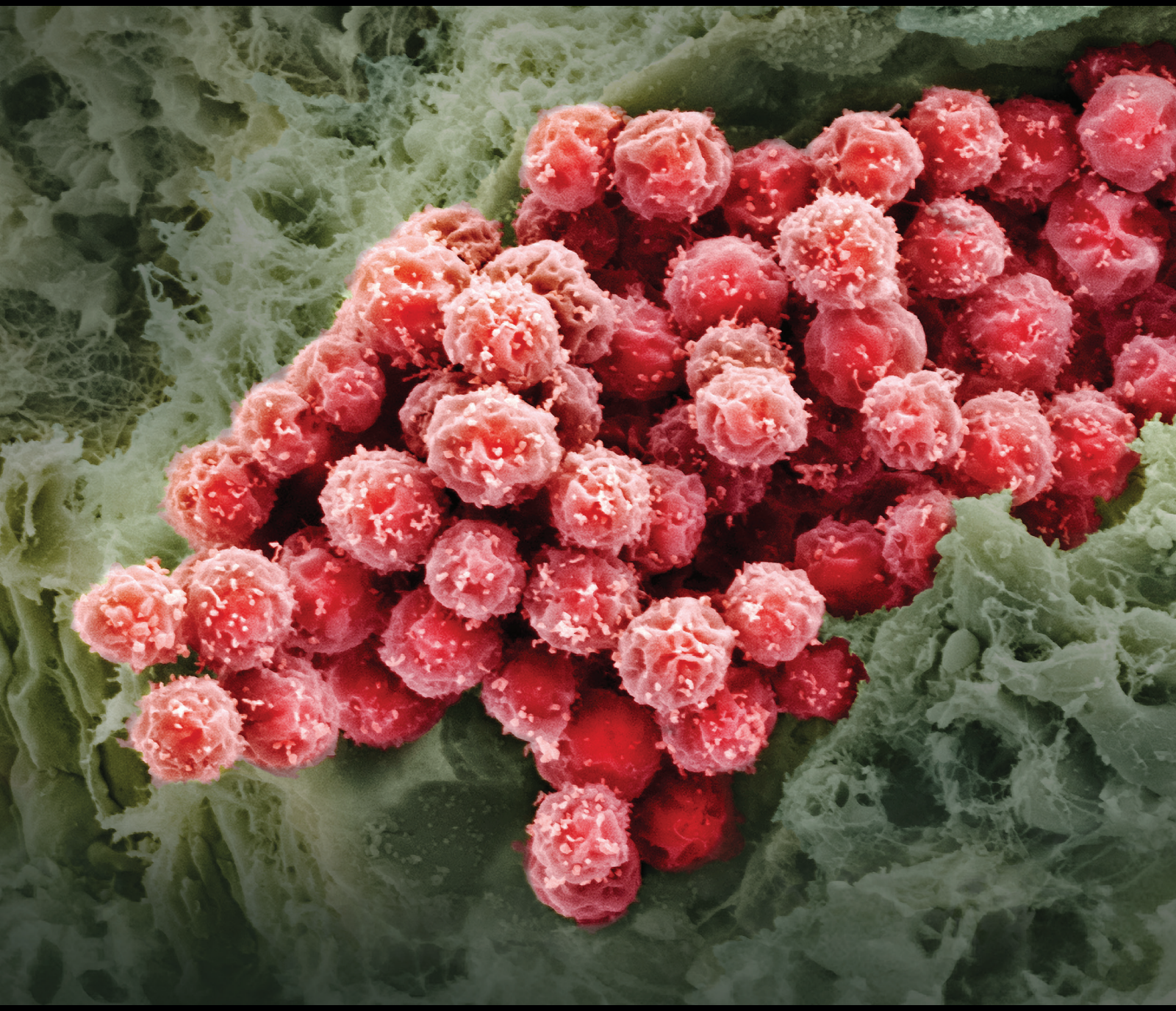
Stem Cells International

Mesenchymal Stem Cell Therapy in Nonhematopoietic Diseases

Guest Editors: Katherine Athayde Teixeira de Carvalho, Gustav Steinhoff, and Juan Carlos Chachques



Mesenchymal Stem Cell Therapy in Nonhematopoietic Diseases

Stem Cells International

Mesenchymal Stem Cell Therapy in Nonhematopoietic Diseases

Guest Editors: Katherine Athayde Teixeira de Carvalho, Gustav Steinhoff, and Juan Carlos Chachques

Editorial Board

Contents

Hindawi Publishing Corporation
Stem Cells International
Volume 2015, Article ID 676903, 2 pages
http://dx.doi.org/10.1155/2015/676903

Editorial

Mesenchymal Stem Cell Therapy in Nonhematopoietic Diseases

Katherine Athayde Teixeira de Carvalho,[1] Gustav Steinhoff,[2] and Juan Carlos Chachques[3]

[1]*Stem Cell Research Laboratory, Pequeno Príncipe Faculty & The Pelé Pequeno Príncipe Research Institute, Silva Jardim 1632, 80.250-200 Curitiba, PR, Brazil*
[2]*Department of Cardiac Surgery, Reference and Translation Center for Cardiac Stem Cell Therapy (RTC), University of Rostock, Schillingallee 35, 18.057 Rostock, Germany*
[3]*Cardiac Bioassist, Pompidou Hospital, University of Paris Descartes, Laboratory of Biosurgical Research, Carpentier Foundation, University of Paris, Leblanc 56, 75.015 Paris, France*

Correspondence should be addressed to Katherine Athayde Teixeira de Carvalho; katherinecarv@gmail.com

Received 17 November 2014; Accepted 17 November 2014

The extensive clinical experiments with stem cells, particularly in the treatment of oncohematological diseases, opened up the possibility of trying out stem cells with nonhematopoietic diseases. Mesenchymal stem cells (MSCs) subsequently emerged as a promising source for the regeneration and repair of various tissues in the treatment of a range of diseases due to their presence in all derived mesenchymal tissues in the adult solid organs as well as in mesoderm from embryonic tissue. This, plus their pluripotentiality and the fact that they were easily obtainable, meant that MSCs represented an important source for studies in regenerative medicine.

MSCs have been shown to be capable of differentiating in various types of mesoderm derived cells as well as ectoderm cells, such as skeletal muscle cells, neurons, cardiomyocytes, osteocytes, chondrocytes, and others. MSCs in specific cultivation conditions *in vitro* and in host transplanted tissue, being niche dependent, arouse clinical interest in stem-cell therapy for regeneration in nonhematopoietic tissue diseases and the prospect of creating a mesenchymal stem-cell bank for research and subsequent clinical use.

In the development of clinical models, always preceded by preclinical studies, there are various requirements to guarantee the safety of therapy with stem cells and their products, including the GMP procedures, cytogenetic control, identification and characterization of cells by immune cytometric analysis, and demonstration of their pluripotentiality for the prospective use of MSC for tissue regeneration in nonhematopoietic diseases [1]. In the study of MSCs, the intrinsic properties—molecular, immunocytochemistry, isolation, expansion, differentiation, and cryopreservation—have made great advances and need further discussion. The fact that MSCs do not express the antigens of histocompatibility means that they can be used in allogeneic transplantation. The development of methods for isolating the subpopulations of MSC fractions together with the perspective with this subpopulation could obtain better results than the total population in replacement therapy for correction of determined specific function [2].

Researchers and physicians are looking into the physiopathology of some diseases in light of the intrinsic cell conditions on the development of each disease and are proposing therapies based on the cells themselves or on cell-based products. Some diseases are triggered in their physiopathology by autoimmune mechanisms that could be stabilized with the paracrine effects of MSCs, such as the anti-inflammatory effect [3]; other diseases are triggered by the senescence of the cells, as in conditions of cellular apoptosis, and the MSC cells could act with the paracrine effects such as antiapoptosis and by cell replacement; there are some diseases which result from the loss of the production of certain molecules and MSCs could differentiate in cells capable of producing the molecule [4]; in other diseases, there is an interruption in signals between the tissue cells, and the MSC paracrine effects could promote the connections through the

production of growth factors [5]. On the other hand, in ischemic diseases, MSCs could be differentiated in specific types of specialized cells, such as vessels and contractile cells, cardiomyocytes [6]; in genetic diseases, MSCs could ensure the delivery of genes for gene therapy [7] and could act in immunomodulation with vaccines [8].

This issue provides discussions of the points described above and takes a look into the future of cell therapy in nonhematopoetic diseases, showing that it is nearer than we expect! It is reality!

Katherine Athayde Teixeira de Carvalho
Gustav Steinhoff
Juan Carlos Chachques

References

[1] S. R. B. R. Sella, J. C. Francisco, T. M. Madalozzo et al., "General requirement for quality and safety in stem cell and cell-based products for translational therapy," *Cell Biology: Research & Therapy*, vol. 3, no. 1, pp. 2–4, 2014.

[2] A. Schade, E. Delyagina, D. Scharfenberg et al., "Innovative strategy for microRNA delivery in human mesenchymal stem cells via magnetic nanoparticles," *International Journal of Molecular Sciences*, vol. 14, no. 6, pp. 10710–10726, 2013.

[3] C. B. Ripoll, M. Flaat, J. Klopf-Eiermann et al., "Mesenchymal lineage stem cells have pronounced anti-inflammatory effects in the twitcher mouse model of Krabbe's disease," *Stem Cells*, vol. 29, no. 1, pp. 67–77, 2011.

[4] I. Linero and O. Chaparro, "Paracrine effect of mesenchymal stem cells derived from human adipose tissue in bone regeneration," *PLoS ONE*, vol. 9, no. 9, Article ID e107001, 2014.

[5] J. Dittmer and B. Leyh, "Paracrine effects of stem cells in wound healing and cancer progression (review)," *International Journal of Oncology*, vol. 44, no. 6, pp. 1789–1798, 2014.

[6] J. C. Chachques, "Development of bioartificial myocardium using stem cells and nanobiotechnology templates," *Cardiology Research and Practice*, vol. 2011, Article ID 806795, 7 pages, 2011.

[7] S. D. Olson, K. Pollock, A. Kambal et al., "Genetically engineered mesenchymal stem cells as a proposed therapeutic for huntington's disease," *Molecular Neurobiology*, vol. 45, no. 1, pp. 87–98, 2012.

[8] R. Blazquez, F. M. Sanchez-Margallo, O. de la Rosa et al., "Immunomodulatory potential of human adipose mesenchymal stem cells derived exosomes on *in vitro* stimulated T cells," *Frontiers in Immunology*, vol. 5, pp. 1–9, 2014.

Hindawi Publishing Corporation
Stem Cells International
Volume 2015, Article ID 146051, 29 pages
http://dx.doi.org/10.1155/2015/146051

Research Article

Human Adult Stem Cells Maintain a Constant Phenotype Profile Irrespective of Their Origin, Basal Media, and Long Term Cultures

Indumathi Somasundaram,[1] Rashmi Mishra,[2] Harikrishnan Radhakrishnan,[3] Rajkumar Sankaran,[4] Venkata Naga Srikanth Garikipati,[5] and Dhanasekaran Marappagounder[6]

[1]*Department of Stem Cells, National Institute of Nutrition, Secunderabad 500 007, India*
[2]*Institute for Biochemistry and Molecular Biology, Ulm University, M24, Level 3, Room 358, Albert-Einstein-Allee 11, 89081 Ulm, Germany*
[3]*Berlin School of Integrative Oncology, Buch, 131254 Berlin, Germany*
[4]*Lifeline RIGID Hospitals, Chennai 600 010, India*
[5]*Stem Cell Therapy Program, Center for Translational Medicine, Temple University School of Medicine, Temple University, Room MERB 9-943, 3500 North Broad Street, Philadelphia, PA 19104, USA*
[6]*Ree Laboratories Private Limited, Andheri West, Mumbai 400 053, India*

Correspondence should be addressed to Dhanasekaran Marappagounder; dhanasekarbio@gmail.com

Received 6 March 2014; Accepted 22 June 2014

Academic Editor: Katherine Athayde Teixeira de Carvalho

The study aims to identify the phenotypic marker expressions of different human adult stem cells derived from, namely, bone marrow, subcutaneous fat, and omentum fat, cultured in different media, namely, DMEM-Low Glucose, Alpha-MEM, DMEM-F12 and DMEM-KO and under long term culture conditions (>P20). We characterized immunophenotype by using various hematopoietic, mesenchymal, endothelial markers, and cell adhesion molecules in the long term cultures (Passages-P1, P3, P5, P9, P12, P15, and P20.) Interestingly, data revealed similar marker expression profiles irrespective of source, basal media, and extensive culturing. This demonstrates that all adult stem cell sources mentioned in this study share similar phenotypic marker and all media seem appropriate for culturing these sources. However, a disparity was observed in the markers such as CD49d, CD54, CD117, CD29, and CD106, thereby warranting further research on these markers. Besides the aforesaid objective, it is understood from the study that immunophenotyping acts as a valuable tool to identify inherent property of each cell, thereby leading to a valuable cell based therapy.

1. Introduction

The ubiquitous existence of multipotent mesenchymal stem cells annexes to be a powerful regenerative tool for its use in cellular therapeutics rendering the replacement of worn out cells [1, 2]. Despite the recent advancement, stem cell therapy is still at its infancy, attributed with several hurdles in regenerative applicability. This might be due to the lack of an ideal source of stem cells that accounts for the functional improvement of the diseased. The isolation and applicability of stem cells derived from the prehistoric source, human bone marrow, and the contemporary source of human adipose tissue has revolutionized the field of regenerative medicine [3–5]. Although these sources outweigh certain uncertainties, stem cell therapeutics in many cases was unsuccessful [6, 7]. The rationale of this failure in terms of stem cell survival, proliferation, and regeneration remains unclear.

Although the reason for the same is not fully understood, researchers combat towards overcoming the recognized barriers such as hyperglycemia, hypoxia and inflammation to

maximize the beneficial effects of MSC in cellular therapeutics [8, 9]. However, yet another potential reason for such failure might be due to the lack of understanding the individual components innate capability that forms the basis of tissue maintenance, repair, and regeneration. This is attributed to the fact that stem cells of adipose tissue and bone marrow reside in a more heterogeneous crude mixture along with the other constituents such as loose connective tissue matrix, endothelial cells, vascular smooth muscle cells, pericytes, leucocytes, mast cells, mesenchymal stem cells, and immune cells such as resident hematopoietic progenitor cells and macrophages [10–12]. The in vitro characterization and maintenance of these heterogenous tissue stem/progenitor cells are critical aspects when assessing their potential for clinical application. It is a well-known fact that stem cells use their receptors for binding other signalling molecules as a way of communication to carry out their functions of self-renewal and differentiation. Despite several attempts of research efforts on revealing their biological properties [10, 13], the phenotypic and functional characteristics of these stem cells, to date, still remain obscure.

The rationale behind this ambiguity relies on the hypothesis that influence of different media and media composition may lead to variations in marker expression [14]. In addition, it is also reported that these markers may or may not be evident at primitive stages or may get lost with expansion in vitro or in vivo [15], thereby identity of inherent population for therapeutic interventions becomes a strenuous task. These discrepancies based on phenotypic characterization of MSCs make its applicability indefinite, thereby demanding a quest for identification of prospective definitive marker profiles of MSCs in vitro. Being in the regenerative medicine epoch of treatment of degenerative diseases, it is important to address this inconclusive tribulation. Hence, identification of prospective markers of most widely used sources such as adipose tissue and bone marrow is of utmost importance to address the following reasons. Firstly, to understand the innate capability of each cell population according to its surface expression pattern, secondly, to advance our understanding of basic biological processes of stem cells during self-renewal and differentiation, that is, their in vivo functionality and finally, to demarcate and develop valuable cell based therapies.

In lieu of the above, this study aimed to identify whether the phenotypic marker expression profiles vary between sources such as bone marrow and subcutaneous fat under different media (DMEM-Low Glucose, Alpha-MEM, DMEM-F12, and DMEM-KO) and under long term culture conditions (>P20). Omentum fat is also included in the study as its immense potency is also underway [16–19].

2. Materials and Methods

2.1. Sampling. The protocol followed for all samples was reviewed and approved by the hospital review board and ethics committee of Lifeline Multispecialty Hospital, Chennai, India. The samples were collected in-house and the research pursuit was explained to the patients followed by obtaining a written informed consent prior to collection of samples.

The omentum fat was collected from patients undergoing exploratory laparotomy. The omentum fat biopsies of 25–40 g were obtained from 4 subjects ($n = 4$) with age group ranging from 28 to 50 and mean BMI of 26.5±2.1 kg/m^2. The collected tissues were processed within 4 hours of removal of fat from patients.

The subcutaneous fat was collected from obese patients undergoing Bariatric surgery in the form of abdominoplasty. Subcutaneous fat of 25–50 g was obtained from 4 subjects ($n = 4$) with age group ranging from 35–55 with a BMI 27.3 ± 1.8 kg/m^2 after completion of surgery. The tissues were quantified and were processed within 4 hours of collection.

Human bone marrow samples were obtained from the iliac crest of patients ($n = 4$) undergoing experimental stem cell therapy for spinal cord injury with mean age of 35.3±3.33 and body mass index (BMI) of 23.5 ± 1.167. All the samples were processed within 2 hours of collection.

2.2. Cell Isolation

2.2.1. Bone Marrow. Mononuclear cells were isolated from bone marrow aspirate by density gradient centrifugation using Ficoll Paque. The aspirates were diluted with twice the amount of PBS (Invitrogen) and layered on to Ficoll Paque (Stemcell Technologies) solution in a centrifuge tube. The layered samples were further centrifuged and the buffy coat layer containing the mononuclear cells was collected. The isolated mononuclear cell suspension was washed with PBS to remove the residual Ficoll content and other contaminants. The erythrocyte content in the isolated pellet was lysed using 0.7% NH_4Cl and lysis reaction was stopped with 0.9% ice cold NaCl. The suspension was centrifuged to obtain the mononuclear cell fraction. The isolated cells were further resuspended in PBS and its total cell count and viability were determined by trypan blue exclusion method.

2.2.2. Adipose Tissue. The surgical samples of obtained subcutaneous fat and omentum fat were washed thrice in wash buffer 1x Phosphate Buffered Saline (PBS) (Hi-Media) containing 1% antibiotic-antimycotic solution (Invitrogen) and were minced into 2-3 mm in diameter. These minced pieces were further digested by 0.075% collagenase type-1 (Hi-Media) solution. 10% Fetal Bovine Serum (FBS) (Invitrogen) was used to inhibit the activity of collagenase. The digested cells were centrifuged at 600 g for 10 minutes at 20°C. The stromal vascular fraction found in the pellet obtained was washed further and subjected to erythrocyte lysis using 0.7% NH_4Cl solution for 5 minutes at room temperature. The cells were subjected to further centrifugation and the pellet recovered was resuspended in PBS. A single cell suspension was obtained after filtration after which the cell viability was evaluated using Trypan Blue staining.

2.3. Cell Culture. Cells isolated from these aforesaid sources were plated at a density of $3 \times 10^5/25\,\text{cm}^2$ flask (Nunc) and cultured in four different filter sterilized media: DMEM-LG (Invitrogen), α-MEM (Invitrogen), DMEM-F12

(Invitrogen), and DMEM-KO (Invitrogen), each of which was supplemented with 10% FBS (Invitrogen) and 1% antibiotic-antimycotic solution. The cells were maintained for 2–4 days before first media change. Standard culture conditions of 37°C, 5% CO_2, and 95% humidity were maintained and 70–80% confluency was obtained. The primary culture was subcultured until passage 20 with media changes twice every week.

2.4. FACS Analysis. Flowcytometric characterization was performed using Becton, Dickinson FACS Aria (BD FACS Aria). Approximately 1×10^6 cells were stained with saturating concentrations of fluorochrome conjugated antibodies, CD34 PE (BD Biosciences), CD45 APC CY7 (BD Biosciences), CD133 APC (e-Biosciences), CD31 FITC (BD Biosciences), HLADR PERCP (BD Biosciences), CD44 FITC (BD Biosciences), CD73 PE (BD Biosciences), CD13 APC (BD Biosciences), CD29 PE (BD Biosciences), CD90 PERCP (e-Biosciences), CD105 APC (e-Biosciences), SSEA4 ALEXAFLOUR (e-Biosciences), CD117 APC (e-Biosciences), ABCG2 PE (e-Biosciences), CD166 PE (BD Biosciences), CD106 FITC (BD Biosciences), CD54 PERCP (BD Biosciences), CD 49d PE (e-Biosciences), and ALDH. The cells were incubated in the dark for 20 minutes at 37°C. The incubated cells were washed thrice with wash flow buffer consisting of phosphate buffer supplemented with 2% (v/v), FBS (Sigma Aldrich), and 0.1% (w/v) sodium azide, NaN_3 (Sigma Aldrich) and resuspended in 500 μL of BD FACS flow and vortexed. BD FACS-DIVA Software was used for sample data acquisition and analysis. The first plot is created with FSC versus SSC in all experiments. The subsequent plots were created using the respective flourochrome (x axis) along with SSC (y axis). The FSC Vs SSC were created to identify the different cell population and to avoid debris. The isotype control was used to set the gates and the analysis regions. The readings of each antibody cocktail in respective tubes were run, analysed, and recorded. All samples were characterized and recorded with a minimum of 10000 events.

2.5. ALDH Analysis. ALDH analysis was performed using Aldehyde dehydrogenase kit (Stem Cell Technologies). Dry ALDEFLUOR reagent was activated by incubating with DMSO followed by incubation with 2 N HCl at room temperature. The incubated mixture was further added with ALDEFLUOR assay buffer and stored at −20°C. Briefly, 1×10^5 cells were recovered by centrifugation and resuspended in assay buffer (1×10^5 cells per mL). The suspended sample was treated with 5 μL of activated ALDH substrate at 37°C in water bath. The incubated sample was further centrifuged and the pellet obtained was resuspended in cold ALDEFLUOR assay buffer. The stained cells were analysed in a flowcytometer with FITC channel. A sample tube containing DEAB (Diethylaminobenzaldehyde—a specific inhibitor of ALDH) was ran as control.

2.6. Statistical Analysis. All data obtained from omentum fat, subcutaneous fat, and bone marrow samples in different media ($n = 4$) were represented as Mean ± Standard Error Mean (SEM). The data were analysed using One Way Analysis of Variance (ANOVA) along with Duncan multiple range test using SPSS 15.0 (SPSS Inc., Chicago, IL, USA). P values were calculated to determine the statistically significant variations. Results were considered statistically significant when $P < 0.05$ and $P < 0.01$.

3. Results

3.1. Comparative Expression Profiles of Collated Surface Antigens. The MSCs of early and later passages from omentum fat, subcutaneous fat, and bone marrow cultured extensively (until P20) in four different media as illustrated (Figure 1) were phenotypically characterized ($n = 4$) for the diverse panel of cell surface marker profiles including mesenchymal stem cell, CD90, CD73, and CD105 as proposed by the International Society for Cell Therapy (ISCT), hematopoietic stem cell, CD34, CD45, and CD133, cell adhesion molecules, CD29, CD49d, CD44, CD166, CD106, CD54, and CD31, and certain unique markers such as CD13, CD117, HLADR, ABCG2, CD140b, SSEA4, and ALDH using flowcytometry.

The dotplots of flowcytometric analysis of these diversified markers for omentum fat (Figures 2 and 3) were illustrated. The comparison of surface antigenic expression profiles of cultured MSCs at early and later passages of these aforesaid sources in DMEM-LG (Figure 4), Alpha-MEM (Figure 5), DMEM-F12 (Figure 6), and DMEM-KO (Figure 7) were comprehended graphically in the form of Mean ± SEM with its statistical significance. Despite the similarities in most of the markers in all media, some inconsistent expressions were identified in markers such as CD117, CD54, and CD49d.

For ease of comparative expression analysis, the articulation of the marker expression was made according to previously specified range and category (Table 1) such as: Remarkable (90–100%), high expression (75–89%), moderate expression (40–74%), low expression (11–39%) and sparse expression (1–10%) [20].

3.2. Comparative Expression Profiles of Categorized Surface Antigens

3.2.1. Hematopoietic Stem Cells Markers. Hematopoietic stem cell markers such as CD34, CD133, CD45, and HLADR were studied for their expression. It is evident from the analysis that these markers were found to be sparsely expressed in early and later passages of all sources in all media except for a slight higher expression of CD45 at early passage of omentum fat MSCs cultured in DMEM-F12.

3.2.2. Mesenchymal Stem Cells Markers. The study revealed a similar remarkable expression of these markers CD90, CD105, and CD73 throughout the long term culture condition of all sources in all media, as defined by ISCT [21]. The SSEA4 showed a sparse expression of all sources in all media. However, an increase in expression was identified in DMEM-LG of all sources.

3.2.3. Cell Adhesion Molecules/Surface Enzymes/Side Population. MSCs innate property of transendothelial migration

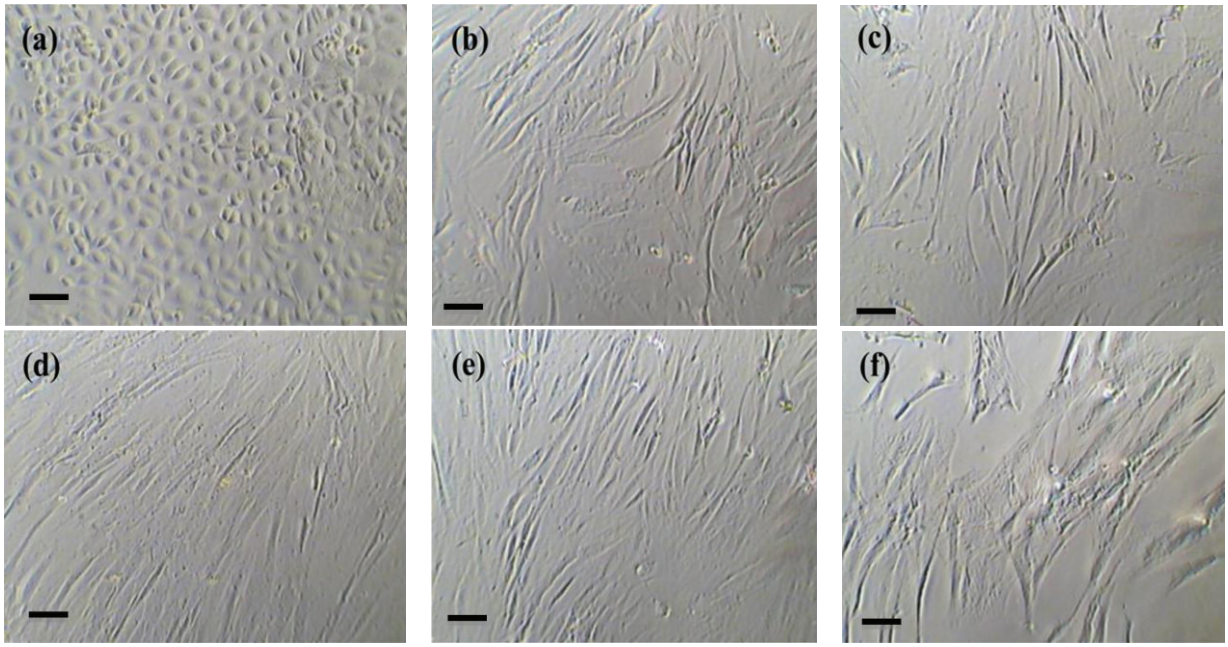


Figure 1: Morphology of cultured mesenchymal stem cells. Representative photomicrographs showing morphology of mesenchymal stem cells at early passage (P3) derived from omentum fat (a), subcutaneous fat (b), and bone marrow (c) and at late passage (P20) derived from omentum fat (d); subcutaneous fat (e) and bone marrow (f), (Scale-20 μm; Magnification at 20x).

and homing is indebted to the presence of cell adhesion molecules. Current study includes the following cell adhesion molecules: CD29, CD44, CD166, CD106, CD31, CD49d, and CD54, surface enzymes: CD13 and ALDH and side population: ABCG2 and CD117. The sparse expressions of CD106 and CD31 were found in all sources of all media. Similarly, the ALDH expression was found to be lower in all sources of all media except for its moderate expression in both early and later passages of DMEM-F12 for OF. Unlike the expressions of MSC specific markers, the study showed a varying expression in the markers such as CD54, CD49d, and CD117 in early and later passages of all sources in all media, except for its similar expressions of CD54 in early passages of SF and BM, CD 49d in early passages of OF and CD117 in both early and later passages of SF in all media as illustrated (Table 1). On the other hand, the markers such as CD29, CD44, CD166, and CD13 showed remarkable expressions throughout the long term culture condition except for its slight decrease in its expression of CD13 at early passage of OF at Alpha-MEM and DMEM-KO.

4. Discussion

Over the past 6 years, there are several reports on variations exhibited in the characterization of cell surface markers at different stages of MSC culture from bone marrow and adipose tissue [3, 10, 13, 20, 22–26], further making cell surface marker expression study an arduous task. The expression profile was identified to change as a function of time in passage and plastic adherence [27, 28]. Hence, there is a lack of thorough understanding of the mechanism underlying stem cell renewal and its functional differentiation. Although, the maintenance of stemness property such as cell proliferation and cell differentiation under long term cultures of different media was studied [9, 19, 29, 30], identification of prospective definitive markers specific to MSCs of the existing contemporary therapeutic adult postnatal sources such as bone marrow and adipose tissue remains elusive. Further, it has not yet been studied what happens to these markers under long term cultures with different media. This formed the basis of our present study.

The impact of different culture media (DMEM-LG, Alpha-MEM, DMEM-F12, and DMEM-KO) exposed to long term culture conditions of MSCs obtained from OF, SF, and BM was analysed in detail for diversified surface antigen until P20 for bone marrow samples and until P25 for adipose tissue samples. Out of the four samples processed from each source, unlike omentum fat and subcutaneous fat, only one sample of bone marrow could grow beyond P20 and rest lost its potential to grow beyond P15. However, on the other hand, the influence of different culture media has not lead to marker expression variations in both early and later passages of all sources except for certain exhibited marker variations seen in CD49d, CD54, and CD117.

