to stop bleeding.[142] The use of autologous freeze-drying platelet-rich plasma (FD-PRP) on bone regeneration in maxillary sinus floor augmentation has been mentioned in a preliminary pilot study.[143] The authors have noted that the results generated in this study suggest that autologous freeze-drying platelet-rich plasma can be used safely for bone engineering in clinical practice. It is also noted that FD-PRP preserves platelet function, cytokine concentration, and functionality.[144]

As for the use of freeze-dried MSCs in clinical practice, there is a limited number of publications. This is primarily associated with the fact that lyophilized MSCs are still considered nonviable, since the freeze-drying process, which includes the removal of water from cells, can disrupt the integrity of cell membranes and change their intracellular components. Additionally, when rehydrated or resolubilized, cells can be further stressed, which can lead to the loss of their viability.

In recent years, a wide range of cryoprotectants and lyoprotectors, including sugars, macromolecules, antioxidants, and chelating agents have been used to prevent damaging processes of lyophilization of MSCs after their rehydration.[145,146]

The most commonly used Trehalose is the trehalose that is injected intracellularly before drying, which significantly increases the viability of lyophilized MSCs after rehydration. However, it is reported that human cells in culture can withstand desiccation for a considerable amount of time even in the absence of trehalose or other exogenously introduced carbohydrate. The authors have tested 12F fibroblast cell lines, Basinger fibroblast cell lines, and human mesenchymal stem cells successfully.[147,148] They have noted that the cells of an organism that are not resistant to desiccation have mechanisms that allow them to resist almost complete desiccation. Additionally, the introduction of trehalose biosynthesis genes made it possible to reversibly dehydrate human cells in culture.

However, it should be noted that the rate of drying, the temperature at which the cells are maintained, the degree of confluence upon drying, and the presence or absence of light have a great influence on the ability to maintain cell viability in the dried state.

The studies concerning the development of a lyophilized off-the-shelf mesenchymal stem cell-derived acellular therapeutics are also interesting. The authors have isolated three distinct MSC+ secretome product (SP) formulations by tangential flow filtration using sucrose, trehalose, and mannitol as lyoprotectants. It is noted that lyophilization is an effective method for maintaining the structural and functional integrity of MSC-SP and can be used to develop a finished therapeutic remedy.[122]

Other authors have used pharmaceutical formulations containing secretome and/or extracellular vesicles, which were extracted from adipose-MSCs and bone marrow-MSCs by combining ultrafiltration (UF) or ultracentrifugation (UC) with lyophilization. As noted by the authors,[149] compared to UC, UF has led to higher protein, lipid, cytokine, and exosomes yields.

The isolation procedure and cell source influenced immunomodulatory activity, which was evaluated in vitro by inhibition of phytohemagglutinin-activated peripheral blood mononuclear cell proliferation, and by modulation of IL-10, IFN-γ and IL-6. The studies related to optimization of a lyoprotector for lyophilization of receptor-targeted Trojan horse liposomes for plasmid DNA delivery should also has to be mentioned.

As it is known, Trojan horse liposomes (THLs) are a form of ligand-targeted nanomedicine, where plasmid DNA is encapsulated within a 100–150 nm pegylated liposome, and the tips of part of the surface pegylated filaments are covalently linked to the receptor – specific monoclonal antibody (MAb) via a thioether bond.[150]

The authors have developed a lyophilization methodology that allows the preservation of the structure and function of THLs after the lyophilization/hydration process. They noted that the optimization of the lyoprotector allows long-term storage of liposomes encapsulated with the receptor-specific monoclonal antibody (MAb) target DNA in a lyophilized state. The method of freeze-drying is also used for the preservation of immunoengineering products.[151]

6. Paracrine Factors of Freeze-Dried MSCs

Given the fact that paracrine factors of MSCs play the main role in the restoration of damaged organs and tissues, why not use the paracrine factors of freeze-dried MSCs.

We would like to draw the reader's attention to the fact that one of the main advantages of freeze-dried MSCs over cryopreserved cells is their increased storage stability. Freeze-dried cell can be stored for many years at room temperature, even at home. They do not require special equipment, reagents, and energy costs. This makes transportation even more convenient.

Our early studies showed that, after lyophilization, MSCs retain >53% of paracrine factors.[152] There are reports that lyophilization ensures the viability of up to 70% of MSCs without the addition of protectors.[91] In addition, after lyophilization, MSCs have been reported to retain >80% of paracrine factors,[123] including VEGF-1, IGF-1, EGF, NO, HGF, bone morphogenetic protein (BMP), keratinocyte growth factor (KGF), from stromal cell-derived factor 1 (SDF-1), monocyte chemoattractant protein-1 (MCP-1) and osteocalcin, osteopontin, bone sialoprotein (BSP), angiopoietin 1 (Ang1), erythropoietin (EPO) and many others.[91,123,153] Unfortunately, there are a limited number of studies describing the mechanisms of interaction of paracrine factors of frozen/dried MSCs after their transplantation with the tissue microenvironment. However, we assume that the effect of freeze-dried paracrine factors on the processes of repair of damaged tissues and organs is similar to the mechanism of action of fresh or cryopreserved MSCs.