These marker variations exhibited in our results in all media was similar to the results of certain previously published [3, 10, 13, 23, 24, 26]. However, further in-depth research is of utmost importance on the cell adhesion molecule that interacts with the cytoskeleton of MSC. This might enhance the understanding of MSC as an instrument of curative therapeutics involved in the applications of neovascularisations, angiogenesis, and treatment of other vascular disorders. This is due to the fact that CD117 serves as an important growth factor that plays a vital role in cell

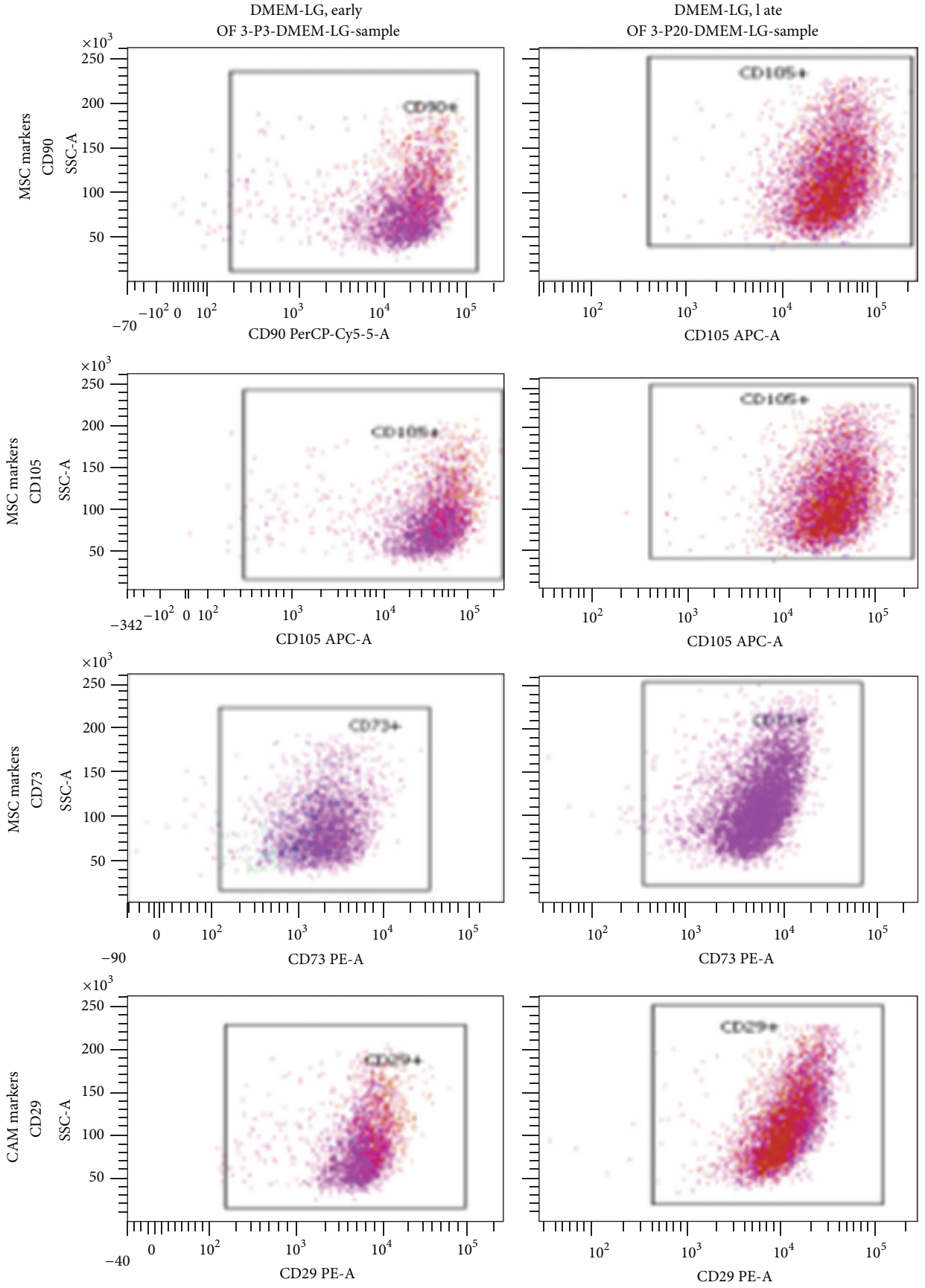


Figure 2: Continued.

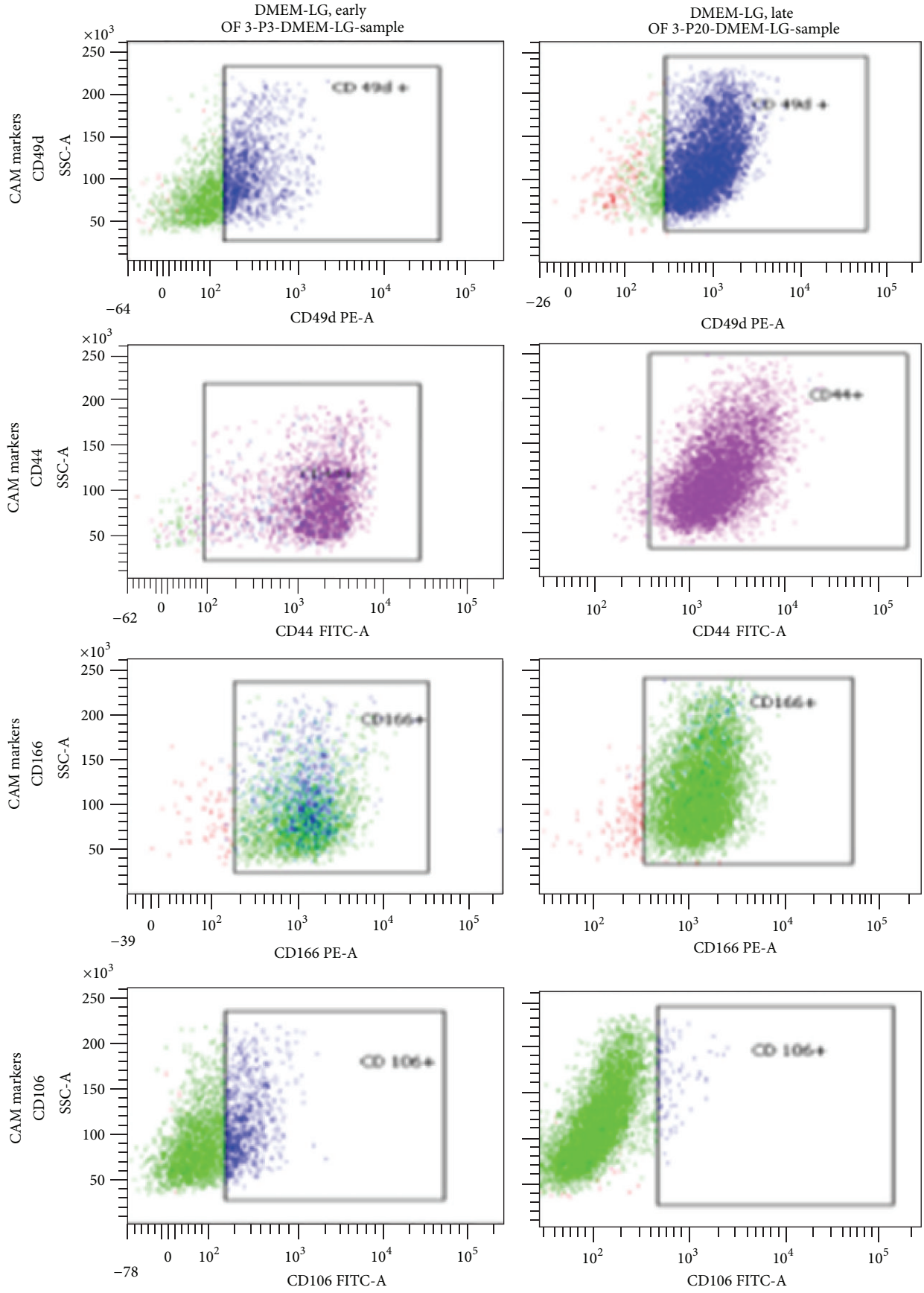


Figure 2: Continued.

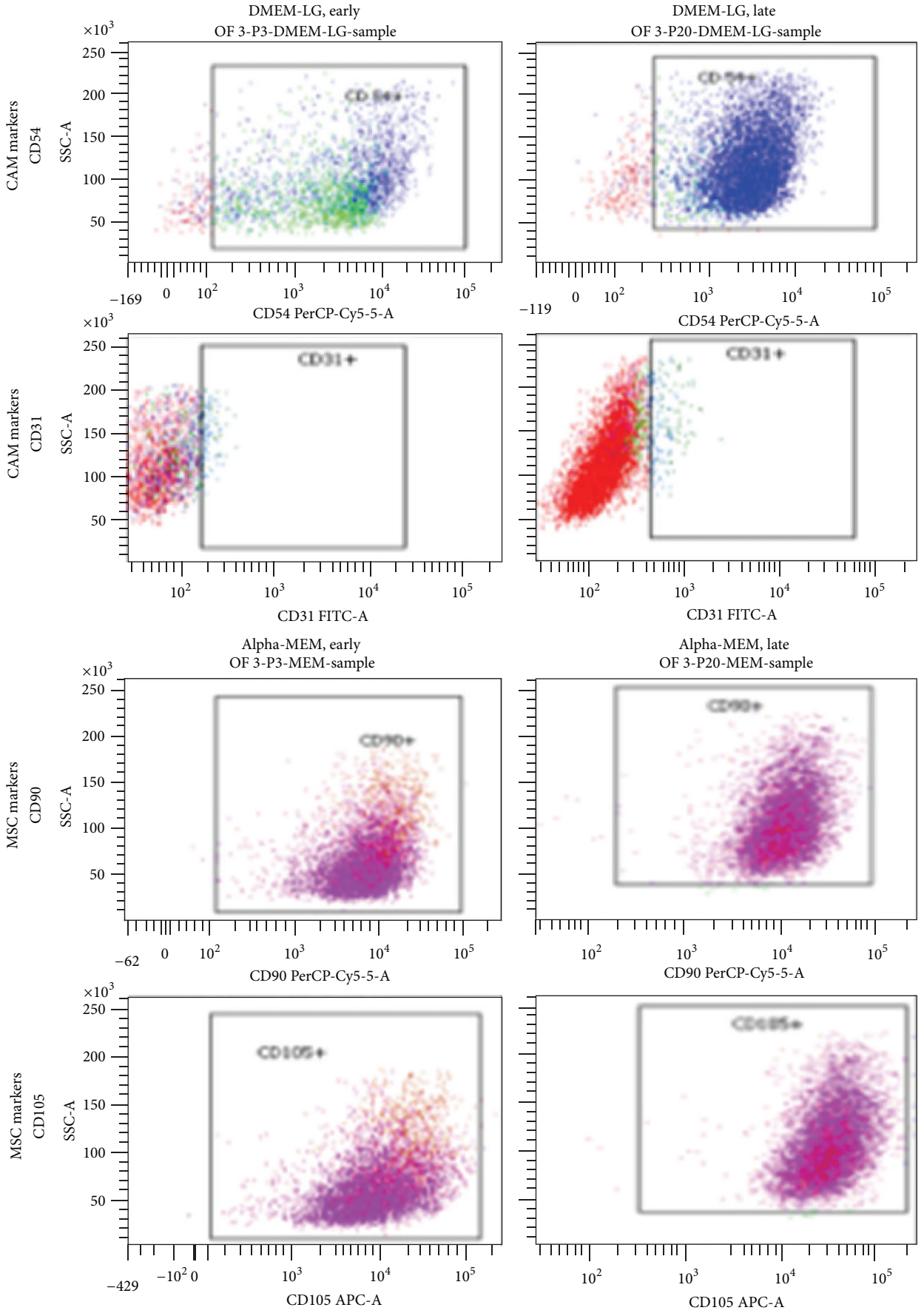


Figure 2: Continued.

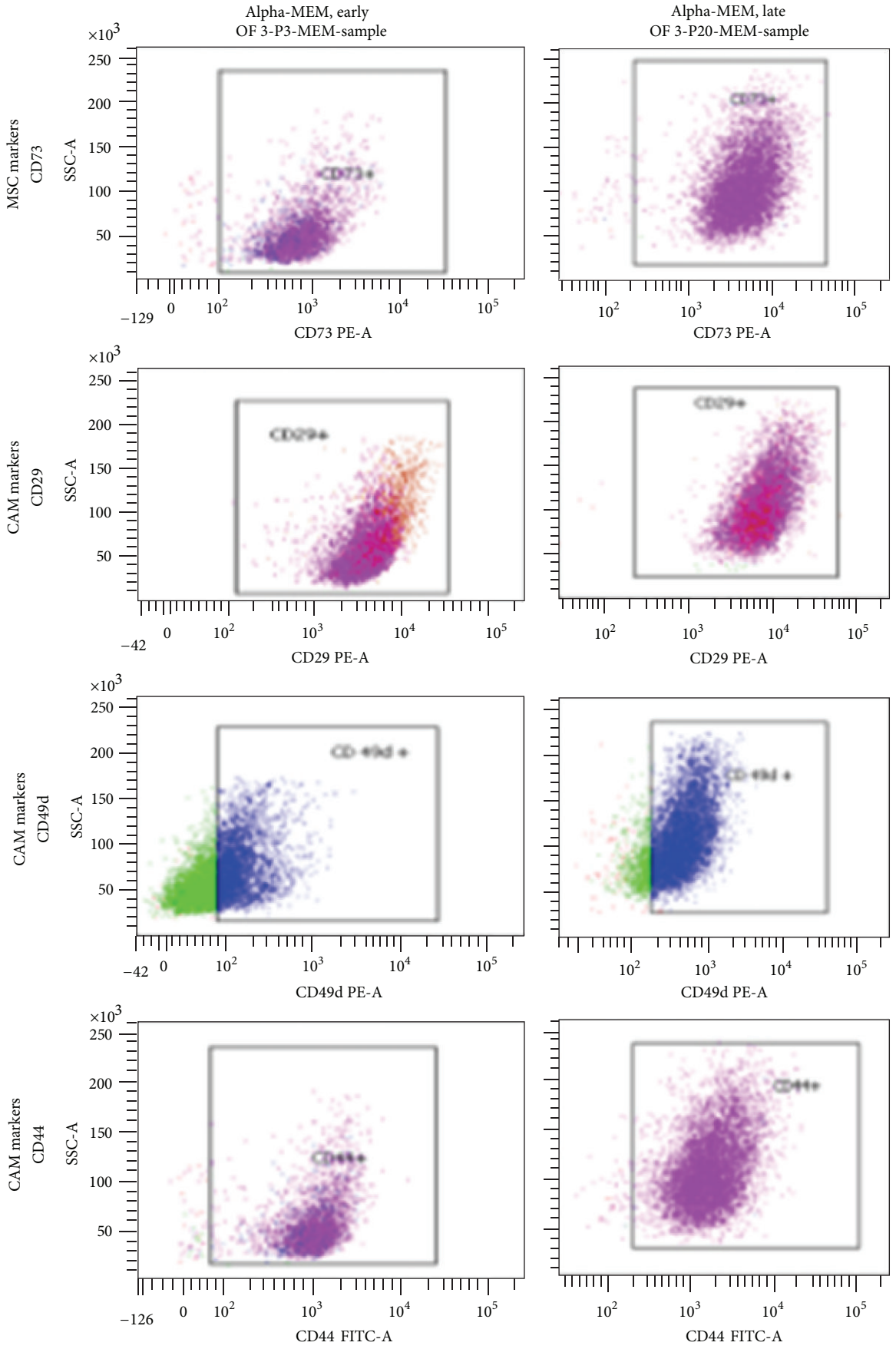


Figure 2: Continued.

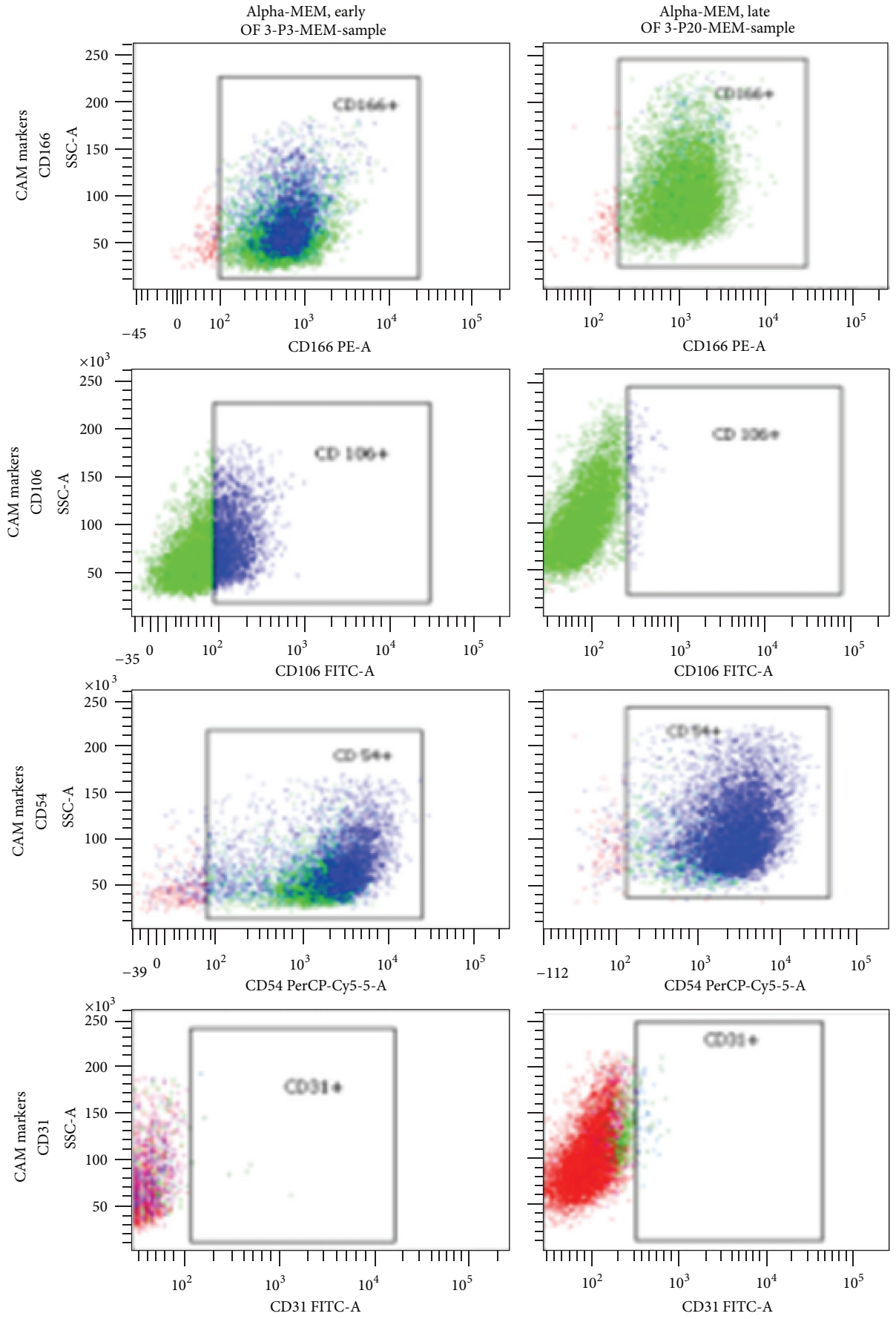


Figure 2: Continued.

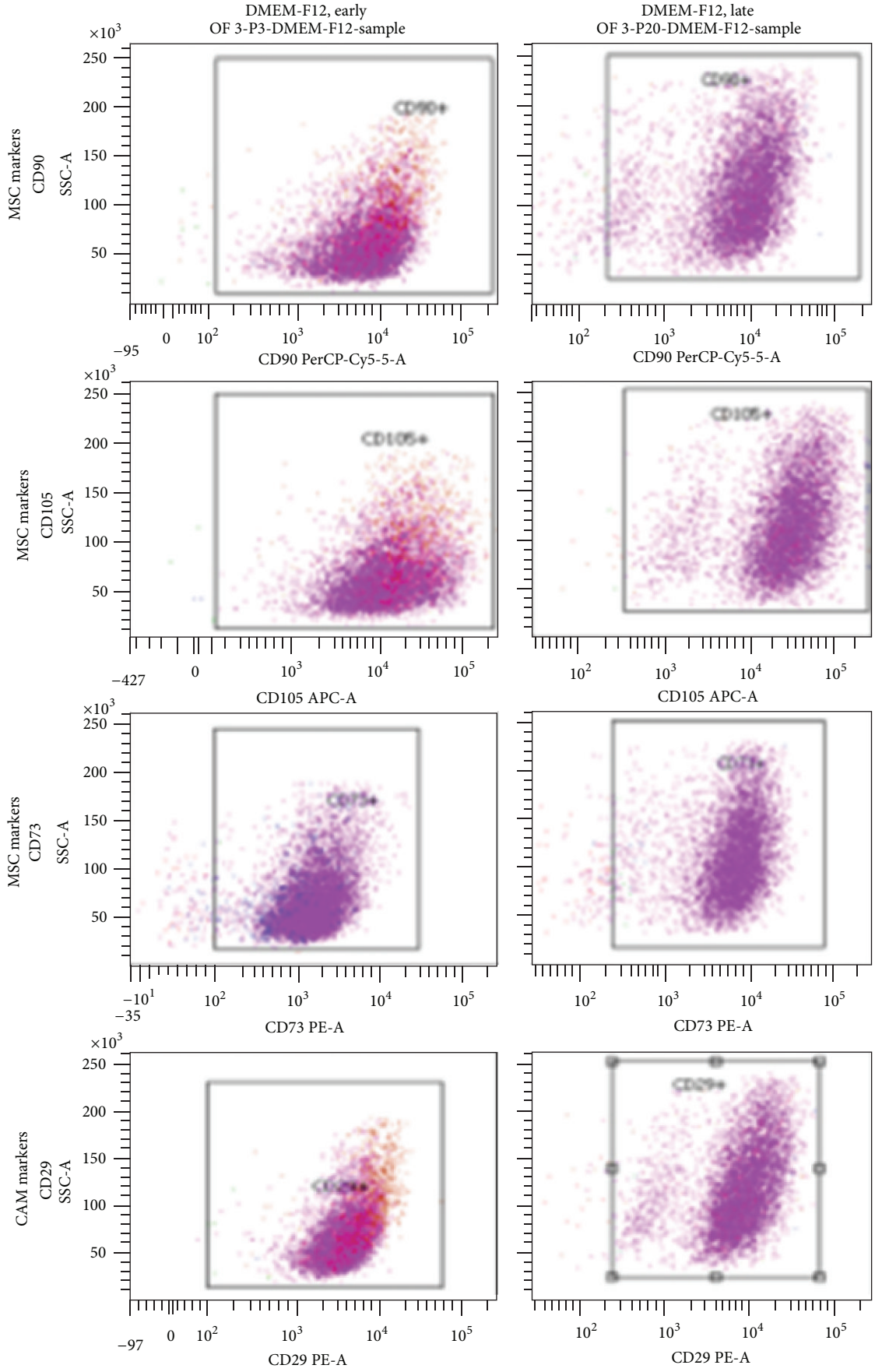


Figure 2: Continued.

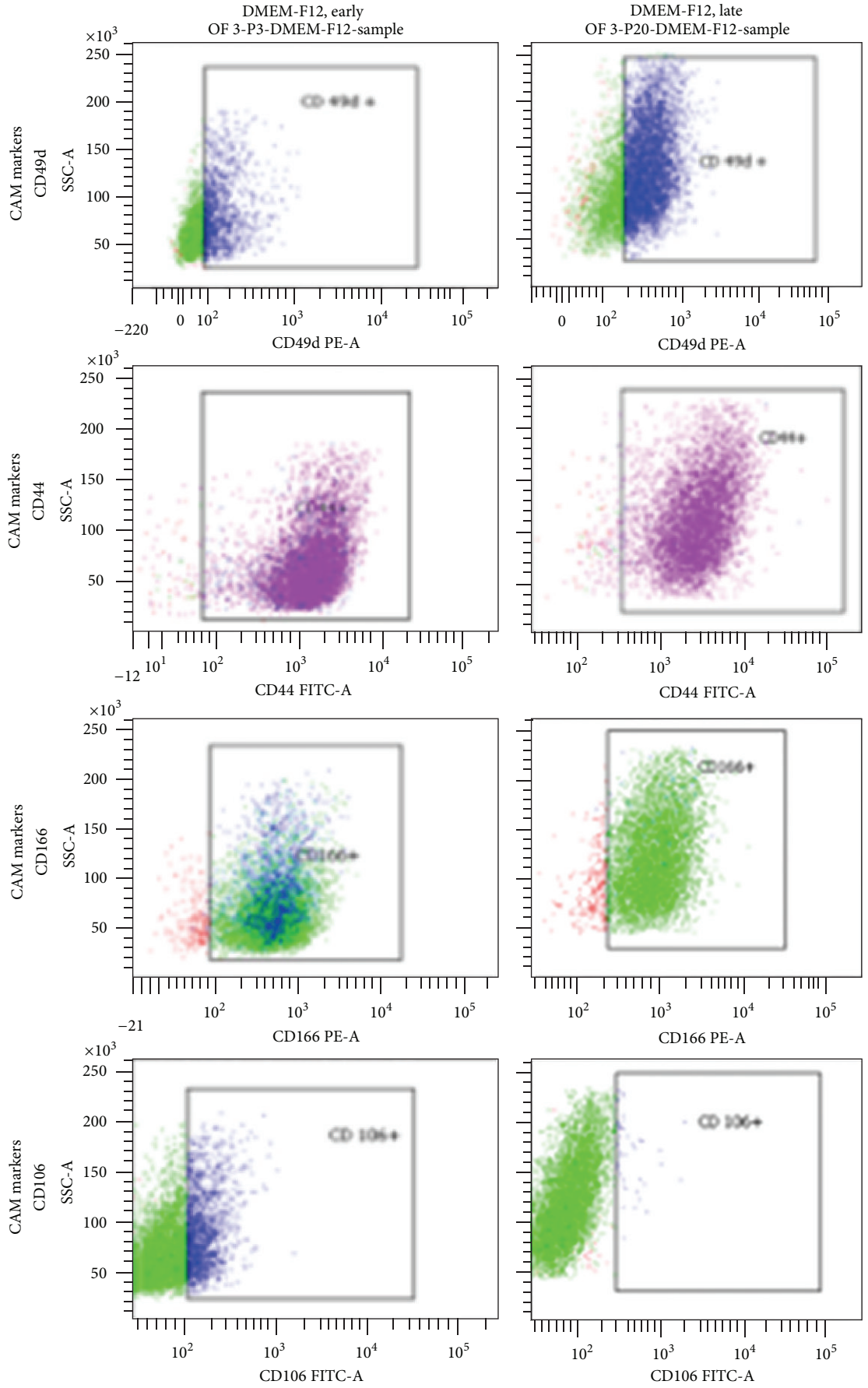


Figure 2: Continued.

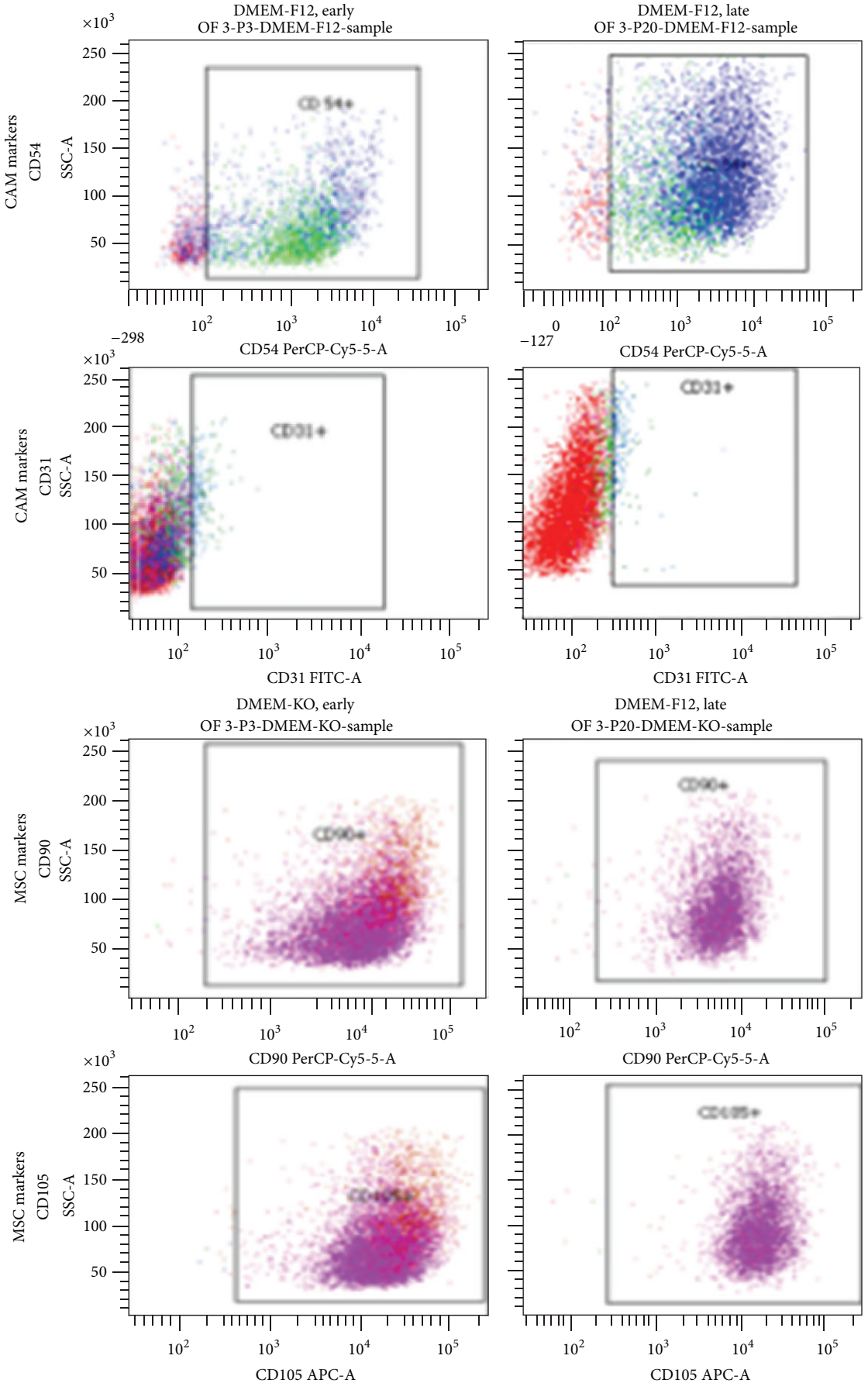


Figure 2: Continued.

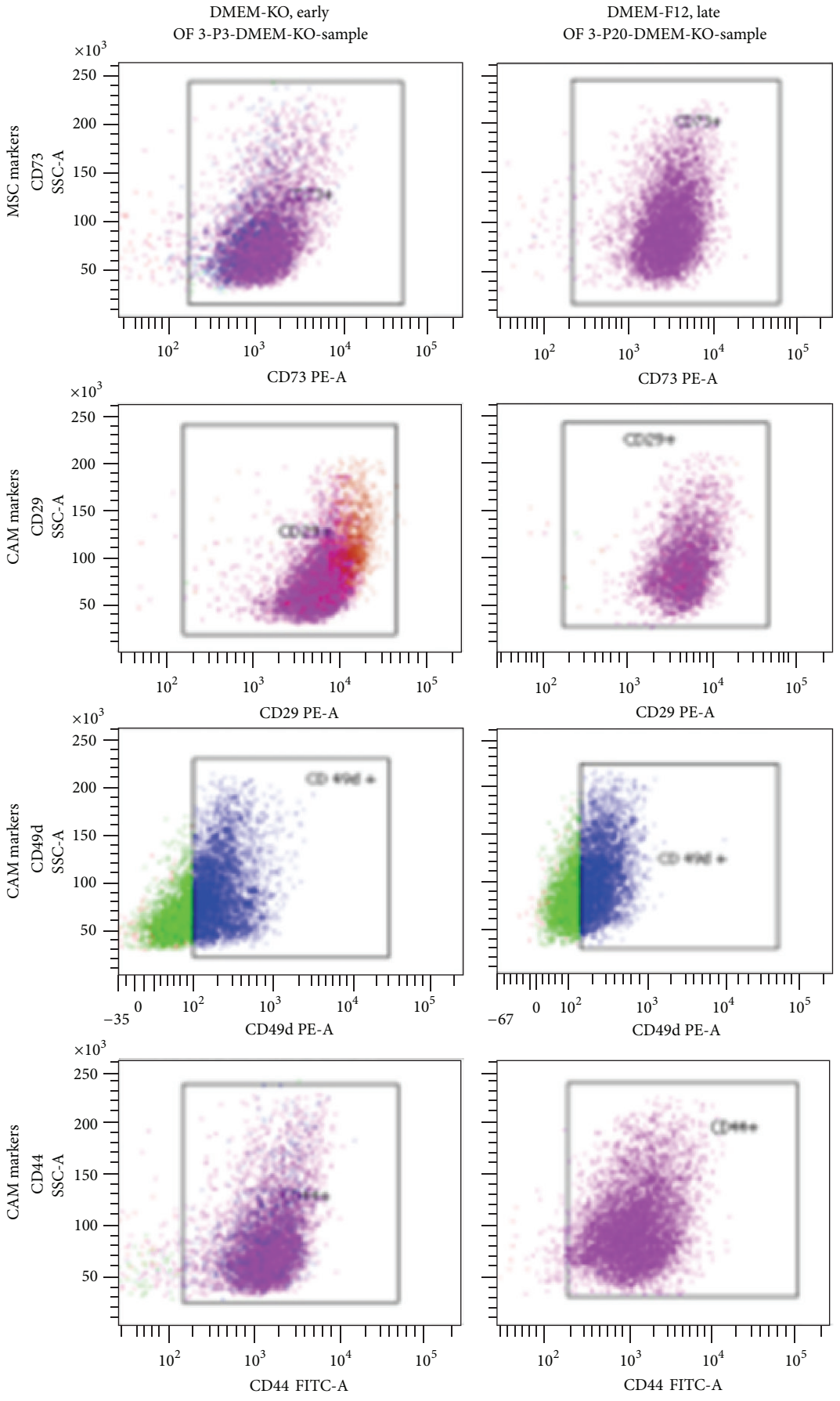


Figure 2: Continued.

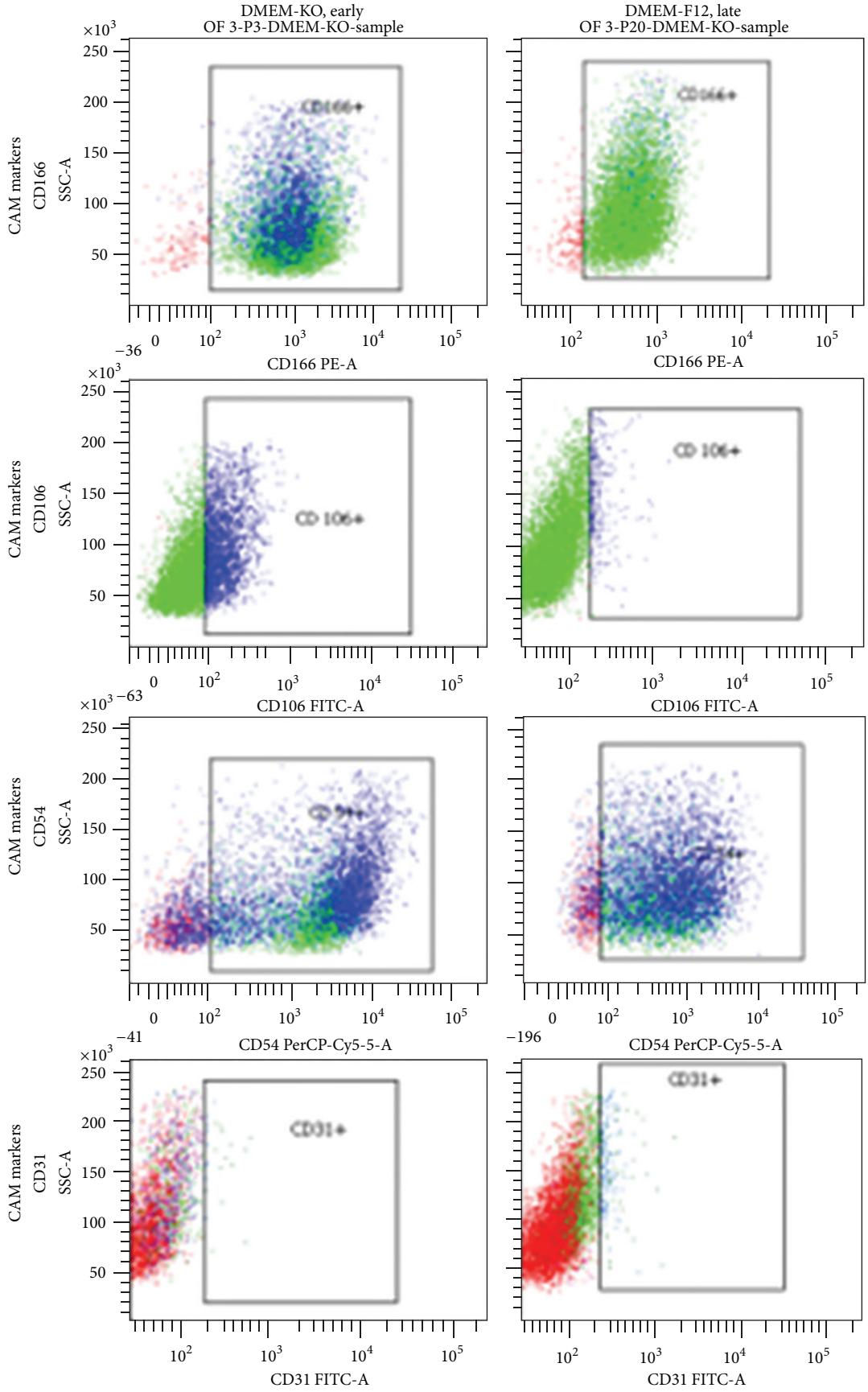


Figure 2: Flowcytometric characterization of MSCs and CAM of omentum fat derived MSC. Characterization of omentum fat derived MSC at early (P3) and late (P20) passages in DMEM-LG, Alpha-MEM, DMEM-F12, and DMEM-KO for CD90, CD105, CD73, CD29, CD49d, CD44, CD166, CD106, CD54, and CD31 using flowcytometry.

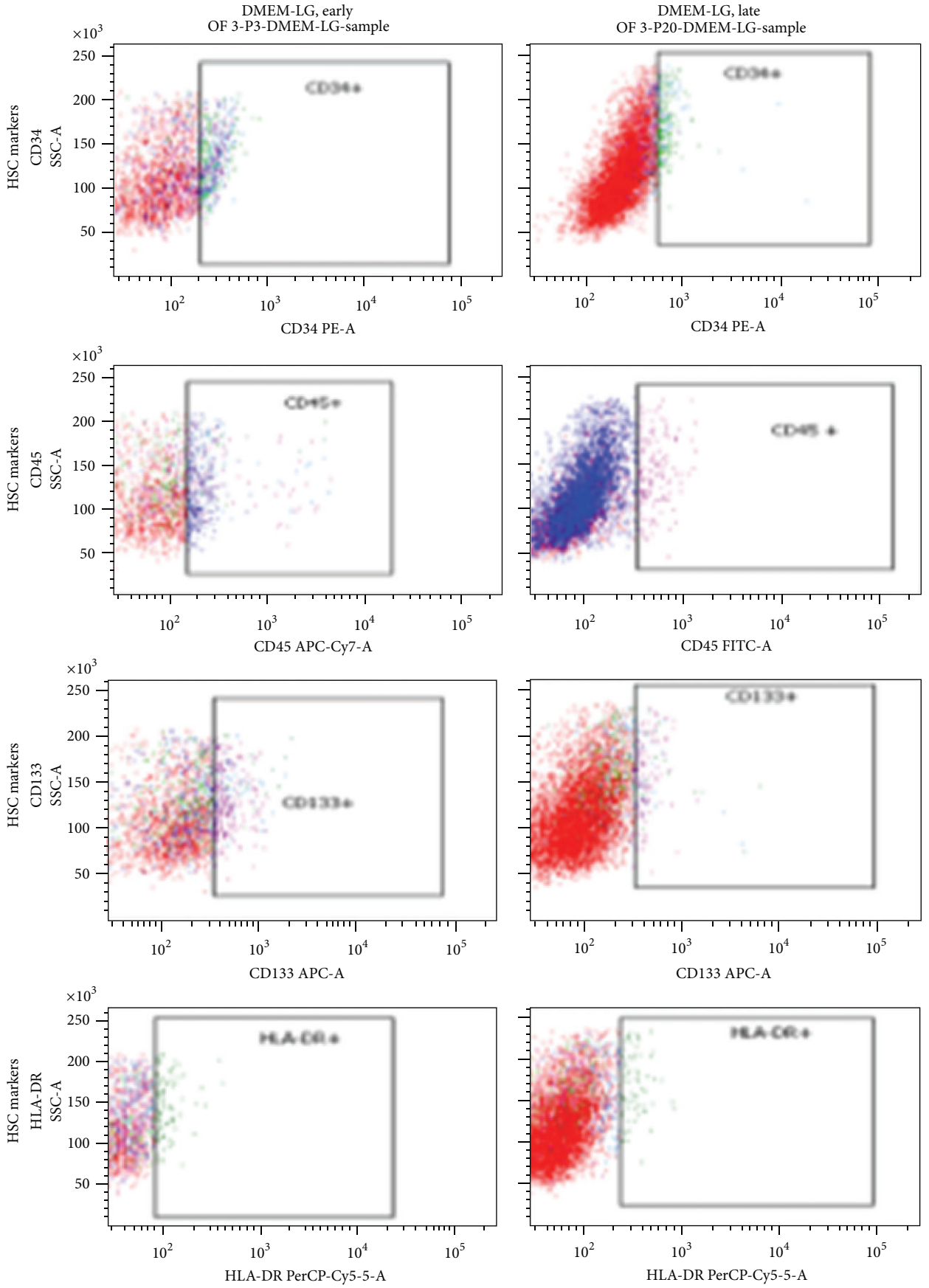


Figure 3: Continued.

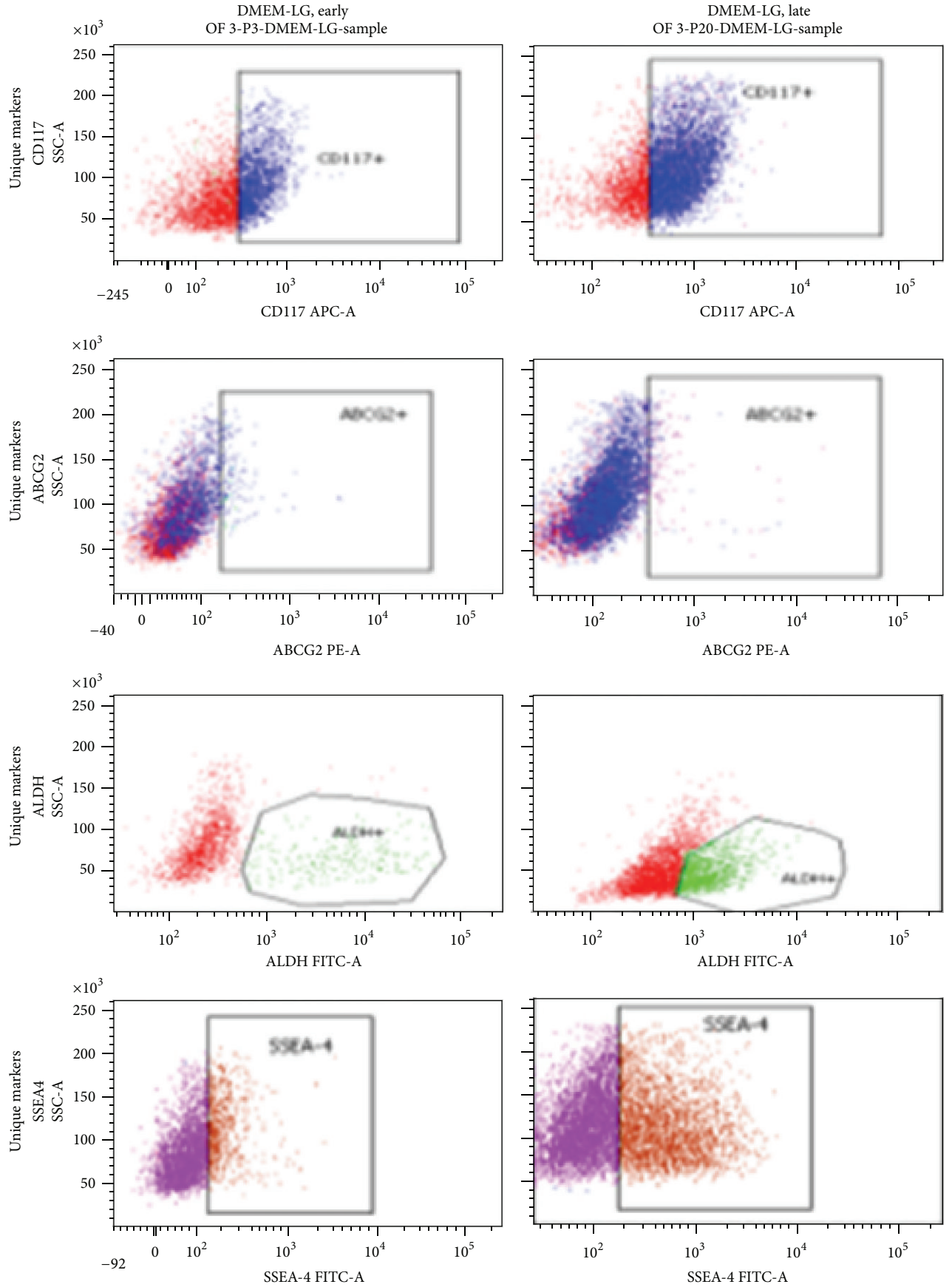


Figure 3: Continued.

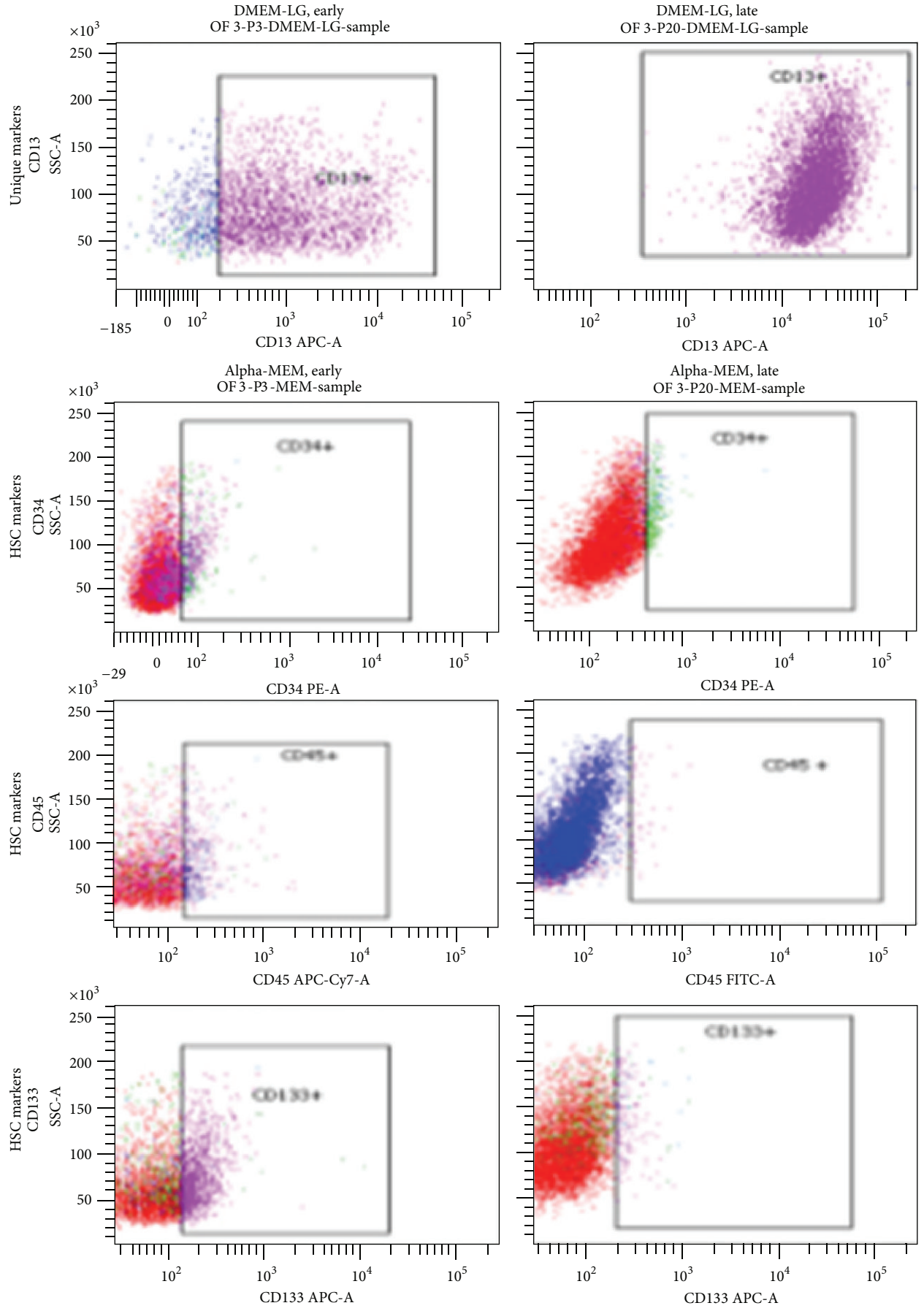


Figure 3: Continued.

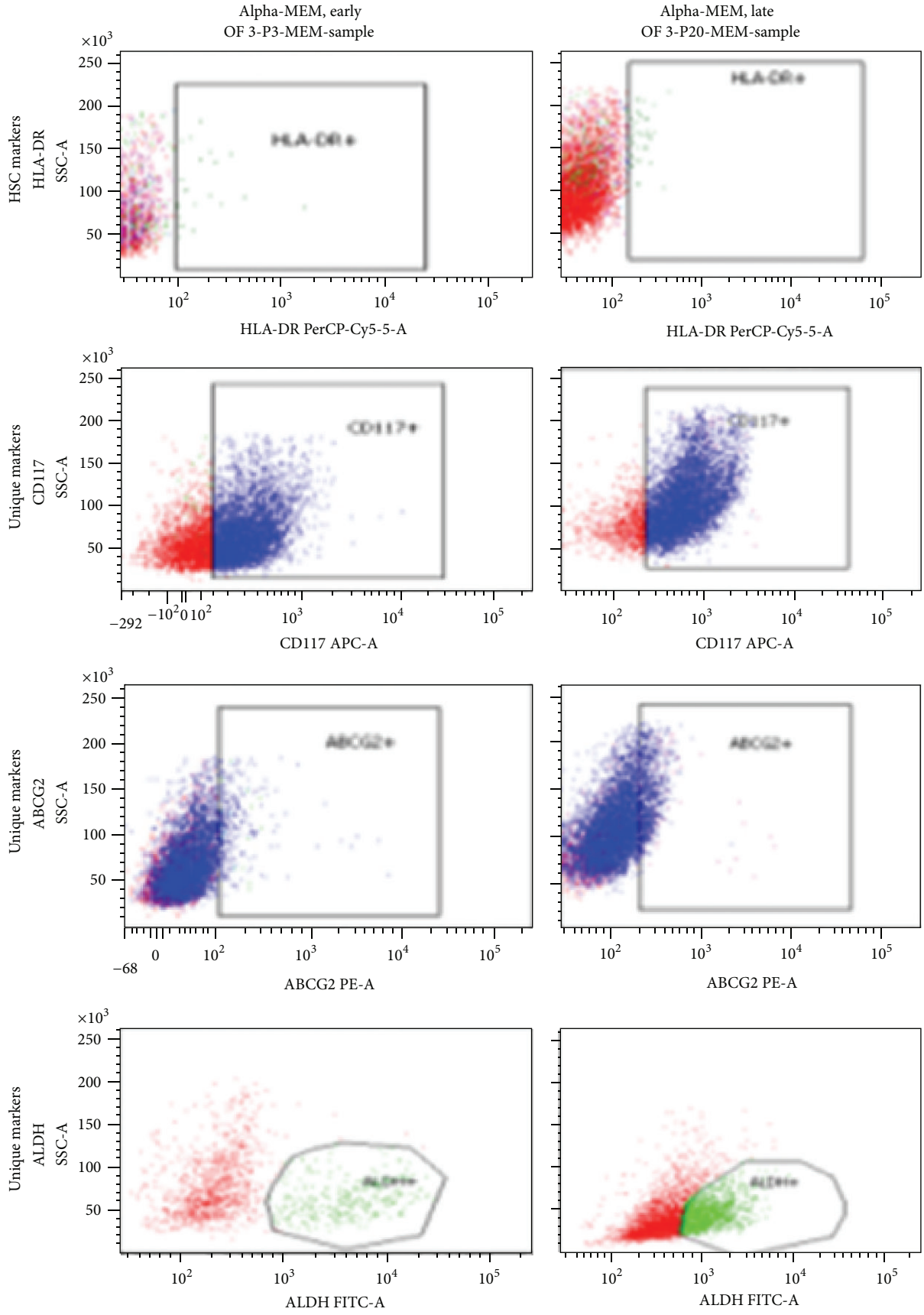


Figure 3: Continued.

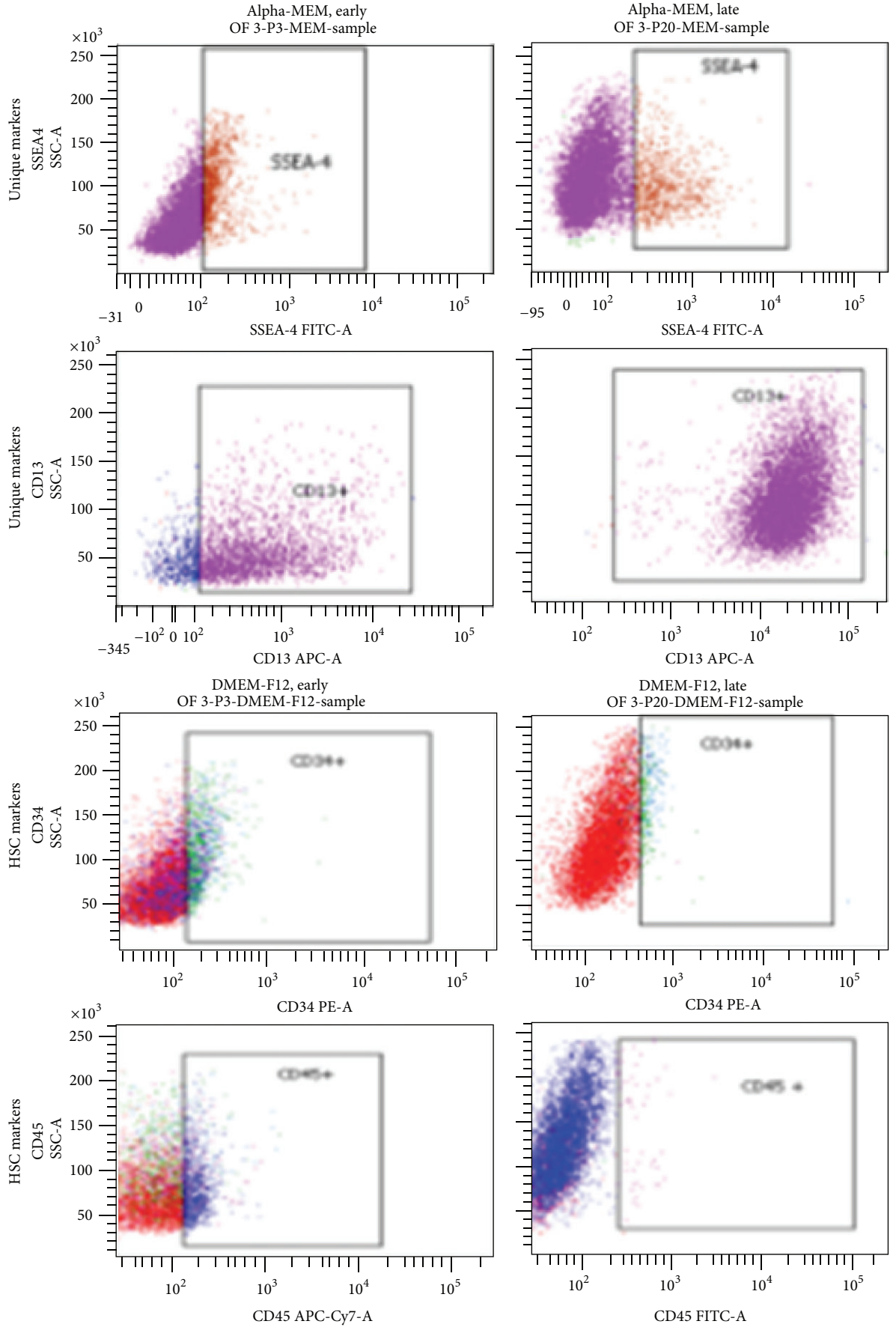


Figure 3: Continued.

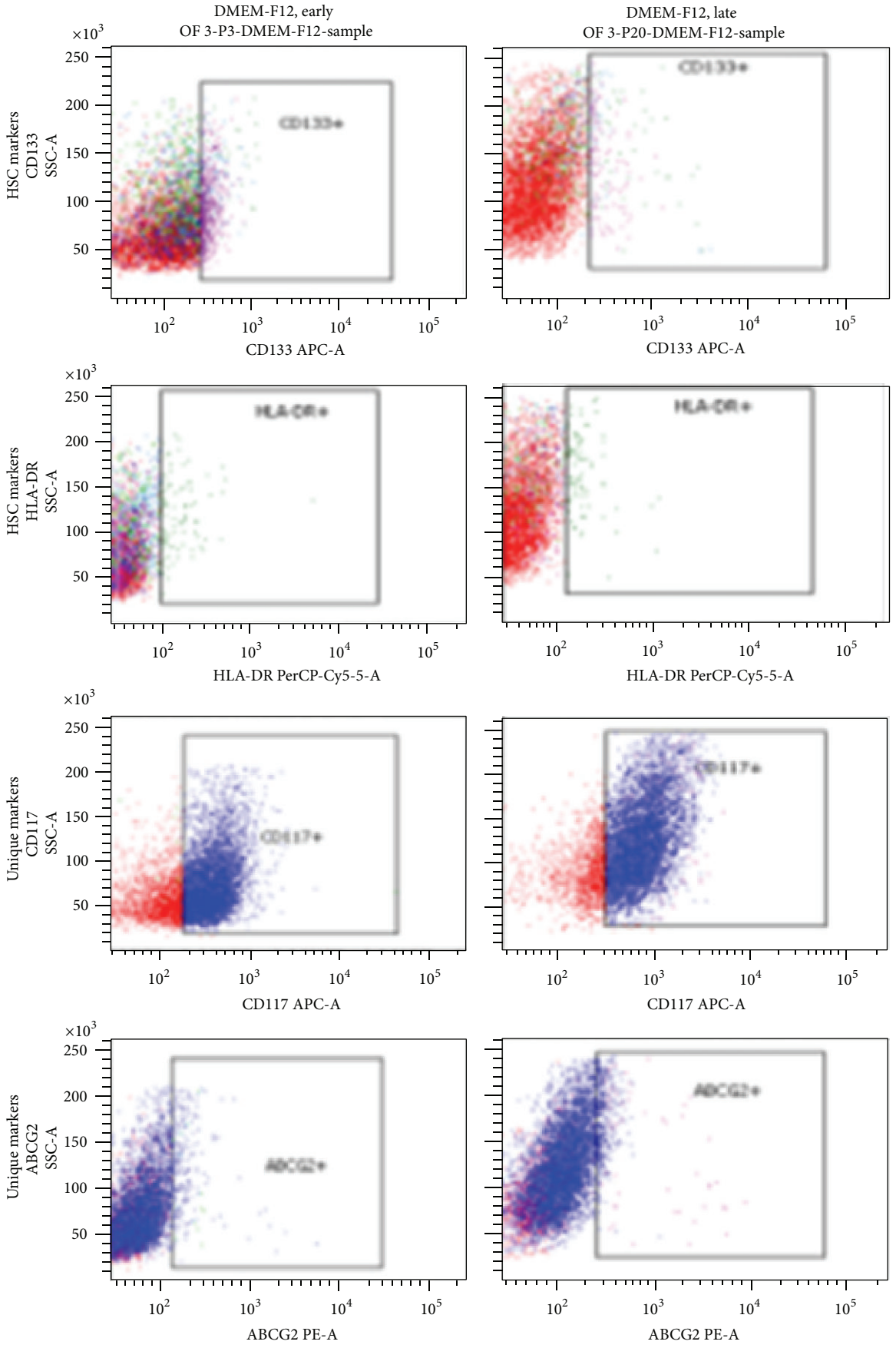


Figure 3: Continued.

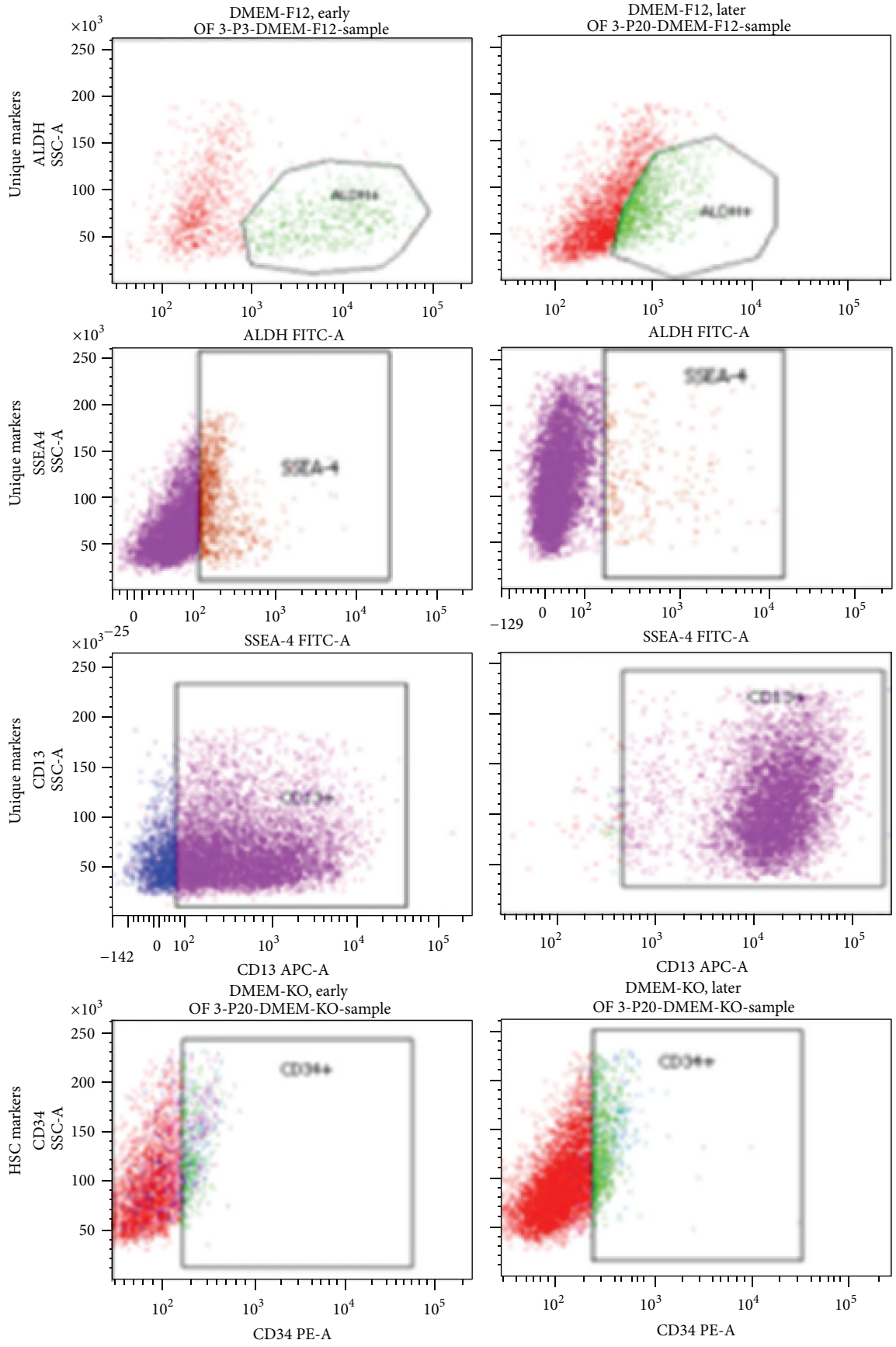


Figure 3: Continued.