In this regard, in our opinion, works related to lyophilized somatic cells are of interest. As the authors note, lyophilized somatic cells that were stored for 3 years at room temperature were able to control embryonic development after injection into enucleated oocytes.[124] Healthy cloned offspring derived from lyophilized somatic cells have also been reported. The authors managed to obtain cloned blastocysts from lyophilized somatic cells and create lines of embryonic stem cells with nuclear transfer.[154] Other authors, who reported that freeze-dried porcine somatic cells subjected to long-term storage at 4°C have almost the same potential for development into blastocysts as non-lyophilized cells,[155] obtained similar results. Freeze-dried human cells stored at room temperature retain several types of RNA in excellent quality for RNA sequencing.[156] In this study, the authors used freeze-dried to stabilize unfractionated human cells in a dried state at room temperature and tested the yield and

integrity of isolated RNA using microfluidic electrophoresis, RT-qPCR, and RNA sequencing. They suggested that freeze-dried human cells is a suitable alternative for long-term stabilization of total RNA in whole human cells for routine diagnostics and high-throughput biomedical research. Other studies have shown that freeze-dried extracellular vesicles from adipose-derived stem cells prevent hypoxia-induced damage to muscle cells.[126] It has also been reported that freeze-dried cord blood-derived mononuclear cells formed colonies after rehydration.[120]

Our preclinical studies have shown the possibility of using freeze-dried BM MSCs for the treatment of radiation wounds.[157] We have developed a bioactive wound dressing (BAWD) that combines decellularized and lyophilized human amniotic membrane seeded with freeze-dried BM MSCs of the rat for the treatment of non-healing wounds. The studies were carried out on inbred Lewis rats with a radiation wound model (dose 60 Gy). On the 20th day after irradiation, the skin around the radiation burn was excised. The wound was treated with biologically active additives. Studies have shown that the nutritional supplement increases the healing rate by 2.5 times. Studies have also shown that the BAWD contains a large number of cytokines and growth factors, including epidermal growth factor, basic fibroblast growth factor, keratinocyte growth factor, VEGF, TGF-α, TGF-β, platelet growth factor, hepatocyte growth factor, and nerve growth factor.

Another preclinical study,[152] in which we used freeze-dried MSCs derived from human placenta (HP MSCs), was performed in the treatment of a rat model of acute respiratory distress syndrome (ARDS). Flow cytometry results showed that cell surface markers of HP MSCs, such as CD73, CD90, and CD105, were highly expressed, while CD31, CD34, HLA-DR, and CD45 showed low expression, consistent with the MSC profiles. Intratracheal injection of lipopolysaccharide increased the expression level of the pro-inflammatory cytokine. In animals with the ARDS model, during the first three days, the levels of inflammatory cytokines TNF-α, IL-1β and IL-6 in the blood serum were significantly increased and remained at the same level during the entire period of observation of the animals. While the intratracheal injection of HP MSCs significantly limited the increase in the level of inflammatory markers.

We have also used freeze-dried MSCs to stimulate the wound-healing process after resection of tongue squamous cell carcinoma in rats.[158] Studies also showed that freeze-dried MSCs retained their unique paracrine factors, which contributed to the acceleration of wound healing processes.

In clinical studies, we used freeze-dried MSCs from a patient with a mandibular bone defect formed after tumor resection. The reconstruction of the mandibular defect was performed using a decellularized three-dimensional cancellous bovine graft (BABG). The decellularized bone graft was loaded with lyophilized MSCs.[153] Analysis of gene expression showed that BABGs containing freeze-dried MBMS express a wide range of growth factors, in particular osteocalcin and osteopontin.

All our preclinical and clinical studies have demonstrated the effectiveness of freeze-dried MSCs, in the restoration of damaged tissues and organs. However, further studies are needed to optimize the therapeutic use of paracrine factors, freeze-dried MSCs, and to better understand their mechanisms of action. Based on the above, it can be concluded, that the paracrine factors of freeze-dried MSCs produce anti-inflammatory cytokines, such as IL-10 and TGF-β, which are involved in the suppression of the activity of such pro-inflammatory immune cells as macrophages and T-cells, and thus can reduce tissue damage and promote their regeneration. Paracrine factors of freeze-dried MSCs also produce angiogenic factors such as VEGF and FGF-2, which promote the proliferation and migration of endothelial cells. In addition to these effects, the paracrine factors of freeze-dried act on progenitor cells to produce growth factors such as BMP-2 and TGF-β, which stimulate proliferation and differentiation, such as progenitor cells into osteoblasts and chondrocytes. Paracrine factors of freeze-dried MSCs also regulate the production and remodeling of the extracellular matrix, which can affect the structure and function of tissues.

7. Conclusion

The analysis of literary and own materials showed the possibility of effective use of stem cells for the restoration of damaged organs and tissues. After freeze-dried, MSCs retain >80% of paracrine factors, the mechanism of action of which on the processes of regeneration of damaged tissues and organs is similar to the mechanism of action of paracrine factors of fresh and cryopreserved MSCs. One of the main advantages of lyophilized MSCs over cryopreserved cells is their increased storage stability. They can be stored for many years at room temperature even at home. They do not require special equipment, reagents, and energy costs, which makes transportation more convenient. However, for the effective use of lyophilized MSCs in clinical practice, a more detailed study of the mechanism of interaction of paracrine factors of lyophilized MSCs with target cells and tissues is required. It is also necessary to identify possible other specific paracrine factors of dried MSCs and develop new therapeutic strategies that will allow the use of lyophilized MSCs for the treatment of various organ and tissue injuries.

Conflict of Interest

The authors declare no conflict of interest.

Keywords

freeze-dried mesenchymal stem cells, Mesenchymal stem cells, paracrine factors of mesenchymal stem cells

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