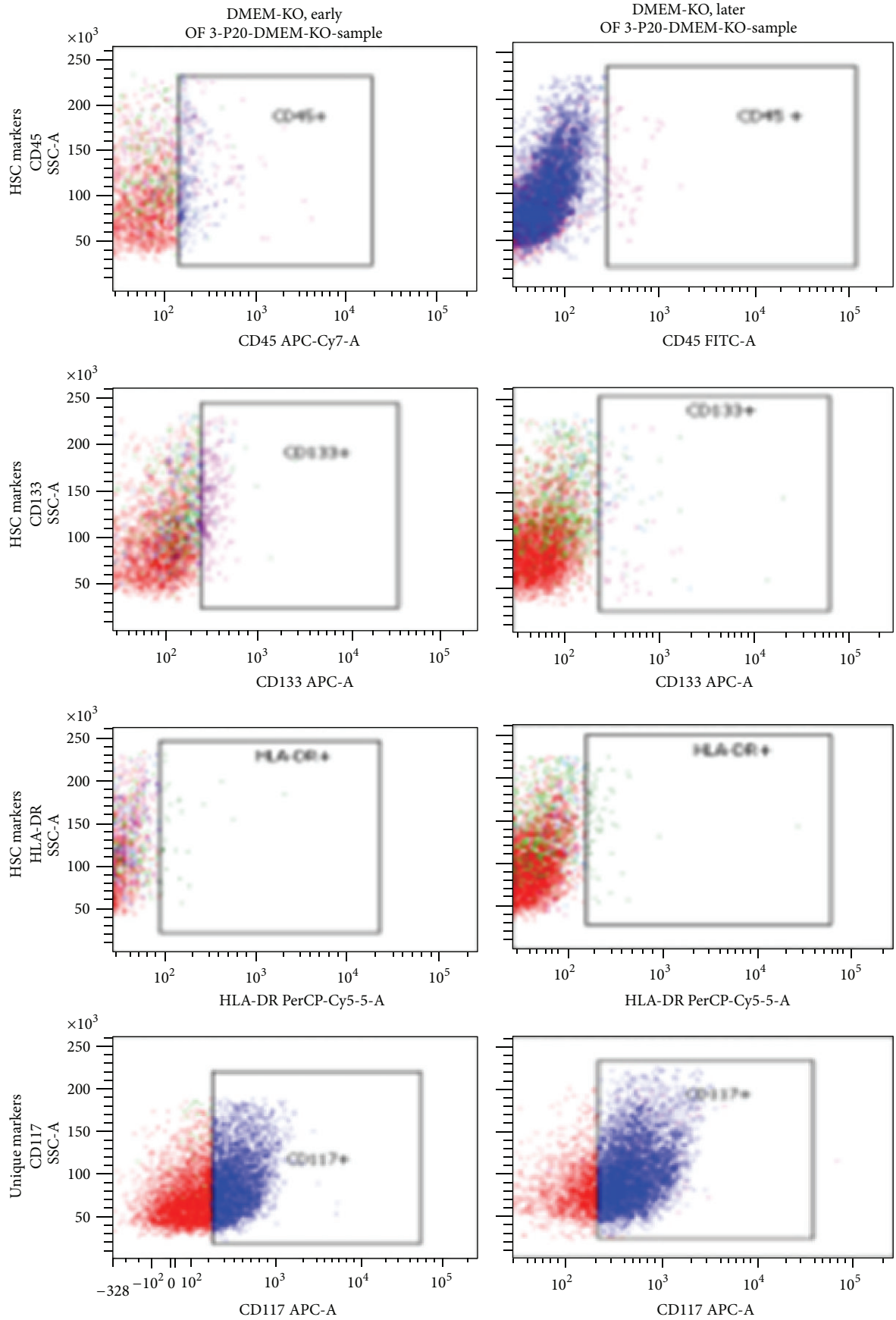


Figure 3: Continued.

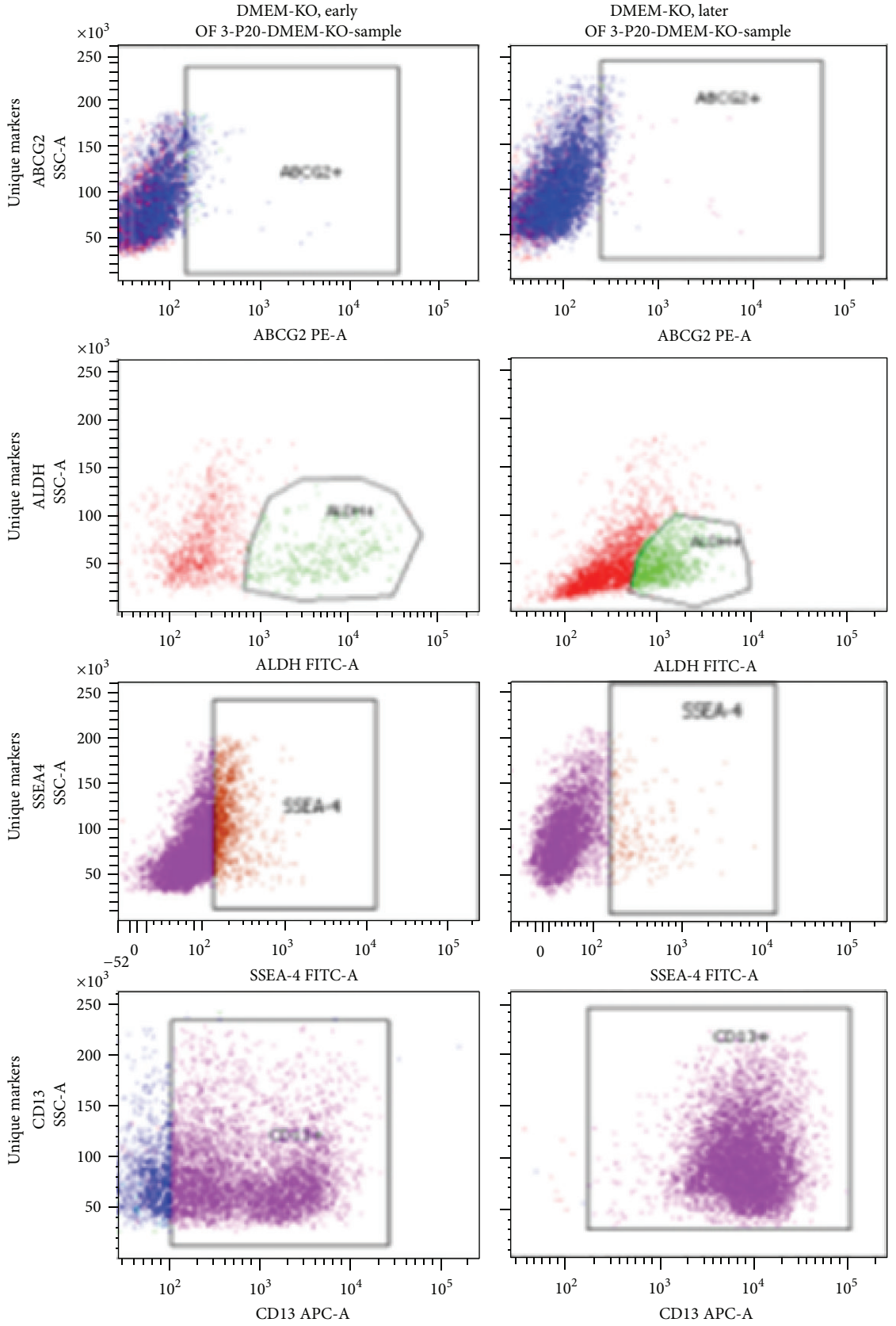


Figure 3: Flowcytometric characterization of HSC and unique markers of omentum fat derived MSC. Characterization of omentum fat derived MSC at early (P3) and late (P20) passages in DMEM-LG, Alpha-MEM, DMEM-F12, and DMEM-KO for CD34, CD45, CD133, HLA-DR, CD117, ABCG2, ALDH, SSEA4, and CD13 using flowcytometry.

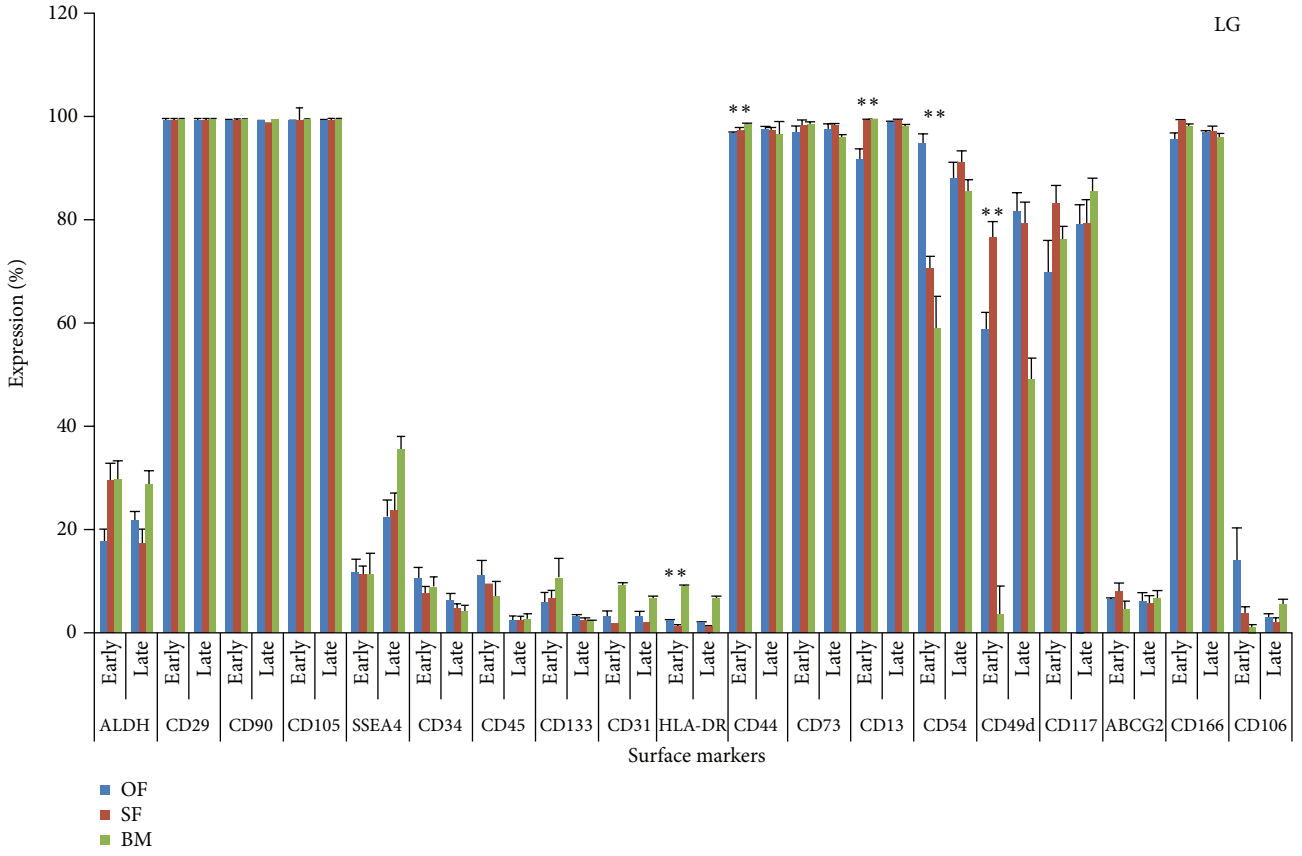


Figure 4: Marker expression profiling of MSCs cultured in DMEM-Low Glucose (DMEM-LG). Comparative Surface antigenic profiling of MSCs derived from omentum fat, subcutaneous fat, and bone marrow at early (P3) and late (P20) passages cultured in DMEM-LG.

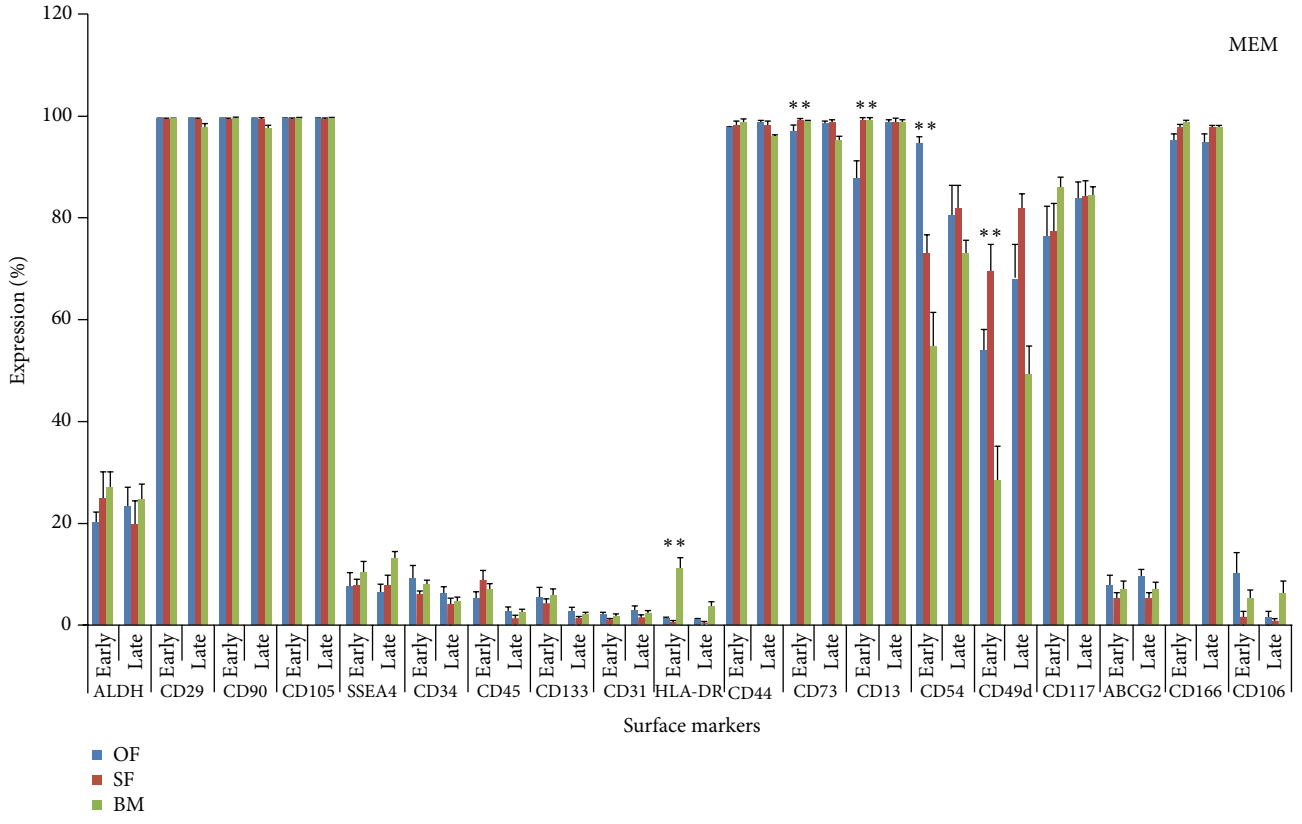


Figure 5: Marker expression profiling of MSCs cultured in Alpha-MEM. Comparative Surface antigenic profiling of MSCs derived from omentum fat, subcutaneous fat, and bone marrow at early (P3) and late (P20) passages cultured in Alpha-MEM.

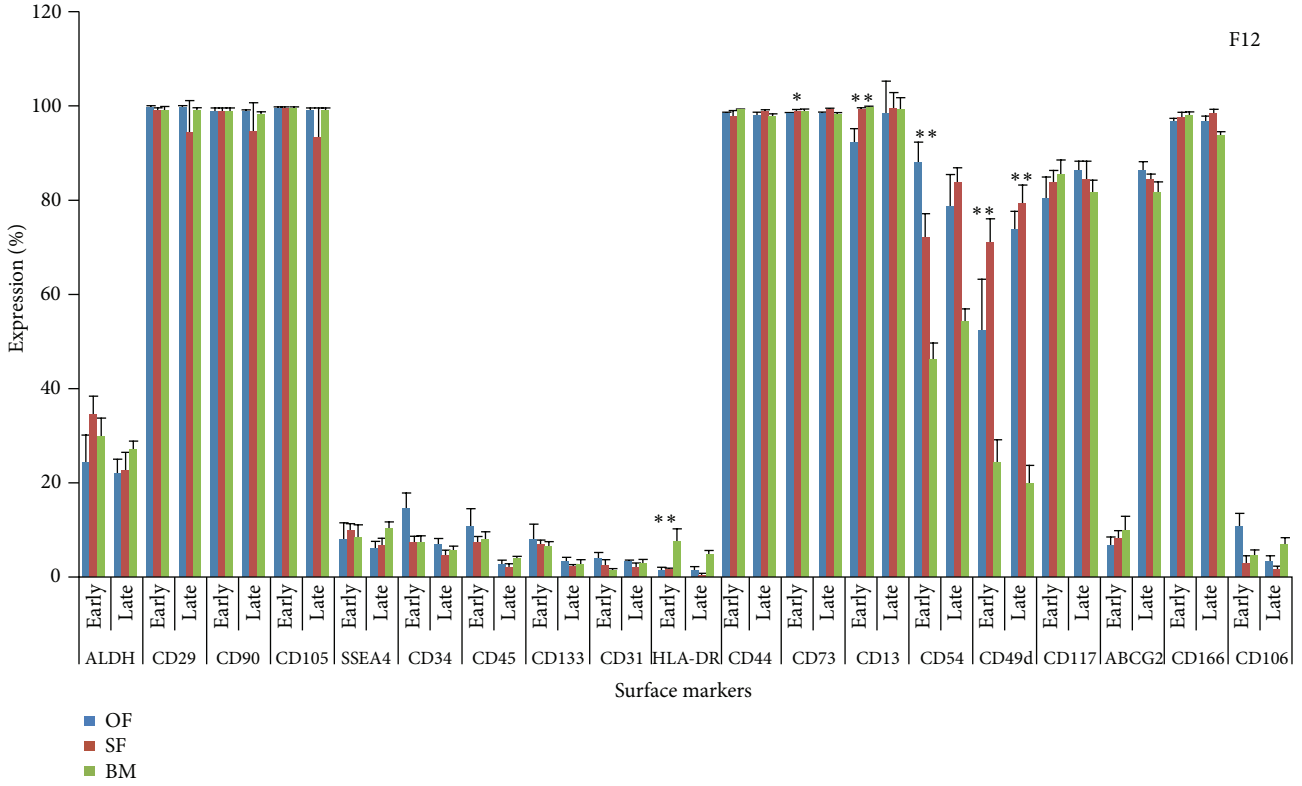


Figure 6: Marker expression profiling of MSCs cultured in DMEM-F12. Comparative Surface antigenic profiling of MSCs derived from omentum fat, subcutaneous fat, and bone marrow at early (P3) and late (P20) passages cultured in DMEM-F12.

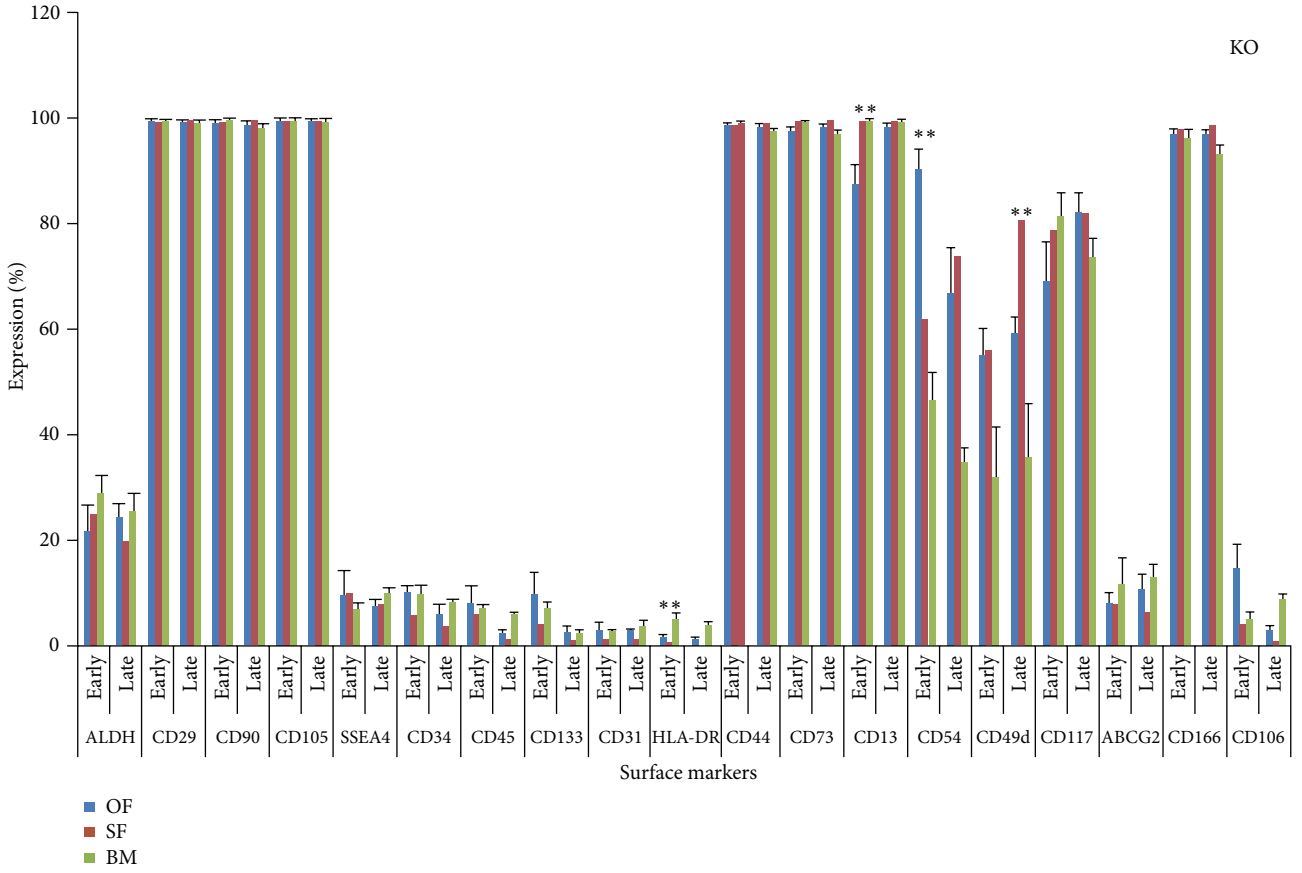


Figure 7: Marker expression profiling of MSCs cultured in DMEM-KO. Comparative Surface antigenic profiling of MSCs derived from omentum fat, subcutaneous fat, and bone marrow at early (P3) and late (P20) passages cultured in DMEM-KO.

Table 1: Comparative analysis of ranges of marker expression profile.

MARKERS	PASSAGE	OF				SF				BM			
		LG	MEM	F12	KO	LG	MEM	F12	KO	LG	MEM	F12	KO
ALDH	EARLY	L	L	M	L	L	L	L	L	L	L	L	L
	LATE	L	L	M	L	L	L	L	L	L	L	L	L
CD29	EARLY	R	R	R	R	R	R	R	R	R	R	R	R
	LATE	R	R	R	R	R	R	R	R	R	R	R	R
CD90	EARLY	R	R	R	R	R	R	R	R	R	R	R	R
	LATE	R	R	R	R	R	R	R	R	R	R	R	R
CD105	EARLY	R	R	R	R	R	R	R	R	R	R	R	R
	LATE	R	R	R	R	R	R	R	R	R	R	R	R
SSEA4	EARLY	S	S	S	S	L	S	S	S	L	S	S	S
	LATE	L	S	S	S	L	S	S	S	L	L	S	S
CD34	EARLY	S	S	L	S	S	S	S	S	S	S	S	S
	LATE	S	S	S	S	S	S	S	S	S	S	S	S
CD45	EARLY	S	S	L	S	S	S	S	S	S	S	S	S
	LATE	S	S	S	S	S	S	S	S	S	S	S	S
CD133	EARLY	S	S	S	S	S	S	S	S	S	S	S	S
	LATE	S	S	S	S	S	S	S	S	S	S	S	S
CD31	EARLY	S	S	S	S	S	S	S	S	S	S	S	S
	LATE	S	S	S	S	S	S	S	S	S	S	S	S
HLA-DR	EARLY	S	S	S	S	S	S	S	S	S	S	S	S
	LATE	S	S	S	S	S	S	S	S	S	S	S	S
CD44	EARLY	R	R	R	R	R	R	R	R	R	R	R	R
	LATE	R	R	R	R	R	R	R	R	R	R	R	R
CD73	EARLY	R	R	R	R	R	R	R	R	R	R	R	R
	LATE	R	R	R	R	R	R	R	R	R	R	R	R
CD13	EARLY	R	H	R	H	R	R	R	R	R	R	R	R
	LATE	R	R	R	R	R	R	R	R	R	R	R	R
CD54	EARLY	R	R	H	R	M	M	M	M	M	M	M	M
	LATE	H	H	H	M	R	H	H	M	M	M	M	L
CD49d	EARLY	M	M	M	M	H	M	M	M	H	L	L	L
	LATE	R	M	H	M	H	H	H	H	S	M	L	L
CD117	EARLY	M	H	H	M	H	H	H	H	M	H	H	H
	LATE	H	H	H	H	H	H	H	H	H	H	H	M
ABCG2	EARLY	S	S	S	S	S	S	S	S	S	S	S	L
	LATE	S	S	S	S	S	S	S	S	S	S	S	L
CD166	EARLY	R	R	R	R	R	R	R	R	R	R	R	R
	LATE	R	R	R	R	R	R	R	R	R	R	R	R
CD106	EARLY	L	S	S	S	S	S	S	S	S	S	S	S
	LATE	S	S	S	S	S	S	S	S	S	S	S	S

Expression ranges: R: remarkable (>90%); H: high (75–89%); M: moderate (40–74%); L: low (11–39%); S: sparse (<10%). Early (P1, P3, and P5), late (P9, P12, P15, and P20); OF: omentum fat; SF: subcutaneous fat; BM: bone marrow; LG: low glucose; MEM: minimum essential media; KO: knock-out.

survival, proliferation, and differentiation [31]. CD54 is an endothelial and leukocyte-associated transmembrane protein long known for its importance in stabilizing cell-cell interactions and facilitating leukocyte endothelial transmigration. As a result of these binding characteristics, CD54 has classically been assigned the function of intercellular adhesion [32]. Similarly, CD49d along with the higher expression of its counterpart, CD29, together forming VLA4 is supposed to play a role in mobilization and homing [20, 23, 33, 34].

However, we found a contrary expression pattern in the markers such as CD49d and CD106 when compared to the previously published reports. The literature reports that adipose tissue MSCs expresses CD49d and not CD106, whereas bone marrow MSCs expresses CD106 but not CD 49d. This reciprocal expression pattern is interesting because CD106 is the cognate receptor of CD49d and both these molecules represent part of a receptor-ligand pair that has an important role in hematopoietic stem cell homing to and mobilization from bone marrow [35–37]. However, our study revealed a

varying expression pattern of CD49d in all sources including bone marrow and a similar sparse expression of CD106 was obtained in all sources, including adipose tissue. Further research on the discrepancies related to CD49, CD29, and CD106 might throw light as these molecules together play a vital interactive role. Besides, CD13 was also identified to be a potent marker that plays a vital role in angiogenesis and migration [20, 38].

Besides, the similar remarkable expression patterns of cell surface markers such as CD90, CD105, CD73, CD29, CD44, CD166, and CD13 and negative expressions of CD34, CD45, CD133, CD31, and HLADR obtained in our study have been detected with highly consistent patterns of expression on the surface of MSCs by different literatures [3, 22, 24, 39, 40]. This coherence in negative expressions supports the fact that these marker expressions were lost with passage; and subsequent expansion will select for a relatively homogeneous cell population compared with the whole cell population [3, 10, 22, 26, 39–41]. The wealth of knowledge on these markers about their crucial migration and homing evokes that these markers impersonate a CAM that performs these aforesaid functions in MSC. Although the existence and functionality of certain MSC specific markers is known, there is uncertainty among the specificity and functionality of several other markers of MSC, thereby demanding further extensive research.

In addition, this study also analysed for the expression of ALDH and ABCG2 in all sources of tissue and different basal media. This is due to the fact that ALDH isozymes involved in drug resistance and retinoic acid generation would be crucial for the protection of stem cells against toxic endogeneuos and exogeneous aldehydes and for their ability to differentiate [42]; it serves as a key marker for the prediction of therapeutic efficacy of MSCs. Similarly, ABCG2 plays a role in protecting stem cells by increasing their survival capacity and proliferation potential, processes which are fundamental for stem cell maintenance and renewal [43]. In search of a novel marker for prospective isolation of tissue specific MSC, notion of cracking MSCs pluripotency was carried by Gang et al. and coworkers for SSEA-4. In coherence with his reports, our study also showed the expressions of SSEA4. Its expression was identified to be more in omentum fat MSCs and in DMEM-LG medium as compared to other source and media, respectively [44].

5. Conclusion

It was demonstrated that the phenotypic characterization of MSCs remained unchanged irrespective of source of tissue, basal media, and extensive culturing. However, further attention on the markers such as CD49d, CD54, CD117, CD29, and CD106 of each source is suggested. Besides, our data clearly shows that any basal media could be used for culturing these sources.

Although this study resolves the enigma that has been circulating all over on the identity of tissue specific cell surface markers, there is a lot more to be explored in all fronts of phenotypic characterization of stem cells for generation of specific MSCs for the specific condition based cell based therapies.

Abbreviations

Alpha-MEM:	Alpha minimal essentialmedia
BMI:	Body mass index
CD:	Cluster of differentiation
DEAB:	Diethylaminobenzaldehyde
DMEM:	Dulbecco's modified eagle's medium
DMSO:	Dimethyl sulfoxide
FBS:	Fetal bovine serum
KO:	Knock out
LG:	Low glucose
MSC:	Mesenchymal stem cells
PBS:	Phosphate buffered saline.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Acknowledgments

The authors thank Neha Kantawala, gastroenterologist, Lifeline RIGID Hospital, and her surgical team, for providing them with the samples. The authors also thank Ms. Nandhini for her assistance in final proof reading. The authors also thank the donors, who volunteered for the study.

References

[1] E. Fuchs and J. A. Segre, "Stem cells: a new lease on life," *Cell*, vol. 100, no. 1, pp. 143–155, 2000.

[2] M. Körbling and Z. Estrov, "Adult stem cells for tissue repair—a new therapeutic concept?" *The New England Journal of Medicine*, vol. 349, no. 6, pp. 570–582, 2003.

[3] S. Gronthos, D. M. Franklin, H. A. Leddy et al., "Surface protein characterization of human adipose tissue-derived stromal cells," *Journal of Cellular Physiology*, vol. 189, no. 1, pp. 54–63, 2001.

[4] H. Deda, M. C. Inci, A. Kurekçi et al., "Treatment of chronic spinal cord injured patients with autologous bone marrow-derived hematopoietic stem cell transplantation: 1-year follow-up," *Cytotherapy*, vol. 10, no. 6, pp. 565–574, 2008.

[5] M. Locke, J. Windsor, and P. R. Dunbar, "Human adipose-derived stem cells: isolation, characterization and applications in surgery," *ANZ Journal of Surgery*, vol. 79, no. 4, pp. 235–244, 2009.

[6] D. Rubio, J. Garcia-Castro, M. C. Martin et al., "Spontaneous human adult stem cell transformation," *Cancer Research*, vol. 65, pp. 3035–3039, 2005.

[7] L. A. Meza-Zepeda, A. Noer, J. A. Dahl, F. Micci, O. Myklebost, and P. Collas, "High-resolution analysis of genetic stability of human adipose tissue stem cells cultured to senescence," *Journal of Cellular and Molecular Medicine*, vol. 12, no. 2, pp. 553–563, 2008.

[8] B. R. Weil, A. M. Abarbanell, J. L. Herrmann, Y. Wang, and D. R. Meldrum, "High glucose concentration in cell culture medium does not acutely affect human mesenchymal stem cell growth factor production or proliferation," *American Journal of*

Physiology: Regulatory Integrative and Comparative Physiology, vol. 296, no. 6, pp. R1735–R1743, 2009.

[9] M. Dhanasekaran, S. Indumathi, J. S. Rajkumar, and D. Sudarsanam, "Effect of high glucose on extensive culturing of mesenchymal stem cells derived from subcutaneous fat, omentum fat and bone marrow," *Cell Biochemistry and Function*, vol. 31, no. 1, pp. 20–29, 2013.

[10] P. A. Zuk, M. Zhu, H. Mizuno et al., "Multilineage cells from human adipose tissue: Implications for cell-based therapies," *Tissue Engineering*, vol. 7, no. 2, pp. 211–228, 2001.

[11] B. Prunet-Marcassus, B. Cousin, D. Caton, M. André, L. Pénicaud, and L. Casteilla, "From heterogeneity to plasticity in adipose tissues: site-specific differences," *Experimental Cell Research*, vol. 312, no. 6, pp. 727–736, 2006.

[12] J. M. Gimble, A. J. Katz, and B. A. Bunnell, "Adipose-derived stem cells for regenerative medicine," *Circulation Research*, vol. 100, no. 9, pp. 1249–1260, 2007.

[13] T. Asahara, H. Masuda, T. Takahashi et al., "Bone marrow origin of endothelial progenitor cells responsible for postnatal vasculogenesis in physiological and pathological neovascularization," *Circulation Research*, vol. 85, no. 3, pp. 221–228, 1999.

[14] P. A. Sotiropoulou, S. A. Perez, M. Salagianni, C. N. Baxevanis, and M. Papamichail, "Characterization of the optimal culture conditions for clinical scale production of human mesenchymal stem cells," *Stem Cells*, vol. 24, no. 2, pp. 462–471, 2006.

[15] B. Roda, P. Reschiglian, A. Zattoni et al., "A tag-less method of sorting stem cells from clinical specimens and separating mesenchymal from epithelial progenitor cells," *Cytometry B: Clinical Cytometry*, vol. 76, no. 4, pp. 285–290, 2009.

[16] A. K. Singh, J. Patel, N. O. Litbarg et al., "Stromal cells cultured from omentum express pluripotent markers, produce high amounts of VEGF, and engraft to injured sites," *Cell and Tissue Research*, vol. 332, no. 1, pp. 81–88, 2008.

[17] S. Baglioni, M. Francalanci, R. Squecco et al., "Characterization of human adult stem-cell populations isolated from visceral and subcutaneous adipose tissue," *The FASEB Journal*, vol. 23, no. 10, pp. 3494–3505, 2009.

[18] M. Toyoda, Y. Matsubara, K. Lin, K. Sugimachi, and M. Furue, "Characterization and comparison of adipose tissue-derived cells from human subcutaneous and omental adipose tissues," *Cell Biochemistry and Function*, vol. 27, no. 7, pp. 440–447, 2009.

[19] D. Marappagounder, I. Somasundaram, R. S. Janvikula, and S. Dorairaj, "Long-term culture optimization of human omentum fat-derived mesenchymal stem cells," *Cell Biology International*, vol. 36, no. 11, pp. 1029–1036, 2012.

[20] M. Dhanasekaran, S. Indumathi, A. Kanmani et al., "Surface antigenic profiling of stem cells from human omentum fat in comparison with subcutaneous fat and bone marrow," *Cytotechnology*, vol. 64, no. 5, pp. 497–509, 2012.

[21] M. Dominici, K. Le Blanc, I. Mueller et al., "Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement," *Cytotherapy*, vol. 8, no. 4, pp. 315–317, 2006.

[22] D. A. de Ugarte, K. Morizono, A. Elbarbary et al., "Comparison of multi-lineage cells from human adipose tissue and bone marrow," *Cells Tissues Organs*, vol. 174, no. 3, pp. 101–109, 2003.

[23] L. Aust, B. Devlin, S. J. Foster et al., "Yield of human adipose-derived adult stem cells from liposuction aspirates," *Cytotherapy*, vol. 6, no. 1, pp. 7–14, 2004.

[24] A. J. Katz, A. Tholpady, S. S. Tholpady, H. Shang, and R. C. Ogle, "Cell surface and transcriptional characterization of human adipose-derived adherent stromal (hADAS) cells," *Stem Cells*, vol. 23, no. 3, pp. 412–423, 2005.

[25] J. B. Mitchell, K. McIntosh, S. Zvonic et al., "Immunophenotype of human adipose-derived cells: temporal changes in stromal-associated and stem cell-associated markers," *Stem Cells*, vol. 24, no. 2, pp. 376–385, 2006.

[26] A. C. Boquest, A. Shahdadfar, J. E. Brinchmann, and P. Collas, "Isolation of stromal stem cells from human adipose tissue.," *Methods in Molecular Biology*, vol. 325, pp. 35–46, 2006.

[27] T. G. Ebrahimian, F. Pouzoulet, C. Squiban et al., "Cell therapy based on adipose tissue-derived stromal cells promotes physiological and pathological wound healing," *Arteriosclerosis, Thrombosis, and Vascular Biology*, vol. 29, no. 4, pp. 503–510, 2009.

[28] H. Mizuno, "Adipose-derived stem and stromal cells for cell-based therapy: current status of preclinical studies and clinical trials," *Current Opinion in Molecular Therapeutics*, vol. 12, no. 4, pp. 442–449, 2010.

[29] M. Dhanasekaran, S. Indumathi, R. P. Lissa, R. Harikrishnan, J. S. Rajkumar, and D. Sudarsanam, "A comprehensive study on optimization of proliferation and differentiation potency of bone marrow derived mesenchymal stem cells under prolonged culture condition," *Cytotechnology*, vol. 65, no. 2, pp. 187–197, 2013.

[30] M. Dhanasekaran, S. Indumathi, M. Rashmi, J. S. Rajkumar, and D. Sudarsanam, "Unravelling the retention of proliferation and differentiation potency in extensive culture of human subcutaneous fat-derived mesenchymal stem cells in different media," *Cell Proliferation*, vol. 45, no. 6, pp. 516–526, 2012.

[31] M. Mireskandari, A. F. Shafaii, G. Kayser, and K. Kayser, "Lack of CD 117 and rare bcl-2 expression in stomach cancer by immunohistochemistry. An immunohistochemical study with review of the literature," *Diagnostic Pathology*, vol. 1, no. 1, article 7, 2006.

[32] D. A. Bleijs, M. E. Binnerts, S. J. van Vliet, C. G. Figdor, and Y. Van Kooyk, "Low-affinity LFA-1/ICAM-3 interactions augment LFA-1/ICAM-1-mediated T cell adhesion and signaling by redistribution of LFA-1," *Journal of Cell Science*, vol. 113, no. 3, pp. 391–400, 2000.

[33] S. Gronthos, A. C. W. Zannettino, S. J. Hay et al., "Molecular and cellular characterisation of highly purified stromal stem cells derived from human bone marrow," *Journal of Cell Science*, vol. 116, no. 9, pp. 1827–1835, 2003.

[34] M. Dhanasekaran, S. Indumathi, R. Poojitha, A. Kanmani, J. S. Rajkumar, and D. Sudarsanam, "Plasticity and banking potential of cultured adipose tissue derived mesenchymal stem cells," *Cell and Tissue Banking*, vol. 14, no. 2, pp. 303–315, 2013.

[35] T. Papayannopoulou, G. V. Priestley, and B. Nakamoto, "Anti-VLA4/VCAM-1-induced mobilization requires cooperative signaling through the kit/mkit ligand pathway," *Blood*, vol. 91, no. 7, pp. 2231–2239, 1998.

[36] T. Südhoff and D. Söhngen, "Circulating endothelial adhesion molecules (sE-selectin, sVCAM-1 and sICAM-1) during rHUG-CSF-stimulated stem cell mobilization," *Journal of Hematotherapy and Stem Cell Research*, vol. 11, no. 1, pp. 147–151, 2002.

[37] E. M. Jung, O. Kwon, K.-S. Kwon et al., "Evidences for correlation between the reduced VCAM-1 expression and hyaluronan synthesis during cellular senescence of human mesenchymal stem cells," *Biochemical and Biophysical Research Communications*, vol. 404, no. 1, pp. 463–469, 2011.

[38] R. Pasqualini, E. Koivunen, R. Kain et al., "Aminopeptidase N is a receptor for tumor-homing peptides and a target for inhibiting angiogenesis," *Cancer Research*, vol. 60, no. 3, pp. 722–727, 2000.

[39] B. Puissant, C. Barreau, P. Bourin et al., "Immunomodulatory effect of human adipose tissue-derived adult stem cells: comparison with bone marrow mesenchymal stem cells," *British Journal of Haematology*, vol. 129, no. 1, pp. 118–129, 2005.

[40] A. M. Parker and A. J. Katz, "Adipose-derived stem cells for the regeneration of damaged tissues," *Expert Opinion on Biological Therapy*, vol. 6, no. 6, pp. 567–578, 2006.

[41] A.-M. Rodriguez, C. Elabd, E.-Z. Amri, G. Ailhaud, and C. Dani, "The human adipose tissue is a source of multipotent stem cells," *Biochimie*, vol. 87, no. 1, pp. 125–128, 2005.

[42] J. S. Moreb, "Aldehyde dehydrogenase as a marker for stem cells," *Current Stem Cell Research and Therapy*, vol. 3, no. 4, pp. 237–246, 2008.

[43] X.-W. Ding, J.-H. Wu, and C.-P. Jiang, "ABCG2: a potential marker of stem cells and novel target in stem cell and cancer therapy," *Life Sciences*, vol. 86, no. 17-18, pp. 631–637, 2010.

[44] E. J. Gang, D. Bosnakovski, C. A. Figueiredo, J. W. Visser, and R. C. R. Perlingeiro, "SSEA-4 identifies mesenchymal stem cells from bone marrow," *Blood*, vol. 109, no. 4, pp. 1743–1751, 2007.

Hindawi Publishing Corporation
Stem Cells International
Volume 2014, Article ID 340257, 26 pages
http://dx.doi.org/10.1155/2014/340257

Review Article

Management of Fibrosis: The Mesenchymal Stromal Cells Breakthrough

Benoît Usunier, Marc Benderitter, Radia Tamarat, and Alain Chapel

Radioprotection and Human Health Division, Institute of Radioprotection and Nuclear Safety, PRP-HOM/SRBE/LR2I, 92260 Fontenay-aux-Roses, France

Correspondence should be addressed to Alain Chapel; alain.chapel@irsn.fr

Received 28 March 2014; Revised 5 June 2014; Accepted 5 June 2014; Published 14 July 2014

Academic Editor: Katherine Athayde Teixeira de Carvalho

Fibrosis is the endpoint of many chronic inflammatory diseases and is defined by an abnormal accumulation of extracellular matrix components. Despite its slow progression, it leads to organ malfunction. Fibrosis can affect almost any tissue. Due to its high frequency, in particular in the heart, lungs, liver, and kidneys, many studies have been conducted to find satisfactory treatments. Despite these efforts, current fibrosis management therapies either are insufficiently effective or induce severe adverse effects. In the light of these facts, innovative experimental therapies are being investigated. Among these, cell therapy is regarded as one of the best candidates. In particular, mesenchymal stromal cells (MSCs) have great potential in the treatment of inflammatory diseases. The value of their immunomodulatory effects and their ability to act on profibrotic factors such as oxidative stress, hypoxia, and the transforming growth factor-β1 pathway has already been highlighted in preclinical and clinical studies. Furthermore, their propensity to act depending on the microenvironment surrounding them enhances their curative properties. In this paper, we review a large range of studies addressing the use of MSCs in the treatment of fibrotic diseases. The results reported here suggest that MSCs have antifibrotic potential for several organs.

1. Introduction

Healthy tissues can be damaged under various conditions by acute or chronic stimuli such as mechanical or chemical injuries, infections, or autoimmune reactions. In most cases, the repair process consists of dead and damaged cells replacement, thus restoring the organ's unimpaired functionality. The first stage of this mechanism, known as the regenerative phase, corresponds to the replacement of damaged cells by cells of the same type, thus ensuring organ functionality. During the second phase, known as fibroplasia or fibrosis, connective tissue replaces degraded normal parenchymal tissue. Unchecked fibrosis leads to substantial remodeling of the ECM (extracellular matrix) with pathological features which results in the formation of permanent scar tissue. Fibrosis may ultimately lead to organ malfunction and death. It mainly originates from chronic inflammation, tissue ischemia, and imbalance in the ECM accumulation/degradation ratio [1].

Most organs are susceptible to fibrotic diseases, generally as a consequence or feature of a preexisting pathology (Figure 1). Obesity, aging, and environmental aggressions are the main causes of fibrogenesis. Fibroproliferative diseases are believed to be responsible for around 45% of deaths in developed countries [2]. Although considerable efforts are being devoted to the search for antifibrotic treatments, there are currently few effective therapies for fibrotic diseases that do not result in severe secondary effects. Anti-inflammatory drugs have been considered as the most promising candidates in clinical trials. A wide range of antioxidants have also been tested. Nevertheless, most drug therapy protocols have failed in achieving sufficient antifibrotic effect.

Thus, cell therapy has recently been put forward as a possibility. In particular, mesenchymal stromal cell (MSC) therapy seems to be a promising treatment. Indeed, preclinical and clinical trials have shown MSCs' ability to improve outcomes in various diseases such as the consequences of radiotherapy [3], autoimmune pathologies [4], neurodegenerative disorders [5], and other etiological agents. Preclinical and clinical studies have also put forward the ability of MSCs to adapt to their environment. Indeed, the regulation of MSCs' secretome is highly influenced by the surrounding

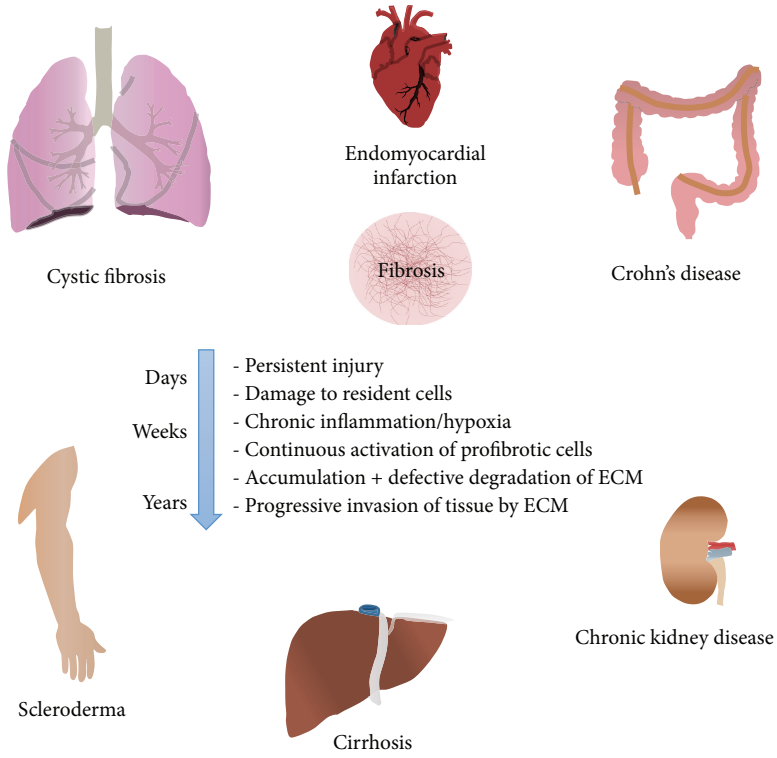


Figure 1: Fibrotic pathologies in various organs. Common features of fibrosis development and progression in various organs and related diseases (ECM: extracellular matrix).

tissue. Therefore, MSC therapy yields different results with different pathologies. Consequently, these effects have led several laboratories to investigate the antifibrotic potential of MSCs.

2. Common Cellular and Molecular Mechanisms of Fibrotic Diseases

2.1. Wound Healing: The Initiation of Fibrosis. Tissue injuries induce damages to resident cells which secrete inflammatory mediators which initiate an antifibrinolytic-coagulation cascade associated with vascular congestion. A temporary ECM is formed to serve as a scaffold for dead cells replacement. Subsequent platelet activation causes the release of various mediators including vasoactive factors (vasodilatation, increased vascular permeability, and edema by plasma exudation), cytokines, and chemokines that enable the recruitment of leukocytes. The formation of a fibrin clot serves as a matrix for cell migration and platelet adhesion. Fibrinolysis is later activated and leads to the dissolution of the fibrin clot replaced by a granulation tissue. Plasmin is released from the fibrin clot and activates the complement system, triggering the release of chemotactic and vasoactive anaphylatoxins [1, 6].

Next, recruited leukocytes home by adhesion to molecules such as selectins, integrins, and immunoglobulins. Phagocytosis of tissue debris, dead cells, and any exogenous organisms is carried out by macrophages and neutrophils. They also produce cytokines and chemokines to recruit endothelial cells necessary for neovascularization. The interaction of fibroblasts, fibrocytes, or other resident cells, such as hepatic stellate cells (HSCs), with the microenvironment induces their differentiation into myofibroblasts which synthesize ECM and growth factors including profibrotic TGF-β1 (transforming growth factor-β1). The secretion of autocrine hormones enables the maturation of myofibroblasts. α-SMA (α-smooth muscle actin) and vimentin expression by myofibroblasts are responsible for their contractile activity [7]. This contractibility is required for the closure of the wound. The formation of this so-called granulation tissue is characterized by the presence of many blood capillaries allowing the supply of nutrients, hormones, and respiratory gas [1, 2, 6].

Finally, the migration and maturation of epithelial and endothelial cells then allow the formation of scar tissue and neovascularization. The provisional ECM is degraded by matrix metalloproteinases (MMPs) once complete tissue replacement is achieved. The subtle equilibrium between MMPs and their inhibitors, tissue inhibitors of metalloproteinases (TIMPs), controls ECM accumulation and degradation throughout the repair process. Thus, it guarantees proper ECM remodeling by inducing a shift in matrix composition. Next, myofibroblasts disappear by apoptosis, triggered by the establishment of a negative activation loop indicating regeneration of the injured tissue [1, 2, 6].

2.2. Specific Fibrosis Mechanisms. Various fibroproliferative pathologies share common features. Fibrosis begins as a normal tissue regeneration process. Resident and recruited cells are activated to produce a provisional ECM facilitating

repair. However, in the case of bacterial infection, ischemia, chronic inflammation, or other persistent stimuli, a constant loop of myofibroblast activation sets in, leading to excessive ECM accumulation. Activated myofibroblasts also produce chemokines to recruit cells from the immune system (macrophages, T- and B-cells, neutrophils, and eosinophils), thus perpetuating chronic inflammation. The pathologic matrix progressively invades the tissue, eventually ending in the presence of a permanent fibrotic scar. Histologically, fibrosis can be defined by two distinct stages. Development corresponds to the onset of matrix accumulation where only scattered fibrosis areas are seen in the tissue, whereas the endpoint is characterized by diffused spans of ECM possibly distributed through the entire tissue. The progressive replacement of dead cells by ECM suppresses organ function and induces stiffness. Ultimately, the best course of treatment for advanced fibrosis is often organ transplantation.

Fibrosis is a complex pathology driven by numerous biological factors such as chronic inflammation and hypoxia. Ionizing radiation, for example, induces endothelial cell death and oxidative stress, resulting in prolonged inflammation and potentially fibrosis. The constant recruitment of inflammatory cells generates an activation loop of myofibroblasts and maintains a steady pool of profibrotic cells.

One of the main molecular agents inducing fibrosis is TGF-β1, mainly synthesized by T-cells during the healing process [8]. TGF-β1 is secreted in a latent form associated with LAP (latency associated peptide). LAP is cleaved to allow the activation of TGF-β1 which is able to bind its receptors TGF-βR1 (transforming growth factor receptor-β1) and TGF-βR2. Therefore, there is a large pool of inactive TGF-β1 in the extracellular environment. Various agents can induce TGF-β1 activation: MMPs [9], reactive oxygen and nitrogen species (ROS and RNS) [10], cytokines [11], or other stimuli such as ionizing radiation [12]. The binding of TGF-β1 to its receptors activates the Smad (small mothers against decapentaplegic homolog) signaling pathway which induces the transcription of various genes, including genes encoding members of the extracellular matrix (collagens mostly) [13]. It also activates the differentiation of fibrocytes toward functional fibroblasts.

EMT (epithelial-to-mesenchymal transition) and EndMT (endothelial-to-mesenchymal transition) are also described as important sources of fibroblasts. Epithelial or endothelial cells assume a spindle shape, lose their cell markers, and express typical fibroblast markers such as FSP-1 (fibroblast specific protein-1), α-SMA, and vimentin [14, 15]. They also acquire the ability to produce collagen and fibronectin (extracellular matrix components) [16]. TGF-β1 has also been shown to decrease the expression and activity of MMPs and increase the expression of TIMPs [17]. Thus, TGF-β1 is considered to be one of the major factors in fibrosis development.

Other growth factors take part in prolonged fibrogenesis. CTGF (connective tissue growth factor) acts synergistically with TGF-β1 to stimulate the signal transduction pathway dependent on TGF-β1 [18]. CTGF can also stimulate the proliferation, migration, and adhesion of fibroblasts and the production of the extracellular matrix [19, 20].

Thus, fibrosis is a multicomponent pathology driven by multiple factors (Figure 2). One of the main issues in treating fibrosis lies in its self-maintenance. Hence, various therapies might be considered depending on the stage of fibrogenesis. Indeed, preventive or curative strategies should differ based on the ECM components and the mechanisms involved. Moreover, combined therapies should be used to simultaneously act on various profibrotic mechanisms and enhance treatment efficacy.

3. Fibrosis Models

Over the years, many models of fibrosis in animals have been developed. Mechanical or chemical procedures are used to mimic damage observed in patients.

Heart. Cardiac fibrosis is characteristic of many heart diseases. Doxorubicin (DOX) or isoproterenol (ISO) is widely used to induce myocardial infarction (MI). It is hypothesized that DOX-induced cardiac damage increases the concentration of reactive oxygen species, thus causing injury to mitochondria, leading to apoptosis and fibrosis [21]. ISO injection directly into the heart produces diffuse myocardial cell death and fibrosis, leading to progressive heart failure [22]. Finally, ligation of the interventricular artery results in ischemia and eventually leads to fibrosis [23].

Kidney. Interstitial fibrosis and glomerulosclerosis are common features of kidney pathologies such as chronic kidney disease (CKD), chronic allograft nephropathy (CAN), or ureteral obstruction. In the reversible unilateral ureteral obstruction (UUO), fibrosis is induced by oxidative stress [24]. Atherosclerotic renal artery stenosis (ARAS) is found among 50% of atherosclerotic patients with other atherosclerotic diseases [25]. In preclinical studies, ARAS is modeled by placing an irritant coil in one of the main renal arteries to induce chronic inflammation [26]. Removal of one or both kidneys and kidney allograft can be performed to create a CAN model [27]. "Nephrectomy + ischemia-reperfusion + cyclosporine" (NIRC) is a recent model mimicking CKD. Oxidative stress caused by ischemia, exacerbated by the immunosuppressive effect of cyclosporine, induces interstitial fibrosis following ischemia-reperfusion [28]. Lastly, in the remnant kidney model (RKM), also called 5/6 nephrectomy (5/6 NX), interstitial fibrosis is induced by removing one kidney and two-thirds of the second. It is hypothesized that subsequent oxidative stress and inflammatory reaction generate fibrosis [29].

Liver. Fibrosis in the liver, or cirrhosis, is the common endpoint of chronic liver diseases. It originates from not only numerous pathologies such as alcoholic liver disease and viral or autoimmune hepatitis but also hepatotoxic drugs and toxins. Carbon tetrachloride (CCl_4) induces irreversible pathologies such as fatty liver, fibrosis, cirrhosis, and cancer and is mainly used in liver damage models [30].

Lungs. Pulmonary fibrosis is an increasingly frequent pathology due to the growing number of smokers and the pollution

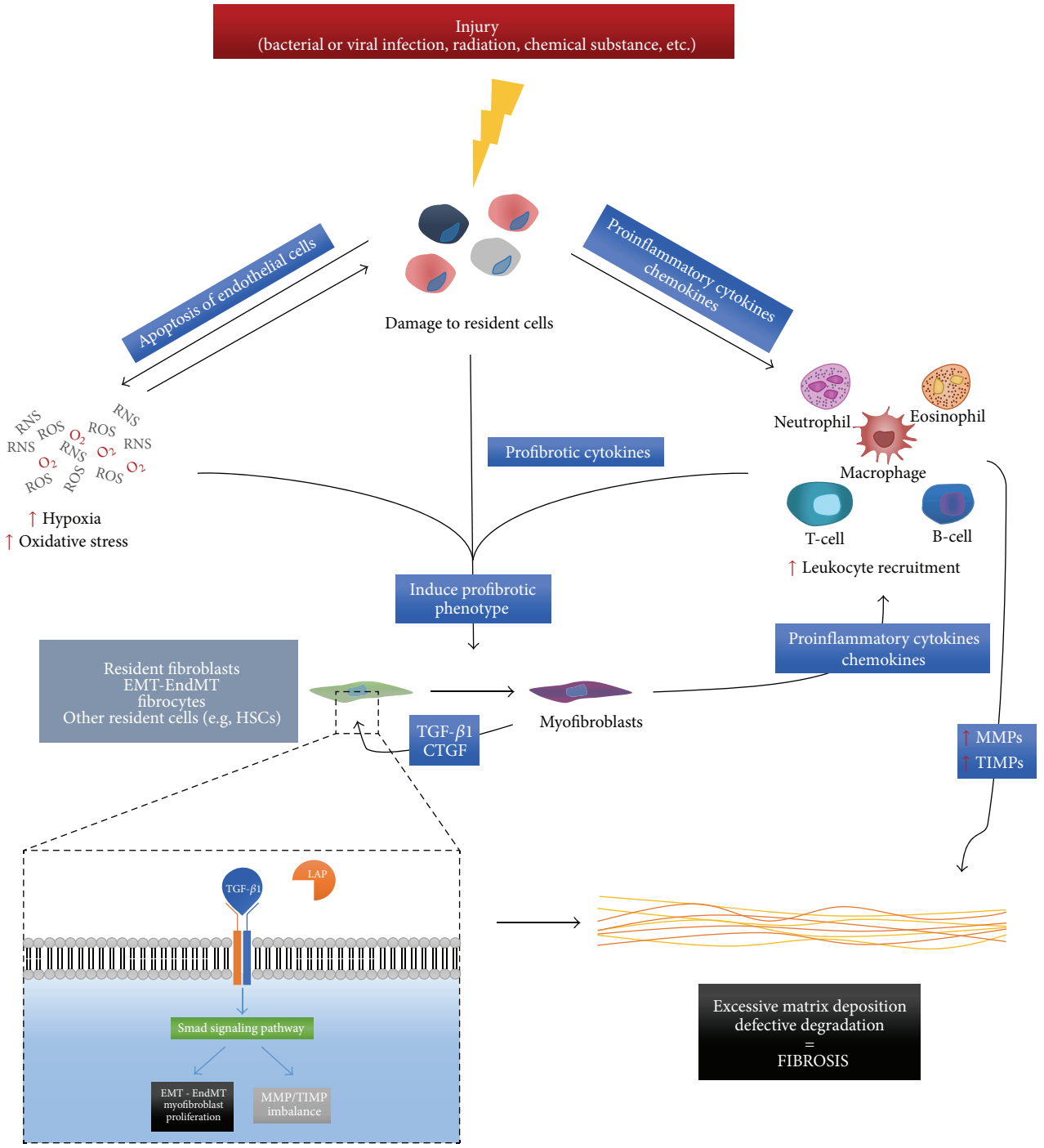


Figure 2: Fibrosis is a multicomponent pathology driven by multiple factors. Fibrotic diseases are driven by multiple factors such as inflammatory reaction, hypoxia, and oxidative stress leading to the activation of the TGF-β1 pathway (DC: dendritic cell, EMT: epithelial-to-mesenchymal transition, LAP: latency associated protein, MMP: matrix metalloproteinase, RNS: reactive nitrogen species, ROS: reactive oxygen species, Smad: small mothers against decapentaplegic homolog, TGF: transforming growth factor, and TIMP: tissue inhibitor of metalloproteinases).

resulting from current lifestyles. The onset of fibrosis in the bleomycin, mainly originating from DNA single and double strand breaks, is a major side effect of this drug which is now widely used in the development of animal models of pulmonary fibrosis [31]. Exposure to silica also induces fibrotic responses. The resulting persistent toxic effect causes chronic inflammation resulting in fibrogenesis [32].

Peritoneum. Peritoneal fibrosis can be initiated by toxins, infectious peritonitis, or incompatible dialysate products.

Chlorhexidine gluconate (CG) was one of the first compounds believed to cause encapsulating peritoneal sclerosis (EPS) during dialysis. Peritoneal exposure to CG leads to an inflammatory reaction causing fibrosis in animal models [33].

Skin. Skin fibrosis is part of a wide range of human disorders including keloids, hypertrophic scars, and scleroderma. Subcutaneous injections of bleomycin produce lesions mimicking scleroderma [34]. Radiation exposure can lead to fibrosis in a number of different organs. Cutaneous radiation-induced fibrosis is caused by a strong inflammatory reaction, apoptosis, and oxidative stress and is a commonly used animal model [35]. Another *in vivo* cutaneous fibrosis model has been developed in mice by producing full-thickness wounds which consequently lead to chronic inflammation [36, 37].

Pancreas. The incidence of chronic pancreatitis is approximately 30 per 100,000 and is increasing over time [38]. Since existing treatments are limited, continuous efforts are being devoted to preclinical studies in animal models. Intravenous administration of dibutyltin dichloride (DBTC) induces damage to the bile duct epithelium. Subsequent inflammation causes fibrosis in the pancreas [39].

Colon-Rectum. 5 to 10% of patients receiving pelvic radiotherapy develop chronic radiation proctopathy due to the high radiosensibility of organs in the radiation field (colon, rectum, and bladder) [3]. Radiation proctopathy is modeled in animals by delivering a high radiation dose to the rectum [40]. Radiation-induced damage to the tissue as well as oxidative stress induces fibrosis in this model.

Common features are characteristic of these animal models of fibrosis. Chemical compounds, physical agents, or surgery procedures are used to induce the initial injury. This protocol is often repeated periodically or maintained over a prolonged time. Subsequent damage to the tissue induce chronic inflammation, oxidative stress, and/or hypoxia necessary to activate resident and recruited cells toward a profibrotic phenotype. In most cases, fibrotic features appear weeks to months after the initial stimulus.

4. Antifibrotic Effects of Mesenchymal Stromal Cells Therapy

MSCs are widely described for their immunoregulatory properties. Nevertheless MSCs' antifibrotic functions are poorly described. Syntheses of *in vivo* study outcomes are described in Table 1 (heart), Table 2 (liver), Table 3 (kidneys), Table 4 (lungs), Table 5 (peritoneum), Table 6 (pancreas), Table 7 (skin), and Table 8 (rectum). The synthesis of *in vitro* study outcomes is shown in Table 9.

4.1. Immunological Aspects. Pathogenic fibrosis results from chronic inflammatory reactions. Recent advances in the immunobiology of MSCs have led to increased interest in their use as a new therapeutic modality to address chronic inflammation associated with fibrosis (Figure 3) [78, 79]. The immunosuppressive effect of MSCs has been extensively studied and documented, particularly because of its value in organ transplantation. MSCs operate on the T and B lymphocytes by blocking them in the G0/G1 phase of the cell cycle, inhibiting the production of immunoglobulins (IgA, IgG, and IgM) and the differentiation of B lymphocytes. MSCs induce a change in polarity in T lymphocytes from a proinflammatory Th1 state to an anti-inflammatory Th2 condition [80, 81]. They act in the differentiation and maturation of dendritic cells and make them tolerogenic [82]. Furthermore, MSCs inhibit the cytotoxic activity of natural killer cells on HLA-1 (human leukocyte antigen-1) negative cells and reduce the production of cytokines: TNF-α (tumor necrosis factor-α), IFN-γ (interferon-γ), and IL-10 (interleukin-10) [83]. Therefore, MSCs are of value for the treatment of diseases with an inflammatory component.

Numerous studies have highlighted the benefits of immunomodulation by MSCs in the treatment of fibrosis. MSC-induced decreased TLR (toll-like receptor) expression suggests their ability to limit chronic inflammation [40]. After the transplantation of MSCs, a decreased infiltration of monocytes/macrophages, neutrophils, and lymphocytes in the tissue was observed in various models [40, 69, 70, 73, 74]. This correlates with the decreased expression of MCP-1 (monocyte chemoattractant protein-1) in some cases [74]. Additionally, underexpression of VCAM-1 (vascular cell adhesion molecule-1) and ICAM-1 (intercellular adhesion molecule-1), involved in leukocyte-endothelial cell interactions, suggests reduced inflammatory cell infiltration [74]. In a model of radiation-induced skin fibrosis, MSCs induced macrophage transition toward a regulatory phenotype, thus limiting chronic inflammation causing fibrosis [76]. Decreased iNOS (nitric oxide synthase) expression after MSC transplantation suggests a reduction of M1 macrophage activity [40]. An increased proportion of anti-inflammatory M2 macrophages were reported after MSC transplantation in a heart fibrosis model [45] and a radiation-induced proctitis model [40]. Microvesicles purified from MSC-conditioned medium, while significantly decreasing the amount of inflammatory cells, produced lower effects compared to MSC transplantation in the lung [69].

MSCs inhibit the expression of IFN-γ, which exerts a proinflammatory effect by inducing overexpression of IL-6 and TNF-α [67]. The decrease in mRNA expression and protein concentration of TNF-α, a profibrotic cytokine, was detected in the tissue after MSC transplantation [40, 58, 60, 65–67, 70, 74, 76, 77]. IL-1α [76], IL-1β [70, 76], and IL-6 [40, 63, 65, 70, 74] are underexpressed in several fibrosis models following MSC injection. Increased expression of anti-inflammatory cytokines IL-4 and IL-10 after MSC transplantation was observed, suggesting the transition of T lymphocytes to a Th2 profile [65]. Similarly, MSCs induced increased IL-10 expression and concentration in a model of cutaneous and rectal radiation-induced fibrosis [40, 76].

Antiapoptotic effects of MSC therapy can also be discussed, as fewer apoptotic events correlate with reduced inflammation. In fibrotic tissues following MSC transplantation, a decrease in apoptotic events was observed [41, 42, 52, 65]. Accordingly, MSCs may protect resident cells, increasing functionality and recovery.

Table 1: MSC treatment on preclinical heart fibrosis models.

References	Organism	Model	Treatment	MSC source	Timing	Quantity	Route	Outcome
[41]	Pig	Ligation	Ligation of the left coronary artery for 90 minutes before reperfusion	BM	30 minutes after reperfusion	3.10^7 cells + atorvastatin	Infarction and peri-infarction zone	(i) Reduced fibrotic area (ii) Reduced inflammation score (iii) Decreased apoptosis (iv) Increased cNOS activity (v) Atorvastatin increases MSC survival
[42]	Rat	DOX	2.5 mg/kg 6 times in 2 weeks	BM	1 week after the first DOX injection	$\sim 5.10^6$ cells or ~1 mL of MSC-conditioned medium	Tail vein	(i) Reduced fibrotic area (similar effect with MSC-conditioned medium) (ii) Increased Bcl-2/Bax ratio (similar effect with MSC-conditioned medium) (iii) High concentration of HGF and IGF-1 in MSC-conditioned medium
[43]	Rat	ISO	170 mg/kg every day for 4 days	BM	4 weeks after the final ISO injection	3.10^6 cells	Intramyocardial	(i) Reduced fibrotic area. (ii) Decreased expression of type I collagen (iii) Decreased expression of pro-MMP-2, active MMP-2, and MMP-9 (iv) Decreased concentration of MMP-2 and MMP-9 (v) Increased concentration of HGF (vi) Increased expression of HGF (sham level)
[44]	Rat	Ligation	Ligation of the interventricular artery	BM (wild type or melatonin treated)	2 weeks after ligation	3 injections of 2.10^6 cells or 3 injections of 50 μL of MSC-conditioned medium	Intramyocardial	(i) Reduced fibrotic area (improved effect with melatonin) (ii) Stimulation of angiogenesis (iii) Increased concentration of HGF (improved effect with melatonin)
[45]	Rat	Ligation	Ligation of the left coronary artery	FM or BM	4 weeks after ligation	Two-layered MSC sheets	Anterior heart wall	(i) Reduced fibrotic area (ii) Increased proportion of M2 macrophages (iii) Increased concentration of VEGF (iv) Increased capillary density in peri-infarct area (v) Some MSCs engrafted 28 days after transplantation (vi) Some engrafted MSCs express α-SMA and/or lectin-I (vii) No significant difference between FM and BM-MSCs

Influence of fibrosis induction methods, MSC source, timing of injection, quantity of MSCs transplanted, and transplantation route. Outcomes are expressed compared to control groups (i.e., groups treated but not transplanted with MSCs) unless stated otherwise (α-SMA: α-smooth muscle actin; BAX: Bcl-2-associated X protein; Bcl-2: B-cell lymphoma 2; BM: bone marrow; DOX: doxorubicin; FM: fetal membrane; HGF: hepatocyte growth factor; IGF: insulin-like growth factor; ISO: isoproterenol; MMP: matrix metalloproteinase; MSC: mesenchymal stromal cell; NOS: nitric oxide synthase; VEGF: vascular endothelial growth factor).

Table 2: MSC treatment on preclinical liver fibrosis models.

References	Organism	Model	Treatment	MSC source	Timing	Quantity	Route	Outcome
[46]	Mouse	CCl_4	1 mL/kg twice a week for 8 weeks + 100 μg/kg IL-6 24 and 48 hours after completion of CCl_4 treatment	BM	52 hours after CCl_4 treatment completion	5 injections of 10^6 cells	Local (liver lobes)	(i) Reduced fibrotic area (ii) Decreased apoptosis (improved by IL-6 treatment) (iii) Decreased expression of markers of apoptosis (Bax, capsase-3, and NF-κB)
[47]	Mouse	CCl_4	0.6 mL/kg twice a week in 1 week	UC	6 weeks after CCl^4 treatment	250 μg of MSC-secreted exosomes		(i) Reduced fibrotic area (ii) Decreased expression of collagens I and III (iii) Decreased expression of TGF-β1 (iv) Decreased concentration of TGF-β1 in serum (v) Decreased phosphorylation of Smad2 (vi) Decreased EMT as evidenced by the decrease in N-cadherin and vimentin positive cells
[48]	Mouse	CCl_4	1 mL/kg twice a week for 8 weeks	BM	4 weeks after the beginning of the CCl_4 treatment	10^6 cells	Tail vein	(i) Reduced fibrotic area (ii) Decreased expression of α-SMA (iii) Decreased expression of collagen type I (iv) Increased expression of MMP-13
[49]	Mouse	CCl_4	1 mL/kg twice a week for 2 or 5 weeks	BM	Immediately following or 1 week after CCl_4 treatment	10^6 cells	Tail vein	(i) Reduced fibrotic area (ii) Decreased expression of TGF-β1 and α-SMA
[50]	Mouse	CCl_4	4 weeks CCl4 treatment followed by 4-week SNP treatment	BM	Following CCl_4 treatment	10^6 cells	Local	(i) Reduced fibrotic area (ii) Decreased expression of NF-kB (iii) Decreased expression of α-SMA (iv) Decreased expression of collagen 1α1 and TIMP-1 (v) Effect improved by SNP treatment
[51]	Mouse	CCl_4	20 mL/kg twice over a 48-hour period	BM		10^6 cells	Tail vein	(i) Reduced fibrotic area (ii) Decreased expression of α-SMA and collagen 1α1 (iii) Injection of FGF2 partially reproduces the effects of MSCs
[52]	Mouse	CCl_4	1 mL/kg twice a week for 8 weeks	AM	After 4 weeks of CCl_4 treatment	10^5 cells	Spleen	(i) Reduced fibrotic area (ii) Increased hepatocyte proliferation (iii) Increased expression of Bcl-2 (iv) Decreased hepatocyte apoptosis (v) Reduced number of α-SMA-positive cells (vi) Increased SOD activity (vii) Increased expression of HGF and Bcl-2

Table 2: Continued.

References	Organism	Model	Treatment	MSC source	Timing	Quantity	Route	Outcome
[53]	Rat	CCl_4	5 mL/kg (first injection) followed by 3 mL/kg twice a week for 12 weeks	AT	2 days after CCl_4 treatment	2.10^6 cells	Tail vein or hepatic portal vein	(i) Reduced fibrotic area (ii) Improvement of the microvasculature (iii) Decreased expression of VEGF
[54]	Rat	CCl_4	0.5 mg/kg twice a week for 4 weeks	BM (wild type or HGF-treated)	Following the first CCl_4 injection	3.10^6 cells	Tail vein	(i) Reduced fibrotic area (improved effect after MSC pretreatment with HGF) (ii) Decreased inflammation
[55]	Rat	CCl_4	0.08 mL/kg twice a week for 6 weeks	BM	Following CCl_4 treatment	3.10^6 cells	IV	Decreased collagen concentration
[56]	Rat	CCl_4	0.5 mg/kg twice a week for 4 weeks	BM	Following CCl_4 treatment	10^6 cells	Hepatic portal vein	Reduced fibrotic area
[57]	Rat	CCl_4	1 mL/kg twice a week for 8 weeks	BM (wild type or adipogenic or hepatogenic differentiation)	4 weeks after the beginning of the CCl_4 treatment	3.10^7 cells	Spleen injection	(i) Reduced fibrotic area (best outcome with undifferentiated MSCs) (ii) Highest expression of MMP-2 and MMP-9 after undifferentiated MSC transplantation
[58]	Rat	CCl_4	1 mL/kg twice a week for 8 weeks	BM (wild type or hepatogenic differentiation)	4 weeks after the beginning of the CCl_4 treatment	5.10^6 cells	Tail vein	(i) Decreased fibrotic area (best effect with predifferentiated MSC + baicalin) (ii) Decreased concentration of TNF-α (best effect with predifferentiated MSC + baicalin) (iii) Decreased concentration of TGF-β1 (best effect with predifferentiated MSC + baicalin) (iv) Decreased collagen concentration (best effect with predifferentiated MSC + baicalin)
[59]	Rat	CCl_4	0.5 mL/kg (first administration) followed by 1 mL/kg twice a week for 8 weeks (gavage)	UC	4 weeks after the beginning of the CCl_4 treatment	5.10^5 cells	Local	(i) Reduced collagen deposition (ii) Decreased concentration of TGF-β1 (iii) Decreased concentration of α-SMA (iv) Increased expression of HGF

Influence of fibrosis induction methods, MSC source, timing of injection, quantity of MSCs transplanted, and transplantation route. Outcomes are expressed compared to control groups (i.e., groups treated but not transplanted with MSCs) unless stated otherwise (α-SMA: α-smooth muscle actin; AM: amniotic membrane; AT: adipose tissue; BAX: Bcl-2-associated X protein; Bcl-2: B-cell lymphoma 2; BM: bone marrow; CCl_4: carbon tetrachloride; EMT: epithelial-to-mesenchymal transition; FGF: fibroblast growth factor; HGF: hepatocyte growth factor; IV: intravenous; MMP: matrix metalloproteinase; MSC: mesenchymal stromal cell; NF-κB: nuclear factor kappa-light-chain-enhancer of activated B-cells; SNP: sodium nitroprusside; SOD: superoxide dismutase; TGF-β: transforming growth factor-β; TIMP: tissue inhibitor of metalloproteinase; TNF-α: tumor necrosis factor-α; UC: umbilical cord; VEGF: vascular endothelial growth factor).

Table 3: MSC treatment on preclinical kidney fibrosis models.

References	Organism	Model	Treatment	MSC source	Timing	Quantity	Route	Outcome
[60]	Mouse	R-UUO	10 days UUO	BM	10 days after UUO	10^6 cells	Renal artery	(i) Decreased expression of TNF-α, TGF-β1, and α-SMA (ii) Increased expression of E-cadherin
[61]	Pig	ARAS	Irritant coil placed in the main renal artery	AT	6 weeks after ARAS	10^6 cells	Local	(i) Reduced fibrotic area (ii) Reduced number of CD163 + macrophages (iii) Increased number of regulatory macrophages (iv) Increased expression of IL-10 (v) Decreased expression of TNF-α (vi) Reduced concentration of MMP-2 (vii) Increased expression of VEGF, FLK-1, and HIF1-α (viii) Reduced MCP-1 positive area
[62]	Rat	Albumin-overload + uninephrectomy	Nephrectomy followed by 5 intraperitoneal injections of BSA (10 mg/g) per weeks during 4 weeks	BM	7 days after the first BSA injection	10^6 cells weekly for 4 weeks	IV	(i) Reduced expression and concentration of MCP-1 and CCL-5 (ii) Reduced expression and concentration of α-SMA (iii) Reduced expression and concentration of collagen IV
[63]	Rat	Allograft	Bi-nephrectomization and single kidney allograft	BM (melatonin treated)	11 weeks after graft	5.10^5 cells	Tail vein	(i) Decreased expression of IL-6, IL-7r, IL-23a, and IL-10 (ii) Decreased concentration of CTGF and α-SMA (iii) Decreased expression of fibronectin (iv) Decreased expression of bFGF
[64]	Rat	NIRC	Excision of the right kidney, 45-minutes ischemia in the left kidney followed by 28-day cyclosporine A treatment	BM	7 or 14 days after ischemia-reperfusion	3.10^6 cells	Local	MSC transplantation 7 days after ischemia reperfusion: (i) Decreased fibrotic area (ii) Decreased expression of collagen types I, III, and IV (iii) Reduced number of α-SMA-positive cells (iv) Decreased activity of MMP-2 (sham level) MSC transplantation 14 days after ischemia reperfusion showed no significant improvement
[65]	Rat	RKM	5/6 nephrectomy	BM	2 weeks after surgical procedure	-2.10^5 cells or -2.10^5 cells every other week (weeks 2, 4, and 6)	IV	(i) Reduced fibrotic area (ii) Increased expression of IL-4 and IL-10 (iii) Decreased expression of IL-6 and TNF-α (iv) Decreased expression of TGF-β1, Smad3, α-SMA, FSP-1, and vimentin (v) Decreased expression of collagen type I, collagen type III, fibronectin, and TIMP-1/MMP-9 ratio (vi) Increased expression of HO-1 (vii) Decreased expression of MCP-1 (viii) Increased expression of HGF

Table 3: Continued.

References	Organism	Model	Treatment	MSC source	Timing	Quantity	Route	Outcome
[66]	Rat	UUO	1 to 4 weeks obstruction	BM	Prior to UUO	10^6 cells	Renal artery	(i) Decreased collagen concentration (ii) Decreased expression of TNF-α (iii) Decreased concentration of TNF-α (iv) Decreased expression of α-SMA (v) Decreased number of FSP-1 positive cells (vi) Increased expression of E-cadherin

Influence of fibrosis induction methods, MSC source, timing of injection, quantity of MSCs transplanted, and transplantation route. Outcomes are expressed compared to control groups (i.e., groups treated but not transplanted with MSCs) unless stated otherwise (α-SMA: α-smooth muscle actin; ARAS: atherosclerotic renal artery stenosis; AT: adipose tissue; BSA: bovine serum albumin; CCL: chemokine ligand; CTGF: connective tissue growth factor; FGF: fibroblast growth factor; FLK: fetal liver kinase; FSP: fibroblast specific protein; HGF: hepatocyte growth factor; HIF: hypoxia-inducible factor; HO-1: heme oxygenase 1; IL: interleukin; IV: intravenous; MCP: monocyte chemoattractant protein; MMP: matrix metalloproteinase; MSC: mesenchymal stromal cell; NIRC: nephrectomy + ischemia-reperfusion + cyclosporine; R-UUO: reversible unilateral ureteral obstruction; TGF-β: transforming growth factor-β; TIMP: tissue inhibitor of metalloproteinase; TNF-α: tumor necrosis factor-α; UUO: unilateral ureteral obstruction; VEGF: vascular endothelial growth factor).

Table 4: MSC treatment on preclinical pulmonary fibrosis models.

References	Organism	Model	Treatment	MSC source	Timing	Quantity	Route	Outcome
[67]	Mouse	Bleomycin	0.15 mg bleomycin administered intranasally	UC	24 h after bleomycin inhalation	10^6 cells	Tail vein	(i) Decreased expression of IL-10, IFN-γ, and TNF-α (ii) Decreased concentration of TGF-β1 (iii) Decreased concentration of pSmad2 (iv) Decreased expression of collagen 1α1 (v) Increased concentration of active MMP-2 (vi) Decreased expression of TIMP-1, TIMP-2, TIMP-3, and TIMP-4
[68]	Mouse	Bleomycin	4 U/kg bleomycin instilled in the tracheal lumen	BM	Immediately following or 1 week after bleomycin inhalation	5.10^5 cells	Jugular vein	(i) Reduced collagen concentration (best effect when MSCs are transplanted immediately after bleomycin treatment) (ii) Decreased expression of MMP-2, MMP-9, and MMP-13
[69]	Mouse	Silica	200 μg/kg twice (day 1 and 4 weeks later) by intratracheal injection	Human BM	12 and 14 weeks after first silica injection	2.10^5 cells or 10 μg of MSC microvesicles	Tail vein	Bronchoalveolar lavage: (i) Reduced number of neutrophils (ii) Reduced number of lymphocytes (iii) Reduced number of macrophages (not significant after microvesicle injection) Lung samples: (i) Reduced fibrotic area (not significant after microvesicle injection) (ii) Reduced concentration of collagen I (iii) Reduced concentration of α-SMA (not significant after microvesicle injection)
[70]	Rat	Bleomycin	3 mg/kg bleomycin instilled intranasally	BM	4 days after bleomycin inhalation	10^6 cells	Tail vein	Bronchoalveolar lavage: (i) Reduced number of neutrophils (ii) Reduced number of lymphocytes (iii) Reduced number of macrophages (iv) Decreased expression of IL-6 and TNF-α Lung samples: (i) Reduced fibrotic area (ii) Decreased expression of IL-1β (iii) Decreased expression of TGF-β (iv) Decreased expression of VEGF (v) Decreased concentration of RNS
[71]	Rat	Bleomycin	5 mg/kg intratracheal perfusion	BM	12 h after bleomycin inhalation	5.10^6 cells	Tail vein	(i) Reduced fibrotic area (ii) Decreased expression of TGF-β1 (iii) Decreased expression of PDGF-A (/1.4) and PDGF-B (iv) Decreased expression of IGF-1 (v) MSCs differentiated in alveolar epithelial cells

Table 4: Continued.

References	Organism	Model	Treatment	MSC source	Timing	Quantity	Route	Outcome
[72]	Rat	Bleomycin	1.28 U instilled intratracheally	BM transfected with HGF expression plasmid	7 days after bleomycin instillation	3.10^6 cells	Intratracheal instillation	(i) Reduced Ashcroft score (fibrosis scoring) (ii) Reduced collagen concentration (iii) Transfected cells yield better results

Influence of fibrosis induction methods, MSC source, timing of injection, quantity of MSCs transplanted, and transplantation route. Outcomes are expressed compared to control groups (i.e. groups treated but not transplanted with MSCs) unless stated otherwise (α-SMA: α-smooth muscle actin; BM: bone marrow; HGF: hepatocyte growth factor; IGF: insulin-like growth factor; IL: interleukin; MMP: matrix metalloproteinase; MSC: mesenchymal stromal cell; PDGF: platelet-derived growth factor; RNS: reactive nitrogen species; pSmad: phosphorylated small mothers against decapentaplegic homolog; TGF-β: transforming growth factor-β; TIMP: tissue inhibitor of metalloproteinase; TNF-α: tumor necrosis factor-α; UC: umbilical cord; VEGF: vascular endothelial growth factor).

Table 5: MSC treatment on preclinical peritoneum fibrosis model.

References	Organism	Model	Treatment	MSC source	Timing	Quantity	Route	Outcome
[73]	Rat	CG	0.1% CG in 2 mL saline injected intraperitoneally	BM	30 minutes after CG injection	10^7 cells	Intraperitoneal	(i) Decreased infiltration of monocytes/macrophages (ii) Reduced number of pSmad2 positive cells (iii) Decreased number of α-SMA and FSP-1 positive cells (iv) Decreased concentration of collagen I and collagen III

Outcomes are expressed compared to control groups (i.e. groups treated but not transplanted with MSCs) unless stated otherwise (α-SMA: α-Smooth Muscle Actin; BM: Bone Marrow; CG: Chlorhexidine Gluconate; FSP: Fibroblast Specific Protein; pSmad: phosphorylated Small Mothers Against Decapentaplegic Homolog).

Table 6: MSC treatment on a preclinical pancreas fibrosis model.

References	Organism	Model	Treatment	MSC source	Timing	Quantity	Route	Results
[74]	Rat	DBTC	8 mg/kg DBTC injected in the tail vein	UC	5 days after DBTC injection	2.10^6 cells	Jugular vein	(i) Reduced inflammatory cell infiltration score (ii) Reduced monocyte/macrophage infiltration (iii) Reduced expression of MCP-1, VCAM-1, ICAM-1, IL-6, and TNF-α (iv) Reduced fibrosis score (v) Reduced expression of TGF-β1 (vi) Reduced concentration of collagen (vii) Reduced number of α-SMA-positive cells

Outcomes are expressed compared to control groups (i.e., groups treated but not transplanted with MSCs) unless stated otherwise (α-SMA: α-smooth muscle actin; DBTC: dibutyltin dichloride; ICAM: intercellular adhesion molecule; IL: interleukin; MCP: monocyte chemoattractant protein; TGF-β: transforming growth factor-β; TNF-α: tumor necrosis factor-α; VCAM: vascular cell adhesion molecule).

MSCs may induce regression in pathophysiological processes associated with fibrosis. These effects are in part mediated by a reduction in chronic inflammation. MSCs likely proceed by a change in immune cell function, an increase in anti-inflammatory cytokines, and a decrease in proinflammatory cytokines and cell apoptosis. These immune mechanisms contribute to a modification of the microenvironment, thus diminishing tissue fibrosis, increasing resident stem cell proliferation, and eventually leading to tissue regeneration.

4.2. The TGF-β1 Pathway. TGF-β1 has been described as one of the major players in fibrosis. Its binding to receptors induces the activation of a signaling cascade leading to the proliferation of phenotypically profibrotic cells such as myofibroblasts. In particular, it induces the EMT and EndMT in part responsible for the proliferation of cells synthesizing ECM. The TGF-β1 signaling pathway is one of the prime targets for antifibrotic therapies and its regulation has been abundantly studied in treatment trials with MSCs. Generally, MSC transplantation reduces the expression and concentration of TGF-β1 [40, 49, 59, 60, 65, 67, 70, 71, 73–76]. The same effect is induced by transplanting exosomes isolated from MSC-conditioned medium [47]. *In vitro*, Ueno et al. showed the inhibition of TGF-β1 overexpression induced by glucose in a coculture model of MSCs and peritoneal mesothelial cells [73]. This effect was associated with the decrease in the phosphorylation of Smad-2, as also shown in an exosome transplantation model [47, 73]. Reduced expression of α-SMA [44, 48, 50, 51, 60, 65, 66, 73] and the lower number of α-SMA positive cells [52, 53, 59, 64, 74, 75] suggest a decrease in the proliferation of myofibroblasts and, to a lesser extent, of TGF-β1-mediated EMT. *In vitro*, a reduced concentration of α-SMA in a coculture of MSCs and HK2 (human kidney 2) cells pretreated with TGF-β1 suggests a direct effect by MSCs on phenotypic changes leading to the accumulation of profibrotic cells [64]. A decreased expression and concentration of CTGF in several models also participate in diminishing profibrotic cells proliferation [40, 63].

Interestingly, several studies have underlined the importance of HGF (hepatocyte growth factor) secreted by MSCs for their antifibrotic effects [44, 47, 73]. MSCs transfected with an HGF expression plasmid yielded better results than nontransfected MSCs in a pulmonary fibrosis model [72]. The use of recombinant HGF partially reproduced the effects of MSCs in a coculture model with albumin-treated proximal tubular epithelial cells (PTECs) [62]. The inhibition of TGF-β1 expression by HGF and its ability to ameliorate the degradation of collagen through the increase in MMP-1 concentration highlights the value of such therapy [84]. Moreover, the increased expression of p-Met, which induces the phosphorylation of c-Met, the HGF membrane receptor, is also part of the action mechanisms of MSCs [59].

Recently, Qi et al. highlighted the importance of TSG-6 (TNF-stimulated gene 6) in the antifibrotic effect of MSCs. In addition to suppressing the secretion of TNF-α by activated

Table 7: MSC treatment on preclinical cutaneous fibrosis models.

References	Organism	Model	Treatment	MSC source	Timing	Quantity	Route	Outcome
[75]	Mouse	Bleomycin	Daily subcutaneous injection of 0.5, 1, 3, or 5 mg/mL bleomycin during 4 weeks	BM	3 hr after bleomycin injection	10^6 cells	Local	(i) Reduced number of macrophages and neutrophils (ii) Decreased expression of TGF-β1 (iii) Reduced number of α-SMA-positive cells (iv) Decreased expression of collagen type I (v) Increased expression of MMP-2, MMP-9, and MMP-13
[76]	Mouse	Radiation-induced	35 grays irradiation	BM (autologous or allogenic)	6 weeks after irradiation	5.10^5 cells	Tail vein	(i) No difference between autologous and allogeneic cells (ii) Reduced fibrotic area (iii) Reduced number of CD68 positive cells and CD80 positive cells (iv) Increased number of CD163 positive cells (v) Modification of macrophages toward a regulatory phenotype (vi) Increased expression of IL-10 (vii) Increased concentration of IL-10 (viii) Decreased expression of IL-1β and Serpine1 (ix) Decreased concentration of IL-1α, IL-1β, and TNF-α (x) Increased expression of PDGF-a
[77]	Mouse	Surgery	Four 6 mm full-thickness wounds on the back	BM	24 hr after surgery	10^6 cells	Local (around the wound)	(i) Decreased concentration of TNF-α (sham level with TSG-6 silenced MSCs) (ii) Decreased secretion of TNF-α by macrophages (no change in the number of macrophages) (iii) TGF-β1 concentration: increased on day 2; decreased on day 5; sham level with TSG-6 silenced MSCs (iv) Increased concentration of TGF-β3 (sham level with TSG-6 silenced MSCs) (v) Decreased α-SMA expression (vi) Decreased concentration of collagens 1α1, 1α2, and 3α1

Influence of fibrosis induction methods, MSC source, timing of injection, quantity of MSCs transplanted, and transplantation route. Outcomes are expressed compared to control groups (i.e., groups treated but not transplanted with MSCs) unless stated otherwise (α-SMA: α-smooth muscle actin; BM: bone marrow; IL: interleukin; MMP: matrix metalloproteinase; MSC: mesenchymal stromal cell; PDGF: platelet-derived growth factor; TGF-β: transforming growth factor-β; TNF-α: tumor necrosis factor-α; TSG-6: TNF-stimulated gene 6).

Table 8: MSC treatment on a preclinical colorectal fibrosis model.

References	Organism	Model	Treatment	MSC source	Timing	Quantity	Route	Results
[40]	Pig	Radiation-induced	High X-ray dose (21 to 29 Grays)	BM	27, 34, and 41 days after irradiation	2.10^6 cells	Ear vein	(i) Reduced fibrotic area (ii) Reduced leukocyte infiltration (iii) Reduced macrophages infiltration (iv) Increased M2 macrophages proportion (v) Reduced expression of iNOS (vi) Reduced expression of TNF-α, IL-6, and IL-8 (vii) Reduced expression of TLR-4 and TLR-5 (viii) Increased expression of IL-10 (ix) Reduced expression of col1a2 and col3a1 (x) Reduced expression of TGF-β1 and CTGF (xi) Decreased collagen-to-MMP-to-TIMP ratio (xii) Increased expression of VEGF in the rectal mucosa (xiii) Reduced expression of angiopoietin and PDGF in the rectal mucosa (xiv) Increased expression of eNOS, VEGF, VEGFR1, and PDGF in the colon

Outcomes are expressed compared to control groups (i.e., groups treated but not transplanted with MSCs) unless stated otherwise (BM: bone marrow; Col: collagen; CTGF: connective tissue growth factor; IL: interleukin; MMP: matrix metalloproteinase; NOS: nitric oxide synthase; PDGF: platelet-derived growth factor; TGF-β: transforming growth factor-β; TGF-βR: transforming growth factor-β receptor; TIMP: tissue inhibitor of metalloproteinase; TLR: toll-like receptor; TNF-α: tumor necrosis factor-α; VEGF: vascular endothelial growth factor; VEGFR: vascular endothelial growth factor receptor).

Table 9: Summary of various *in vitro* studies using MSC-conditioned medium or MSCs cocultured with cells of interest.

References	Culture conditions	Cell type	MSC source	Outcome
[44]	MSC-conditioned medium	Cardiac fibroblasts	BM	(i) Reduced collagens I and III deposit (ii) Decreased viability (iii) Decreased expression of α-SMA (iv) Increased of MMP-2 and MMP-9 activity (v) Increased expression of MT1-MMP (vi) Decreased expression of TIMP-2 MMP-2 −/− MSC-conditioned medium: (i) No change in collagen concentration Incubation with anti-HGF antibody: (i) Reduced MMP-2 and MMP-9 activity (ii) Decreased expression of MMP-2 (iii) Increased expression of TIMP-2
[64]	MSC-conditioned medium	TGF-β1-treated HK2	BM	(i) Decreased concentration of α-SMA (ii) Increased concentration of E-cadherin
[51]	Coculture: MSCs	Fibrotic hepatocytes	BM	Increased secretion of FGF2
[73]	Coculture: MSCs in Transwell	HPMCs	BM	(i) Decreased expression of TGF-β1 (ii) Decreased expression of fibronectin (iii) Decreased concentration of pSmad2 (iv) Decreased expression of α-SMA
[77]	Coculture LPS + IFN-γ-treated MSCs	Activated macrophages	BM	(i) Reduced concentration of TNF-α and IL-12 (ii) Reduced concentration of NO
[62]	MSCs in Transwell Pretreatment of one or both cell types with HSA	PTECs	BM	(i) Reduced expression of TNF-α, IL-6, IL-8, MCP-1, and CCL-5 (ii) Inhibition of NF-κB nuclear translocation (iii) Reduced EMT (iv) Increased expression and concentration of HGF and TSG-6 by MSCs exposed to HSA (v) Recombinant HGF or TSG-6 partially reproduces MSCs'effects

Influence of culture conditions on the outcome. Outcomes are expressed compared to control groups (i.e., groups treated without the use of MSC treatment) unless stated otherwise (α-SMA: α-smooth muscle actin; BM: bone marrow; CCL: chemokine ligand; EMT: epithelial-to-mesenchymal transition; FGF: fibroblast growth factor; HGF: hepatocyte growth factor; HK2: human kidney 2; HPMC: human peritoneal mesothelial cells; HAS: human serum albumin; HGF: hepatocyte growth factor; IFN-γ: interferon-γ; IL: interleukin; LPS: lipopolysaccharide; MCP: monocyte chemoattractant protein; MMP: matrix metalloproteinase; MSC: mesenchymal stromal cell; NF-κB: nuclear factor kappa-light-chain-enhancer of activated B-cells; NO: nitric oxide; proximal tubular epithelial cell; pSmad: phosphorylated small mothers against decapentaplegic homolog; TGF-β: transforming growth factor-β; TIMP: tissue inhibitor of metalloproteinase; TNF-α: tumor necrosis factor-α; TSG-6: TNF-stimulated gene 6).

macrophages, this protein induces a change in the TGF-β1/TGF-β3 balance, from a profibrotic high ratio to an antifibrotic low ratio [77]. These results are confirmed in a coculture model in which recombinant TSG-6 partially reproduced the effects of MSCs [62].

4.3. Hypoxia/Oxidative Stress. Accumulation of ECM in the tissue, death of endothelial cells, and increased levels of reactive oxygen and nitrogen species (ROS and RNS, resp.) result in hypoxia and oxidative stress during fibrosis. These factors lead to increased apoptosis and activation of TGF-β1. The improved vascularization of tissue and a more effective neutralization of oxidizing radicals would therefore enhance the effectiveness of antifibrotic therapies.

MSCs' ability to relieve oxidative stress has already been shown in several works. First, they seem to increase the expression and concentration of enzymes responsible for scavenging free radicals, such as NQO1 (NADPH quinone oxidoreductase 1), Gr (glutathione reductase), GPx (glutathione peroxidase), and HO-1 (heme oxygenase 1) [85, 86]. Nrf2 (nuclear factor (erythroid-derived 2)-like 2) activation is protective against oxidative stress and induces SOD (superoxide dismutase) production which decreases ROS concentration in the liver. MSC treatment correlates with an increase in Nrf2 and SOD which might reduce ROS accumulation, thus decreasing oxidative stress [87]. In a coculture model, an increased survival of cerebellar neurons is correlated with the secretion of SOD3 by MSCs [88].

MSC-mediated angiogenesis has also been demonstrated. MSCs are able to secrete a large range of angiogenic factors such as VEGF (vascular endothelial growth factor), FGF-2 (fibroblast growth factor-2), and MCP-1 [89–91]. Some studies also suggest their ability to promote endothelial cell proliferation [92, 93]. The reduced expression of VEGF, associated with improved microcirculation in the tissue after MSC transplantation, was observed [53]. Mias et al. showed a stimulation of angiogenesis following treatment with MSCs

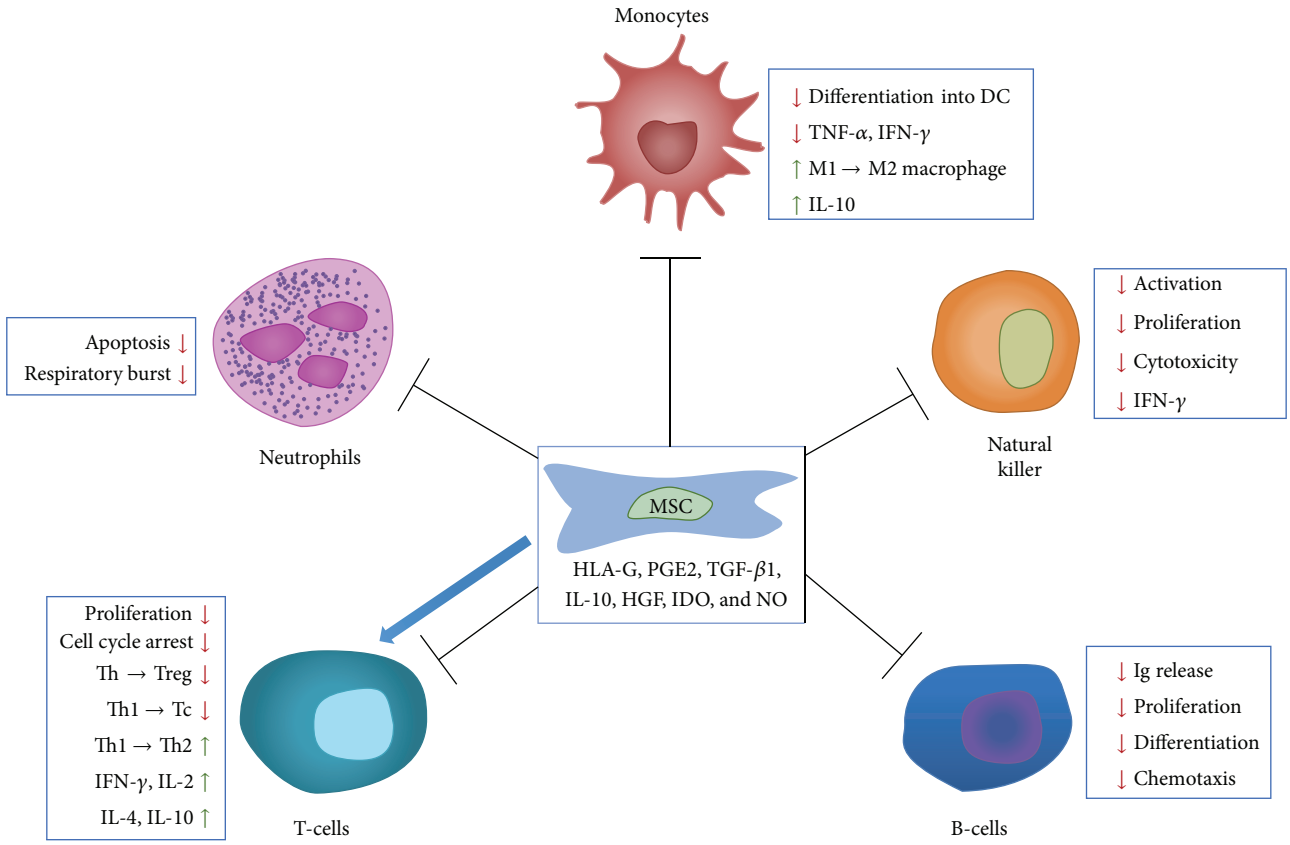


Figure 3: MSCs exert various effects on immune cells. A summary of MSC-mediated effects on the immune response. Various factors secreted by MSC exert an inhibitory effect on cells of the immune system which are involved in the fibrotic process (HGF: hepatocyte growth factor, HLA: human leukocyte antigen, IDO: indoleamine 2,3-dioxygenase, IFN-γ: interferon-γ, Ig: immunoglobulin, IL: interleukin, MSC: mesenchymal stromal cell, NO: nitric oxide, PGE2: prostaglandin E2, Tc: cytotoxic T-cell, TGF-β: transforming growth factor-β, TNF-α: tumor necrosis factor-α, Th: helper T-cell, and Treg: regulatory T-cell).

[44]. The transplantation of MSC sheets into the scarred myocardium increased neovascularization in a myocardial infarction model [45]. The authors also reported evidences of MSCs differentiating to participate in the formation of new vascular structures.

Conversely, an increased expression of VEGF posttreatment, with the concomitant overexpression of HIF-1α, was shown in a renal fibrosis model, indicating elevated tissue hypoxia [61]. HIF-1α (hypoxia-inducible factor-1α) stimulates the expression of VEGF under hypoxic conditions. In the same way, in a radiation-induced proctitis model, the overexpression of VEGF was accompanied by a reduction in angiopoietin and PDGF expression [40]. It can be hypothesized that insufficient angiogenesis in these models induces these variations. This gene expression profile may reflect proangiogenic signals mediated by MSCs. The evaluation of tissue vascularization would give better insights into MSCs effect on angiogenesis in these models.

MSCs may therefore act in different ways on hypoxia and oxidative stress by increasing angiogenesis in the tissue and by improving the inactivation of ROS and RNS. This feature, contributing to the inhibition of LAP cleavage from TGF-β1 and reduction of apoptosis, could contribute to MSCs' antifibrotic effects.

4.4. Matrix Remodeling. Excess production of ECM and the failure to degrade it are the hallmark of fibrosis. Thus, the ultimate goal in case of fibrotic diseases is to restore a nonpathological healing process, by inhibiting ECM production and enabling the degradation of its various components. Indeed, the imbalance of MMPs, responsible for the degradation of ECM, and TIMPs, their inhibitor, results in improper ECM remodeling, hence preventing restoration to a nonpathological matrix.

In different fibrosis models, a decreased expression and concentration of collagen, the main component of the ECM, were found after MSC transplantation [41, 43–46, 48–53, 56, 58, 59, 64–68, 71, 73, 76]. This effect is also obtained after transplanting microvesicles or exosomes secreted into an MSC culture, suggesting a paracrine control of MSCs on ECM degradation [47, 69].

Changes in the expression and concentration of MMPs and TIMPs have also been studied. After MSC transplantation, the increased expression of MMP-2, MMP-9, MMP-13, and MMP-14 has been observed in several fibrosis models [48, 65, 75]. Following the addition of MSC-conditioned culture medium to a culture of heart fibroblasts, an increase in the activity of MMP-2 and MMP-9 was found [44]. Conversely, several studies have shown reduced expression,

concentration, or activity of MMPs. Accordingly, Alfrano et al. noted the decreased activity of MMP-2 after transplantation in the NIRC model [64]. In some fibrosis models, MMP-2, MMP-9, and MMP-13 have a lower expression and concentration following treatment with MSCs [43, 61, 68]. However, these variations suggest restoration to levels similar to untreated controls.

MSCs seem to have a repressive effect on the expression of TIMPs such as TIMP-1 [50, 65]. A reduction in the concentration of TIMP 1 to 4 was shown after MSC transplantation [67]. In an *in vitro* model, a decrease in the expression of TIMP-2 was observed, suggesting that MSCs have a paracrine effect [44]. Finally, Linard et al. demonstrated a tendency toward the resolution of fibrosis by calculating the collagen-to-MMP-to-TIMP ratio, a marker of fibrosis evolution [40, 94].

MMP and TIMP expression are impaired in fibrotic pathologies. In fact, lower TIMP expression is generally associated with fibrosis resolution. In cases of heart failure, an increased expression of MMPs has been observed in the initial and final phase [95, 96]. It has been shown that increased MMP-2 activity is associated with pathological ECM remodeling in the kidney [97]. Thus, decreased activity following MSC therapy suggests a transition to a nonpathological state. On the contrary, it has been shown that MMP-2 is implicated in alveolar regeneration, which could explain its increased activity after transplantation in a pulmonary fibrosis model [98]. Finally, as certain MMPs activate latent TGF-β1, a decrease in their concentration would result in a lesser activation of downstream effectors. Taken together with a decreased fibrotic area and ECM component (collagen, fibronectin, etc.) expression, these results indicate a change in ECM composition, close to that observed in nonpathological animals. Hence, MSCs seem to improve ECM quality, allowing the appearance of a microenvironment favorable to tissue regeneration.

4.5. Transplantation Conditions. Various transplantation conditions have been assessed in the studies reported in this work including MSC activation and the optimization of MSC delivery. First, melatonin has been shown to improve MSC survival after transplantation, as well as having proangiogenic abilities [99, 100]. In both occurrences of this treatment, melatonin-treated MSCs exerted increased beneficial effects compared to nontreated cells, as evidenced by reduced ECM deposit and inflammation [44, 63]. Qiao et al. showed potentiation in predifferentiated MSCs treated with baicalin, which possesses anti-inflammatory and antioxidant properties [58]. Cotreatment with atorvastatin increased the survival and efficacy of MSCs [41].

Multiple transplantation timings have been compared to investigate their respective effect. Alfarano et al. showed that transplantation 7 days after ischemia-reperfusion was more effective on ECM deposition, myofibroblast proliferation, and MMP activity in their model compared to transplantation after 14 days [64]. In bleomycin-induced lung fibrosis, Ortiz et al. also observed greater effectiveness from MSCs when transplanted earlier [68].

Interestingly, Ishikane et al. demonstrated that the transplantation of fetal membrane or bone-marrow-derived MSCs yielded similar results on myocardial infarction [45].

In two different studies, the value of MSC differentiation before transplantation was observed. In the rat model of CCl_4-induced fibrosis, opposite effects were reported. Hardjo et al. showed a higher potential for nondifferentiated MSCs, compared to adipogenic and hepatogenic differentiation, on ECM accumulation and MMP expression [57]. Conversely, in the exact same model, Qiao et al. found that hepatogenic predifferentiation had no significant influence on the effect of MSCs [58].

Recently, new delivery procedures have been studied to improve MSCs engraftment in fibrotic tissues. MSCs grown in two-layered sheets and transplanted in a rat model of myocardial infarction were found in significant number 28 days after transplantation. Part of these cells showed evidences of differentiation, participating in neovascularization of the infarct [45]. Indeed, MSC homing in the damaged tissue is generally transient, which could explain the decreased long-term benefit often observed. Embedding MSCs in scaffolds or biomaterials could improve their beneficial effects [101, 102].

5. MSC Clinical Trials

In clinical settings, the transplantation of MSCs has been studied on numerous pathologies. A systematic review of clinical trials evaluated the safety of MSC injections. Thirty-six studies were included representing 1012 patients. The meta-analysis did not reflect any serious complications related to MSC injections. Only a transient fever was highlighted (reviewed in [103]). Around 30 clinical trials are currently registered worldwide for evaluating MSC therapy for fibrosis (http://clinicaltrials.gov). Liver and pulmonary fibrosis are most widely represented, but some occurrences of renal and vocal fold treatment exist. MSCs engraft preferentially in the lungs and liver which is the reason for a higher number of clinical trials on these organs [104]. In most of these studies, only organ functionality is evaluated but not fibrosis markers. Thus, it is not clear whether the improvement of the symptoms and quality of life is due to fibrosis reduction or the amelioration of other pathological features.

Bone-marrow-derived MSCs improve liver function in patients with liver cirrhosis as evidenced by phase I clinical trials [105–107]. The Model for End-stage Liver Disease (MELD) score is used to evaluate the mortality risk in patients with end-stage liver disease (reviewed in [108]). The mean MELD score is significantly lower after MSC injection compared to placebo controls. In patients with decompensated liver fibrosis, MSCs significantly improved quality of life as evidenced by the increase in physical and mental component scales [105] and through the SF-36 questionnaire [109]. Inducing hepatic differentiation prior to MSC injection improved liver function in treated patients [106]. Finally, fibrosis markers were measured on 30 patients during a phase I trial [107]. Laminin, hyaluronic acid, and type IV collagen

were significantly decreased 48 weeks after intervention. On the other hand, HGF, an antifibrotic growth factor, was increased after 48 weeks, as compared to nontreated patients. Based on these clinical trials [107], it appears that MSCs may exert an antifibrotic effect on liver cirrhosis.

The results of a phase I study show the ability of MSCs to reduce allograft rejection after renal transplantation [110]. MSCs decreased graft rejection by exerting immunosuppression and probably by preventing interstitial fibrosis. The absence of a placebo control in this trial did not permit the comparison and identification of the specific effect of MSCs. Thus, it is necessary to gather additional clinical data.

MSC therapy has proven to be effective in patients suffering from complications following acute myocardial infarction [111, 112]. In the first trial, functional testing showed an improvement in both heart and lung functions. There was evidence that MSC treatment led to reverse remodeling, which could be correlated with fibrosis reduction [113]. Six months after treatment, global symptom scores were significantly better in the MSC group versus the placebo group [111]. In the second study, MSC treatment reduced symptoms associated with ischemic cardiomyopathy. There was also evidence of reverse remodeling concomitant with infarct size reduction, probably linked to reduced fibrosis [112].

Pelvic radiation disease (PRD) is induced in 5 to 10% of patients within 10 years after abdominopelvic radiotherapy. Fibrosis to the colon and rectum is the main characteristic of late complications of radiotherapy. Since no satisfactory treatment exists for PRD and given the results of MSC therapy on radiation-induced burns [114], the curative potential of MSCs is being evaluated in clinical trials for PRD treatment. In particular, 4 patients suffering from serious intestinal radiation-induced lesions following overdosage of radiotherapy have been treated. The systemic administration of MSCs resulted in efficient analgesic and anti-inflammatory effects as well as hemorrhage reduction [3]. These results indicate the potential of MSC to diminish the adverse effect of radiotherapy and possibly radiation-induced fibrosis.

Based on these clinical trials, MSC therapy has proven to be safe and effective in patients suffering from diseases associated with fibrosis without the adverse effect of MSC transplantation. Nevertheless, there is a need for randomized trials (phase 3) to gather statistically significant data and to demonstrate MSCs' efficacy in limiting fibrosis.

6. MSC Therapy versus the Current Management of Fibrosis

The future of MSC therapy for fibrotic diseases mostly relies on a comparison with current management strategies. Results from preclinical and clinical trials highlight the ability of MSCs to act on fibrosis through different mechanisms: (i) immunosuppression, (ii) inhibition of the TGF-β1 pathway, (iii) reduction of hypoxia and oxidative stress, and (iv) restoration of ECM degradation. Thus, the potential of MSC therapy lies in the ability to act simultaneously on various fibrogenesis parameters. There are currently several therapy protocols for fibrotic therapies under assessment in clinical trials. Most of those treatments are designed to act on a single pathway underlying fibrosis development and progression, unlike cell therapy.

Presently, therapy protocols for fibroproliferative diseases mostly consist of symptomatic treatments. For example, patients with idiopathic pulmonary fibrosis (IPF) are often prescribed oxygen therapy and vaccination against viral and bacterial infections of the airways is recommended, if any exists. Likewise, antifibrotic strategies in the liver are most effective when they are able to cure the underlying disease. Many anti-inflammatories and antioxidants have been unsuccessful candidates for fibrosis treatment [115]. Ultimately, organ transplantation is required to ensure the survival of patients with fibrosis.

The first example of clinically used pharmacological antifibrotic agent is pirfenidone, which acts on TGF-β1 activity and inflammation and which has antioxidative properties [116]. It has been approved for the treatment of IPF in Europe, Canada, South Korea, and Japan. Preclinical studies have shown its ability to suppress TGF-β1 gene expression and to significantly reduce its concentration in lavage fluid in models of pulmonary fibrosis [117]. Pirfenidone is also effective in animal models of heart [118], kidney [119], liver [120], and radiation-induced fibrosis [121]. The FDA has not yet approved pirfenidone for pulmonary fibrosis based on a lack of efficacy and survival benefit, especially in long-term clinical trials [122]. Moreover, a meta-analysis of clinical trial results shows that pirfenidone induces adverse gastrointestinal, neurological, and dermatological adverse effects [123].

Other antifibrotic drugs are currently being examined for clinical use (reviewed in [6, 124]). Those pharmacological agents are mainly anti-inflammatory drugs and inhibitors of the TGF-β1 signaling pathway acting on different molecular targets. Despite the fact that some of these drugs have been evidenced to exert antifibrotic effects in animal models, there is a lack of clinical data that may lead to their approval.

Although some pharmacological compounds have proven to be effective, the necessity to use multiple drugs for the treatment of fibrosis is increasingly recognized. Furthermore, MSCs specifically home to damaged tissues and are able to behave depending on the surrounding environment, delivering transiently and locally specific molecules necessary for restoring tissue homeostasis. Conversely, drugs affect every organ, regardless of its pathological state. There is a need for more clinical data on MSC therapy to ascertain its effectiveness and safety. However, while inducing minor side effects, MSCs have shown promising antifibrotic effects, regardless of the organ, and should be considered as a major candidate.

7. Conclusion

Altogether, the objective analysis of the literature supports the antifibrotic effect of MSCs. It is sometimes argued that MSCs could have profibrotic properties because they are likely to acquire a myofibroblastic phenotype *in vitro* [125]

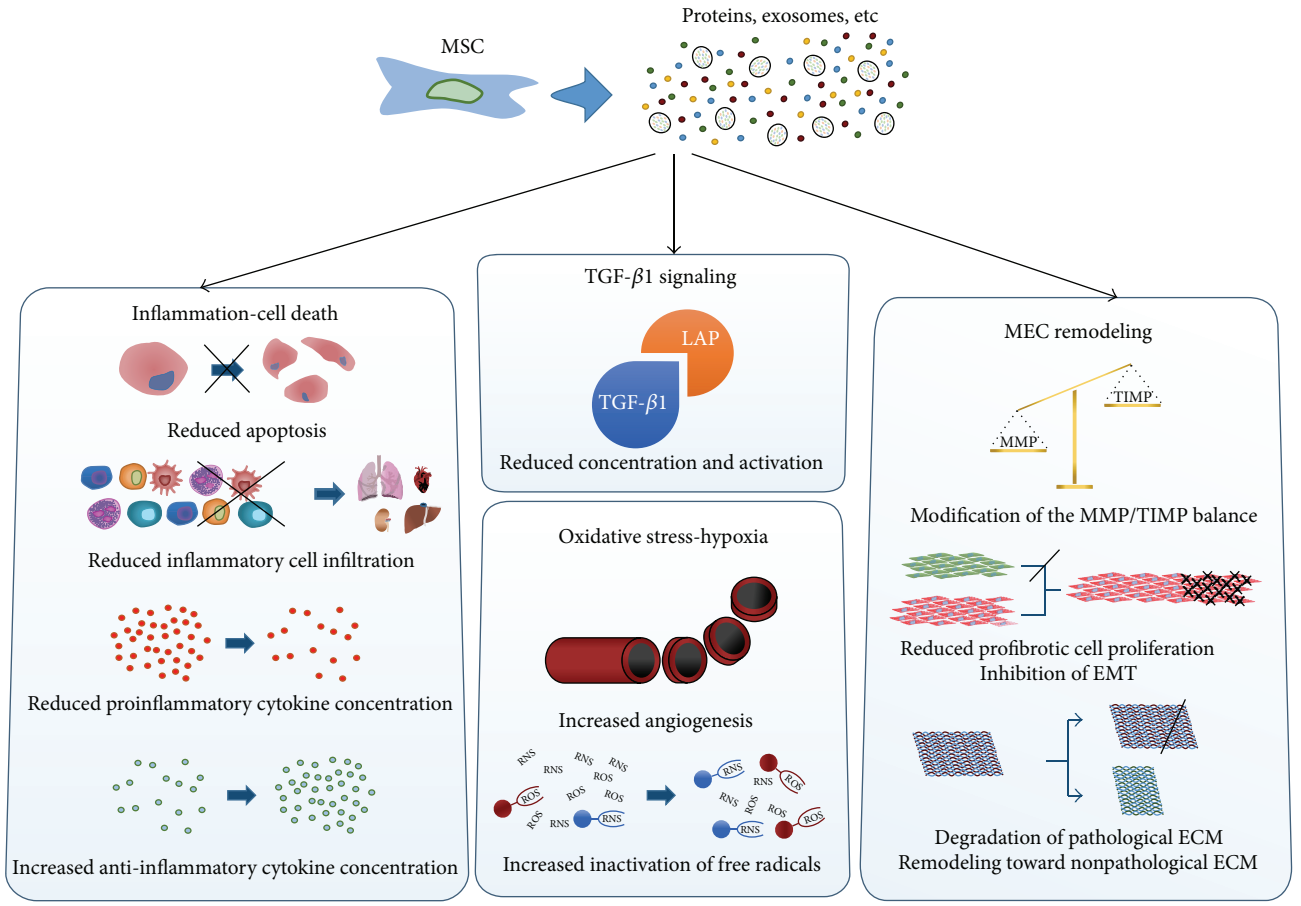


Figure 4: Common outcome of MSC therapy for various fibrotic diseases. Based on the studies reported in this work, several mechanisms have been underlined, mostly concerning inflammatory reaction and apoptosis, oxidative stress/hypoxia modulation, and extracellular matrix remodeling. It appears that MSC secretome activates a wide range of antifibrotic pathways (ECM: extracellular matrix, EMT: epithelial-to-mesenchymal transition, LAP: latency associated protein, MMP: matrix metalloproteinase, MSC: mesenchymal stromal cell, TGF-β: transforming growth factor-β, and TIMP: tissue inhibitor of metalloproteinase).

or that the mesenchymal origin of myofibroblasts [126] indicates profibrotic properties. Nevertheless, there is, to our knowledge, no example showing MSC transplantation to have a profibrotic effect on a developing or established disease.

Since fibrosis is a very complex multicomponent process, it can be hypothesized that MSCs act through different secreted factors on multiple pathways (Figure 4). This assumption is supported by the fact that the role of transplanted MSC depends mainly on the surrounding environment.

The principal mode of action of MSCs may be exerted mainly through inhibition of the TGF-β1 signaling pathway, mainly by blocking the passage from its latent form to its active form. The reduction of inflammation in the tissue, the improvement of angiogenesis, and the reduced oxidative stress seem to be responsible for this effect. The decrease in the concentration of activated TGF-β1 would lead to reduced EMT and myofibroblast proliferation, consequently shifting the balance between synthesis and degradation of the ECM. Furthermore, results suggest that MSCs possess the ability to inhibit TGF-β1 mRNA as well as protein synthesis [47, 58]. Thus, they would act on two different levels, preventing injury-triggered TGF-β1 overexpression and modifying the surrounding microenvironment to lessen the concentration of TGF-β1-activating factors.

Another interesting and extensively studied feature of MSC therapy against fibrotic diseases is their immunomodulatory ability. In numerous studies reported here, MSCs seem to reduce immune cell homing in the damaged tissue [61, 70]. This could in part explain the decrease in proinflammatory cytokines mRNA expression and production. Most notably, TNF-α and IFN-γ, two major profibrotic cytokines, were underexpressed in several studies reported here [66, 67]. These observations are consistent with the implementation of an antifibrotic "virtuous circle" in which fewer immune cells migrate to damaged tissues, hence reducing proinflammatory cytokines production. By inhibiting the acute inflammatory reaction, it is conceivable that MSCs reduce the consequent chronic inflammation.

Reduced hypoxia and oxidative stress are also an important effect of MSCs in this context [52, 77]. In fact, high ROS and RNS concentrations, combined with low oxygen

intake, further increase TGF-β1 activation. It also induces apoptosis in resident cells, resulting in increasingly elevated inflammation. The ability of MSCs to improve the neutralization of free radicals, already described in other models, is supplemented by indications of improved angiogenesis [44, 45]. The resulting improvement in tissue vasculature reduces ischemia, allowing better regeneration of the injured organ.

As expected, the inhibition of the TGF-β1 signaling pathway induces a substantial remodeling of the ECM toward a nonpathological state. The decreased expression and concentration of ECM components, associated with the restoration of the MMP/TIMP balance, improve the quality of the connective tissue [43, 64]. This can mostly be explained by a lower profibrotic cell population (myofibroblasts mainly). This allows for better homing of the cell types necessary for regeneration of the damaged tissue, suggesting the possibility of reversing fibrosis under the influence of MSCs.

MSCs seem to have a paracrine effect highlighted by the results obtained in studies using MSC-conditioned medium [42, 44]. Several factors have been put forward as mediating this effect. First, HGF, an antifibrotic mediator which also has antiapoptotic properties, should be mentioned. MSC therapy combined with antibodies against HGF greatly reduces the effects of the treatment and recombinant HGF administration partially reproduces the effects of MSCs [44, 62]. The treatment of fibrosis by HGF has already been assessed in earlier studies and has shown great potential [127]. Moreover, TSG-6, a recently discovered protein highlighted for its immunosuppressant effect, seems to play a major role in the antifibrotic action of MSCs [62]. The use of antibodies or gene silencing methods significantly reduces MSCs' ability to alleviate fibrosis. Indeed, TSG-6 has been demonstrated to inhibit the secretion of TNF-α by macrophages and to alter the TGF-β1/TGF-β3 balance toward an antifibrotic ratio [77].

The study of MSC transplantation conditions also needs extensive investigation. Data reported in this paper indicate that the pretreatment of MSCs to potentiate their effect may yield better outcomes. Equally, transplantation timing after injury is of great importance. In fact, results suggest that earlier therapies improve the efficacy of MSCs on fibrosis [49, 64]. This is to be expected, as inhibition of the acute inflammatory reaction by MSCs would prevent the onset of chronic inflammation. MSC source is also an important factor to be considered. It has been reported in this paper that fetal membrane and bone-marrow-derived MSCs were equally effective [45]. The comparison between different sources of MSCs is an important matter considering the fact that some tissues, such as adipose tissue, are easier to harvest and/or contain higher numbers of stem cells. Moreover, the value of predifferentiation is to be further investigated as contrary data have been gathered. In any case, supplementary studies need to be conducted to confirm these effects. Finally, although preclinical data suggest the strong antifibrotic effect of MSCs [41, 57, 70], most studies were carried on the early stages of fibrosis development. Since fibrosis is often diagnosed in more advanced phases, assessment of the effects of MSCs on established fibrosis is required in order to consider the routine use of MSC therapy on such pathologies.

These observations highlight the great potential of MSCs in the treatment of fibrotic diseases. Given these results, MSCs seem to act in the same way, regardless of the organ, and no occurrence of profibrotic effects has been reported. However, the mechanisms by which MSCs act on fibrosis have not yet been clearly elucidated and additional studies are needed. Besides, concerns about effects promoting certain pathologies, such as cancer, are still preventing their routine clinical use. Thus, emphasizing many pathways triggered by MSC homing is of great importance. Furthermore, the regulation of phenotypic changes in MSCs needs to be thoroughly evaluated. As described previously, exposing MSCs to profibrotic stimuli may trigger various changes in their secretome, probably leading to variable responses. Understanding the relative implication of the factors influencing MSC phenotype would provide valuable insight into potentiation and possible adverse effects. In addition, it has been shown that microvesicles or exosomes secreted by MSCs partially reproduce their effect [47, 69]. Describing their composition and elucidating the triggers influencing their content are essential. The importance of MSC homing to damaged tissues also needs to be addressed, mostly in terms of cell-to-cell contacts and microenvironment influence. Although few reports show the importance of engraftment and the differentiation of MSCs [45], these processes are likely to play a role in the beneficial effects of cell therapy. Also, optimal treatment protocols remain to be established. First, the timing of MSC transplantation surely influences the success of the therapy. The immunomodulatory effect of MSCs should in fact be most effective when transplantation is undergone during the acute inflammatory reaction to prevent the installation of chronic inflammation. Based on the results reported in this review, it is unclear whether MSCs could reverse fibrosis in its more advanced stage and fully restore tissue homeostasis. Nonetheless, MSC therapy for the treatment of fibrosis in any organ should be strongly considered and studied as it shows promising potential.

Abbreviations

5/6 NX: 5/6 nephrectomy
α-SMA: α-Smooth muscle actin
AM: Amniotic membrane
ARAS: Atherosclerotic renal artery stenosis
AT: Adipose tissue
BAX: Bcl-2-associated X protein
Bcl-2: B-cell lymphoma 2
BM: Bone marrow
BSA: Bovine albumin serum
CAN: Chronic allograft nephropathy
CCL: Chemokine ligand
CCl_4: Carbon tetrachloride
CG: Chlorhexidine gluconate
CKD: Chronic kidney disease
Col: Collagen
CsA: Cyclosporine A
CTGF: Connective tissue growth factor
DBTC: Dibutyltin dichloride
DC: Dendritic cell

DOX: Doxorubicin
ECM: Extracellular matrix
EMT: Epithelial-to-mesenchymal transition
EndMT: Endothelial-to-mesenchymal transition
EPS: Encapsulating peritoneal sclerosis
FGF: Fibroblast growth factor
FLK: Fetal liver kinase
FM: Fetal membrane
FSP: Fibroblast specific protein
GPx: Glutathione peroxidase
Gr: Glutathione reductase
HGF: Hepatocyte growth factor
HIF: Hypoxia-inducible factor
HK2: Human kidney 2
HLA: Human leukocyte antigen
HO-1: Heme oxygenase 1
HPMC: Human peritoneal mesothelial cells
HSA: Human serum albumin
HSC: Hepatic stellate cell
ICAM: Intercellular adhesion molecule
IDO: Indoleamine 2,3-dioxygenase
IFN-γ: Interferon-γ
Ig: Immunoglobulin
IGF: Insulin-like growth factor
IL: Interleukin
IPF: Idiopathic pulmonary fibrosis
ISO: Isoproterenol
IV: Intravenous
LAP: Latency associated protein
LPS: Lipopolysaccharide
MCP: Monocyte chemoattractant protein
MI: Myocardial infarction
MMP: Matrix metalloproteinase
MSC: Mesenchymal stromal cell
NF-κB: Nuclear factor kappa-light-chain-enhancer of activated B-cells
NIRC: Nephrectomy + ischemia-reperfusion + cyclosporine
NK: Natural killer
NO: Nitric oxide
NOS: Nitric oxide synthase
NQO1: NADPH quinone oxidoreductase 1
Nrf2: Nuclear factor (erythroid-derived 2)-like 2
PDGF: Platelet-derived growth factor
pSmad: Phosphorylated small mothers against decapentaplegic homolog
PGE2: Prostaglandin E2
PTEC: Proximal tubular epithelia cell
RKM: Remnant kidney model
RNS: Reactive nitrogen species
ROS: Reactive oxygen species
R-UUO: Reversible unilateral ureteral obstruction
Smad: Small mothers against decapentaplegic homolog
SNP: Sodium nitroprusside
SOD: Superoxide dismutase
Tc: Cytotoxic T-cell
TGF-β: Transforming growth factor-β
Th: Helper T-cell
TGF-βR: Transforming growth factor-β receptor
TIMP: Tissue inhibitor of metalloproteinase
TLR: Toll-like receptor
TNF-α: Tumor necrosis factor-α
Treg: Regulatory T-cell
TSG-6: TNF-stimulated gene 6
UC: Umbilical cord
UUO: Unilateral ureteral obstruction
VCAM: Vascular cell adhesion molecule
VEGF: Vascular endothelial growth factor
VEGFR: Vascular endothelial growth factor receptor.

Conflict of Interests

The authors declare that they have no conflict of interests regarding the publication of this paper.

References

[1] V. Kumar, A. K. Abbas, and N. Fausto, "Tissue renewal and repair: regeneration, healing, and fibrosis," in *Pathologic Basis of Disease*, V. K. A. K. Abbas and N. Fausto, Eds., Elsevier Saunders, Philadelphia, Pa, USA, 2005.

[2] T. A. Wynn, "Cellular and molecular mechanisms of fibrosis," *Journal of Pathology*, vol. 214, no. 2, pp. 199–210, 2008.

[3] A. Chapel, S. Francois, L. Douay, M. Benderitter, and J. Voswinkel, "New insights for pelvic radiation disease treatment: multipotent stromal cell is a promise mainstay treatment for the restoration of abdominopelvic severe chronic damages induced by radiotherapy," *World Journal of Stem Cells*, vol. 5, no. 4, pp. 106–111, 2013.

[4] J. Voswinkel, S. Francois, N. Gorin, and A. Chapel, "Gastrointestinal autoimmunity: Preclinical experiences and successful therapy of fistulizing bowel diseases and gut Graft versus host disease by mesenchymal stromal cells," *Immunologic Research*, vol. 56, no. 2-3, pp. 241–248, 2013.

[5] S. C. Schwarz and J. Schwarz, "Translation of stem cell therapy for neurological diseases," *Translational Research*, vol. 156, no. 3, pp. 155–160, 2010.

[6] T. A. Wynn and T. R. Ramalingam, "Mechanisms of fibrosis: therapeutic translation for fibrotic disease," *Nature Medicine*, vol. 18, no. 7, pp. 1028–1040, 2012.

[7] G. Gabbiani, "The biology of the myofibroblast," *Kidney International*, vol. 41, no. 3, pp. 530–532, 1992.

[8] T. A. Wynn, "Common and unique mechanisms regulate fibrosis in various fibroproliferative diseases," *Journal of Clinical Investigation*, vol. 117, no. 3, pp. 524–529, 2007.

[9] Q. Yu and I. Stamenkovic, "Cell surface-localized matrix metalloproteinase-9 proteolytically activates TGF-β and promotes tumor invasion and angiogenesis," *Genes and Development*, vol. 14, no. 2, pp. 163–176, 2000.

[10] M. H. Barcellos-Hoff and T. A. Dix, "Redox-mediated activation of latent transforming growth factor-β1," *Molecular Endocrinology*, vol. 10, no. 9, pp. 1077–1083, 1996.

[11] R. M. Lyons, J. Keski-Oja, and H. L. Moses, "Proteolytic activation of latent transforming growth factor-β from fibroblast-conditioned medium," *Journal of Cell Biology*, vol. 106, no. 5, pp. 1659–1665, 1988.

[12] E. J. Ehrhart, P. Segarini, M. L.-S. Tsang, A. G. Carroll, and M. H. Barcellos-Hoff, "Latent transforming growth factor β1 activation in situ: quantitative and functional evidence after low-dose γ-irradiation," *The FASEB Journal*, vol. 11, no. 12, pp. 991–1002, 1997.

[13] F. Verrecchia, M. Chu, and A. Mauviel, "Identification of Novel TGF-β/Smad Gene Targets in Dermal Fibroblasts using a Combined cDNA Microarray/Promoter Transactivation Approach," *Journal of Biological Chemistry*, vol. 276, no. 20, pp. 17058–17062, 2001.

[14] S. N. Flier, H. Tanjore, E. G. Kokkotou, H. Sugimoto, M. Zeisberg, and R. Kalluri, "Identification of epithelial to mesenchymal transition as a novel source of fibroblasts in intestinal fibrosis," *Journal of Biological Chemistry*, vol. 285, no. 26, pp. 20202–20212, 2010.

[15] E. M. Zeisberg, O. Tarnavski, M. Zeisberg et al., "Endothelial-to-mesenchymal transition contributes to cardiac fibrosis," *Nature Medicine*, vol. 13, no. 8, pp. 952–961, 2007.

[16] F. Rieder and C. Fiocchi, "Intestinal fibrosis in IBD—a dynamic, multifactorial process," *Nature Reviews Gastroenterology and Hepatology*, vol. 6, no. 4, pp. 228–235, 2009.

[17] S. Barrientos, O. Stojadinovic, M. S. Golinko, H. Brem, and M. Tomic-Canic, "Growth factors and cytokines in wound healing," *Wound Repair and Regeneration*, vol. 16, no. 5, pp. 585–601, 2008.

[18] J. G. Abreu, N. I. Ketpura, B. Reversade, and E. M. De Robertis, "Connective-tissue growth factor (CTGF) modulates cell signalling by BMP and TGF-β," *Nature Cell Biology*, vol. 4, no. 8, pp. 599–604, 2002.

[19] M. R. Duncan, K. S. Frazier, S. Abramson et al., "Connective tissue growth factor mediates transforming growth factor β-induced collagen synthesis: Downregulation by cAMP," *FASEB Journal*, vol. 13, no. 13, pp. 1774–1786, 1999.

[20] B. S. Weston, N. A. Wahab, and R. M. Mason, "CTGF mediates TGF-β-induced fibronectin matrix deposition by upregulating active $\alpha5\beta1$ integrin in human mesangial cells," *Journal of the American Society of Nephrology*, vol. 14, no. 3, pp. 601–610, 2003.

[21] E. H. Choi, N. Lee, H. J. Kim et al., "Schisandra fructus extract ameliorates doxorubicin-induce cytotoxicity in cardiomyocytes: altered gene expression for detoxification enzymes," *Genes and Nutrition*, vol. 2, no. 4, pp. 337–345, 2008.

[22] J. R. Teerlink, J. M. Pfeffer, and M. A. Pfeffer, "Progressive ventricular remodeling in response to diffuse isoproterenol-induced myocardial necrosis in rats," *Circulation Research*, vol. 75, no. 1, pp. 105–113, 1994.

[23] T. Nishikimi, K. Uchino, and E. D. Frohlich, "Effects of α1-adrenergic blockade on intrarenal hemodynamics in heart failure rats," *The American Journal of Physiology: Regulatory Integrative and Comparative Physiology*, vol. 262, no. 2, part 2, pp. R198–R203, 1992.

[24] A. L. Cochrane, M. M. Kett, C. S. Samuel et al., "Renal structural and functional repair in a mouse model of reversal of ureteral obstruction," *Journal of the American Society of Nephrology*, vol. 16, no. 12, pp. 3623–3630, 2005.

[25] V. H. Urbieta-Caceres, R. Lavi, X.-Y. Zhu et al., "Early atherosclerosis aggravates the effect of renal artery stenosis on the swine kidney," *The American Journal of Physiology*, vol. 299, no. 1, pp. F135–F140, 2010.

[26] L. O. Lerman, R. S. Schwartz, J. P. Grande, P. F. Sheedy II, and J. C. Romero, "Noninvasive evaluation of a novel swine model of renal artery stenosis," *Journal of the American Society of Nephrology*, vol. 10, no. 7, pp. 1455–1465, 1999.

[27] I. Herrero-Fresneda, J. Torras, A. Vidal, N. Lloberas, J. M. Cruzado, and J. M. Grinyó, "Reduction of postischemic immune inflammatory response: an effective strategy for attenuating chronic allograft nephropathy," *Transplantation*, vol. 79, no. 2, pp. 165–173, 2005.

[28] R. Chaaya, C. Alfarano, C. Guilbeau-Frugier et al., "Pargyline reduces renal damage associated with ischaemia-reperfusion and cyclosporin," *Nephrology Dialysis Transplantation*, vol. 26, no. 2, pp. 489–498, 2011.

[29] C. Fleck, D. Appenroth, P. Jonas et al., "Suitability of 5/6 nephrectomy (5/6NX) for the induction of interstitial renal fibrosis in rats: influence of sex, strain, and surgical procedure," *Experimental and Toxicologic Pathology*, vol. 57, no. 3, pp. 195–205, 2006.

[30] L. W. D. Weber, M. Boll, and A. Stampfl, "Hepatotoxicity and mechanism of action of haloalkanes: carbon tetrachloride as a toxicological model," *Critical Reviews in Toxicology*, vol. 33, no. 2, pp. 105–136, 2003.

[31] C. A. Claussen and E. C. Long, "Nucleic acid recognition by metal complexes of bleomycin," *Chemical Reviews*, vol. 99, no. 9, pp. 2797–2816, 1999.

[32] B. B. Moore and C. M. Hogaboam, "Murine models of pulmonary fibrosis," *The American Journal of Physiology—Lung Cellular and Molecular Physiology*, vol. 294, no. 2, pp. L152–L160, 2008.

[33] B. J. R. Junor, J. D. Briggs, and M. A. Forwell, "Sclerosing peritonitis–the contribution of chlorhexidine in alcohol," *Peritoneal Dialysis Bulletin*, vol. 5, no. 2, pp. 101–104, 1985.

[34] T. Yamamoto, S. Takagawa, I. Katayama et al., "Animal model of sclerotic skin. I: Local injections of bleomycin induce sclerotic skin mimicking scleroderma," *Journal of Investigative Dermatology*, vol. 112, no. 4, pp. 456–462, 1999.

[35] J. A. Horton, E. J. Chung, K. E. Hudak et al., "Inhibition of radiation-induced skin fibrosis with imatinib," *International Journal of Radiation Biology*, vol. 89, no. 3, pp. 162–170, 2013.

[36] T. Peters, A. Sindrilaru, B. Hinz et al., "Wound-healing defect of $CD18^{-/-}$ mice due to a decrease in TGF-β_1 and myofibroblast differentiation," *The EMBO Journal*, vol. 24, no. 19, pp. 3400–3410, 2005.

[37] R. J. Berry, G. Wiernik, T. J. S. Patterson, and J. W. Hopewell, "Excess late subcutaneous fibrosis after irradiation of pig skin, consequent upon the application of the NSD formula," *British Journal of Radiology*, vol. 47, no. 557, pp. 277–281, 1974.

[38] N. Hazra and M. Gulliford, "Evaluating pancreatitis in primary care: a population-based cohort study," *The British Journal of General Practice*, vol. 64, no. 622, pp. e295–e301, 2014.

[39] J. Merkord, L. Jonas, H. Weber, G. Kröning, H. Nizze, and G. Hennighausen, "Acute interstitial pancreatitis in rats induced by dibutyltin dichloride (DBTC): pathogenesis and natural course of lesions," *Pancreas*, vol. 15, no. 4, pp. 392–401, 1997.

[40] C. Linard, E. Busson, V. Holler et al., "Repeated autologous bone marrow-derived mesenchymal stem cell injections improve radiation-induced proctitis in pigs," *Stem Cells Translational Medicine*, vol. 2, no. 11, pp. 916–927, 2013.

[41] L. Song, Y. J. Yang, Q. T. Dong et al., "Atorvastatin enhance efficacy of mesenchymal stem cells treatment for swine myocardial infarction via activation of nitric oxide synthase," *PLoS ONE*, vol. 8, no. 5, Article ID e65702, 2013.

[42] S. M. Gorji, A. A. K. Malekshah, M. B. Hashemi-Soteh, A. Rafiei, K. Parivar, and N. Aghdami, "Effect of mesenchymal stem cells on doxorubicin-induced fibrosis," *Cell Journal*, vol. 14, no. 2, pp. 142–151, 2012.

[43] L. Li, Y. Zhang, Y. Li et al., "Mesenchymal stem cell transplantation attenuates cardiac fibrosis associated with isoproterenol-induced global heart failure," *Transplant International*, vol. 21, no. 12, pp. 1181–1189, 2008.

[44] C. Mias, O. Lairez, E. Trouche et al., "Mesenchymal stem cells promote matrix metalloproteinase secretion by cardiac fibroblasts and reduce cardiac ventricular fibrosis after myocardial infarction," *Stem Cells*, vol. 27, no. 11, pp. 2734–2743, 2009.

[45] S. Ishikane, H. Hosoda, K. Yamahara et al., "Allogeneic transplantation of fetal membrane-derived mesenchymal stem cell sheets increases neovascularization and improves cardiac function after myocardial infarction in rats," *Transplantation*, vol. 96, no. 8, pp. 697–706, 2013.

[46] G. A. Nasir, S. Mohsin, M. Khan et al., "Mesenchymal stem cells and Interleukin-6 attenuate liver fibrosis in mice," *Journal of Translational Medicine*, vol. 11, no. 1, article 78, 2013.

[47] T. Li, Y. Yan, B. Wang et al., "Exosomes derived from human umbilical cord mesenchymal stem cells alleviate liver fibrosis," *Stem Cells and Development*, vol. 22, no. 6, pp. 845–854, 2013.

[48] V. Rabani, M. Shahsavani, M. Gharavi, A. Piryaei, Z. Azhdari, and H. Baharvand, "Mesenchymal stem cell infusion therapy in a carbon tetrachloride-induced liver fibrosis model affects matrix metalloproteinase expression," *Cell Biology International*, vol. 34, no. 6, pp. 601–605, 2010.

[49] B. Fang, M. Shi, L. Liao, S. Yang, Y. Liu, and R. C. Zhao, "Systemic infusion of FLK1+ mesenchymal stem cells ameliorate carbon tetrachloride-induced liver fibrosis in mice," *Transplantation*, vol. 78, no. 1, pp. 83–88, 2004.

[50] G. Ali, S. Mohsin, M. Khan et al., "Nitric oxide augments mesenchymal stem cell ability to repair liver fibrosis," *Journal of Translational Medicine*, vol. 10, no. 1, article 75, 2012.

[51] R. Pan, P. Wang, L. Xiang, and J. Shao, "Delta-like 1 serves as a new target and contributor to liver fibrosis down-regulated by mesenchymal stem cell transplantation," *Journal of Biological Chemistry*, vol. 286, no. 14, pp. 12340–12348, 2011.

[52] D. Zhang, M. Jiang, and D. Miao, "Transplanted human amniotic membrane-derived mesenchymal stem cells ameliorate carbon tetrachloride-induced liver cirrhosis in mouse," *PLoS ONE*, vol. 6, no. 2, Article ID e16789, 2011.

[53] Y. Wang, F. Lian, J. Li et al., "Adipose derived mesenchymal stem cells transplantation via portal vein improves microcirculation and ameliorates liver fibrosis induced by CCl4 in rats," *Journal of Translational Medicine*, vol. 10, no. 1, article 133, 2012.

[54] S. Oyagi, M. Hirose, M. Kojima et al., "Therapeutic effect of transplanting HGF-treated bone marrow mesenchymal cells into CCl4-injured rats," *Journal of Hepatology*, vol. 44, no. 4, pp. 742–748, 2006.

[55] M. T. Abdel Aziz, H. M. Atta, S. Mahfouz et al., "Therapeutic potential of bone marrow-derived mesenchymal stem cells on experimental liver fibrosis," *Clinical Biochemistry*, vol. 40, no. 12, pp. 893–899, 2007.

[56] Y. Chang, J. Liu, P. Lin et al., "Mesenchymal stem cells facilitate recovery from chemically induced liver damage and decrease liver fibrosis," *Life Sciences*, vol. 85, no. 13-14, pp. 517–525, 2009.

[57] M. Hardjo, M. Miyazaki, M. Sakaguchi et al., "Suppression of carbon tetrachloride-induced liver fibrosis by transplantation of a clonal mesenchymal stem cell line derived from rat bone marrow," *Cell Transplantation*, vol. 18, no. 1, pp. 89–99, 2009.

[58] H. Qiao, Y. Tong, H. Han et al., "A novel therapeutic regimen for hepatic fibrosis using the combination of mesenchymal stem cells and baicalin," *Pharmazie*, vol. 66, no. 1, pp. 37–43, 2011.

[59] P. Tsai, T. Fu, Y. A. Chen et al., "The therapeutic potential of human umbilical mesenchymal stem cells from Wharton's jelly in the treatment of rat liver fibrosis," *Liver Transplantation*, vol. 15, no. 5, pp. 484–495, 2009.

[60] Z. M. Bai, X. D. Deng, J. D. Li et al., "Arterially transplanted mesenchymal stem cells in a mouse reversible unilateral ureteral obstruction model: in vivo bioluminescence imaging and effects on renal fibrosis," *Chinese Medical Journal*, vol. 126, no. 10, pp. 1890–1894, 2013.

[61] B. Ebrahimi, A. Eirin, Z. Li et al., "Mesenchymal stem cells improve medullary inflammation and fibrosis after revascularization of swine atherosclerotic renal artery stenosis," *PLoS ONE*, vol. 8, no. 7, Article ID e67474, 2013.

[62] H. J. Wu, W. H. Yiu, R. X. Li et al., "Mesenchymal stem cells modulate albumin-induced renal tubular inflammation and fibrosis," *PLoS One*, vol. 9, no. 3, Article ID e90883, 2014.

[63] M. Franquesa, E. Herrero, J. Torras et al., "Mesenchymal stem cell therapy prevents interstitial fibrosis and tubular atrophy in a rat kidney allograft model," *Stem Cells and Development*, vol. 21, no. 17, pp. 3125–3135, 2012.

[64] C. Alfarano, C. Roubeix, R. Chaaya et al., "Intraparenchymal Injection of bone marrow mesenchymal stem cells reduces kidney fibrosis after ischemia-reperfusion in cyclosporine-immunosuppressed rats," *Cell Transplantation*, vol. 21, no. 9, pp. 2009–2019, 2012.

[65] P. Semedo, M. Correa-Costa, M. A. Cenedeze et al., "Mesenchymal stem cells attenuate renal fibrosis through immune modulation and remodeling properties in a rat remnant kidney model," *Stem Cells*, vol. 27, no. 12, pp. 3063–3073, 2009.

[66] H. Asanuma, B. A. Vanderbrink, M. T. Campbell et al., "Arterially delivered mesenchymal stem cells prevent obstruction-induced renal fibrosis," *Journal of Surgical Research*, vol. 168, no. 1, pp. e51–e59, 2011.

[67] Y. Moodley, D. Atienza, U. Manuelpillai et al., "Human umbilical cord mesenchymal stem cells reduce fibrosis of bleomycin-induced lung injury," *American Journal of Pathology*, vol. 175, no. 1, pp. 303–313, 2009.

[68] L. A. Ortiz, F. Gambelli, C. McBride et al., "Mesenchymal stem cell engraftment in lung is enhanced in response to bleomycin exposure and ameliorates its fibrotic effects," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 100, no. 14, pp. 8407–8411, 2003.

[69] M. Choi, T. Ban, and T. Rhim, "Therapeutic use of stem cell transplantation for cell replacement or cytoprotective effect of microvesicle released from mesenchymal stem cell," *Molecules and Cells*, vol. 37, no. 2, pp. 133–139, 2014.

[70] S. Lee, A. Jang, Y. Kim et al., "Modulation of cytokine and nitric oxide by mesenchymal stem cell transfer in lung injury/fibrosis," *Respiratory Research*, vol. 11, article 16, 2010.

[71] F. Zhao, Y. F. Zhang, Y. G. Liu et al., "Therapeutic effects of bone marrow-derived mesenchymal stem cells engraftment on bleomycin-induced lung injury in rats," *Transplantation Proceedings*, vol. 40, no. 5, pp. 1700–1705, 2008.

[72] A. Gazdhar, N. Susuri, K. Hostettler et al., "HGF expressing stem cells in usual interstitial pneumonia originate from the bone marrow and are antifibrotic," *PLoS ONE*, vol. 8, no. 6, Article ID e65453, 2013.

[73] T. Ueno, A. Nakashima, S. Doi et al., "Mesenchymal stem cells ameliorate experimental peritoneal fibrosis by suppressing

inflammation and inhibiting TGF-β1 signaling," *Kidney International*, vol. 84, no. 2, pp. 297–307, 2013.

[74] C. H. Zhou, M. L. Li, A. L. Qin et al., "Reduction of fibrosis in dibutyltin dichloride-induced chronic pancreatitis using rat umbilical mesenchymal stem cells from Wharton's jelly," *Pancreas*, vol. 42, no. 8, pp. 1291–1302, 2013.

[75] Y. Wu, S. Huang, J. Enhe et al., "Bone marrow-derived mesenchymal stem cell attenuates skin fibrosis development in mice," *International Wound Journal*, 2013.

[76] J. A. Horton, K. E. Hudak, E. J. Chung et al., "Mesenchymal stem cells inhibit cutaneous radiation-induced fibrosis by suppressing chronic inflammation," *Stem Cells*, vol. 31, no. 10, pp. 2231–2241, 2013.

[77] Y. Qi, D. Jiang, A. Sindrilaru et al., "TSG-6 Released from intradermally injected mesenchymal stem cells accelerates wound healing and reduces tissue fibrosis in murine full-thickness skin wounds," *Journal of Investigative Dermatology*, vol. 134, no. 2, pp. 526–537, 2014.

[78] P. Mok, C. Leong, and S. Cheong, "Cellular mechanisms of emerging applications of mesenchymal stem cells," *Malaysian Journal of Pathology*, vol. 35, no. 1, pp. 17–32, 2013.

[79] N. G. Singer and A. I. Caplan, "Mesenchymal stem cells: Mechanisms of inflammation," *Annual Review of Pathology: Mechanisms of Disease*, vol. 6, pp. 457–478, 2011.

[80] A. Corcione, F. Benvenuto, E. Ferretti et al., "Human mesenchymal stem cells modulate B-cell functions," *Blood*, vol. 107, no. 1, pp. 367–372, 2006.

[81] A. Keating, "How do mesenchymal stromal cells suppress T cells?" *Cell Stem Cell*, vol. 2, no. 2, pp. 106–108, 2008.

[82] F. Bifari, V. Lisi, E. Mimiola, A. Pasini, and M. Krampera, "Immune modulation by mesenchymal stem cells," *Transfusion Medicine and Hemotherapy*, vol. 35, no. 3, pp. 194–204, 2008.

[83] P. A. Sotiropoulou, S. A. Perez, A. D. Gritzapis, C. N. Baxevanis, and M. Papamichail, "Interactions between human mesenchymal stem cells and natural killer cells," *Stem Cells*, vol. 24, no. 1, pp. 74–85, 2006.

[84] Y. Taniyama, R. Morishita, M. Aoki et al., "Angiogenesis and antifibrotic action by hepatocyte growth factor in cardiomyopathy," *Hypertension*, vol. 40, no. 1, pp. 47–53, 2002.

[85] Y. T. Chen, C. K. Sun, Y. C. Lin et al., "Adipose-derived mesenchymal stem cell protects kidneys against ischemia-reperfusion injury through suppressing oxidative stress and inflammatory reaction," *Journal of Translational Medicine*, vol. 9, article 51, 2011.

[86] C. K. Sun, C. H. Yen, Y. C. Lin et al., "Autologous transplantation of adipose-derived mesenchymal stem cells markedly reduced acute ischemia-reperfusion lung injury in a rodent model," *Journal of Translational Medicine*, vol. 9, no. 1, article 118, 2011.

[87] S. Francois, M. Mouiseddine, B. Allenet-Lepage et al., "Human mesenchymal stem cells provide protection against radiation-induced liver injury by antioxidative process, vasculature protection, hepatocyte differentiation, and trophic effects," *BioMed Research International*, vol. 2014, Article ID 151679, 14 pages, 2014.

[88] K. Kemp, K. Hares, E. Mallam, K. J. Heesom, N. Scolding, and A. Wilkins, "Mesenchymal stem cell-secreted superoxide dismutase promotes cerebellar neuronal survival," *Journal of Neurochemistry*, vol. 114, no. 6, pp. 1569–1580, 2010.

[89] T. Kinnaird, E. Stabile, M. S. Burnett et al., "Marrow-derived stromal cells express genes encoding a broad spectrum of arteriogenic cytokines and promote in vitro and in vivo arteriogenesis through paracrine mechanisms," *Circulation Research*, vol. 94, no. 5, pp. 678–685, 2004.

[90] S. C. Hung, R. R. Pochampally, S. C. Chen, S. C. Hsu, and D. J. Prockop, "Angiogenic effects of human multipotent stromal cell conditioned medium activate the PI3K-Akt pathway in hypoxic endothelial cells to inhibit apoptosis, increase survival, and stimulate angiogenesis," *Stem Cells*, vol. 25, no. 9, pp. 2363–2370, 2007.

[91] J. Niu, A. Azfer, O. Zhelyabovska, S. Fatma, and P. E. Kolattukudy, "Monocyte chemotactic protein (MCP)-1 promotes angiogenesis via a novel transcription factor, MCP-1-induced protein (MCPIP)," *The Journal of Biological Chemistry*, vol. 283, no. 21, pp. 14542–14551, 2008.

[92] T. Kinnaird, E. S. Burnett, M. Shou et al., "Local delivery of marrow-derived stromal cells augments collateral perfusion through paracrine mechanisms," *Circulation*, vol. 109, no. 12, pp. 1543–1549, 2004.

[93] J. Rehman, D. Traktuev, J. Li et al., "Secretion of angiogenic and antiapoptotic factors by human adipose stromal cells," *Circulation*, vol. 109, no. 10, pp. 1292–1298, 2004.

[94] C. Strup-Perrot, D. Mathé, C. Linard et al., "Global gene expression profiles reveal an increase in mRNA levels of collagens, MMPs, and TIMPs in late radiation enteritis," *American Journal of Physiology: Gastrointestinal and Liver Physiology*, vol. 287, no. 4, pp. G875–G885, 2004.

[95] D. Reinhardt, H. H. Sigusch, J. Henße, S. C. Tyagi, R. Körfer, and H. R. Figulla, "Cardiac remodelling in end stage heart failure: Upregulation of matrix metalloproteinase (MMP) irrespective of the underlying disease, and evidence for a direct inhibitory effect of ACE inhibitors on MMP," *Heart*, vol. 88, no. 5, pp. 525–530, 2002.

[96] D. Vanhoutte, M. Schellings, Y. Pinto, and S. Heymans, "Relevance of matrix metalloproteinases and their inhibitors after myocardial infarction: a temporal and spatial window," *Cardiovascular Research*, vol. 69, no. 3, pp. 604–613, 2006.

[97] S. Cheng, A. S. Pollock, R. Mahimkar, J. L. Olson, and D. H. Lovett, "Matrix metalloproteinase 2 and basement membrane integrity: a unifying mechanism for progressive renal injury," *The FASEB Journal*, vol. 20, no. 11, pp. 1898–1900, 2006.

[98] T. Yaguchi, Y. Fukuda, M. Ishizaki, and N. Yamanaka, "Immunohistochemical and gelatin zymography studies for matrix metalloproteinases in bleomycin-induced pulmonary fibrosis," *Pathology International*, vol. 48, no. 12, pp. 954–963, 1998.

[99] C. Mias, E. Trouche, M. H. Seguelas et al., "Ex vivo pretreatment with melatonin improves survival, proangiogenic/ mitogenic activity, and efficiency of mesenchymal stem cells injected into ischemic kidney," *Stem Cells*, vol. 26, no. 7, pp. 1749–1757, 2008.

[100] H. K. Yip, Y. C. Chang, C. G. Wallace et al., "Melatonin treatment improves adipose-derived mesenchymal stem cell therapy for acute lung ischemia-reperfusion injury," *Journal of Pineal Research*, vol. 54, no. 2, pp. 207–221, 2013.

[101] C. L. Visage, O. Gournay, N. Benguirat et al., "Mesenchymal stem cell delivery into rat infarcted myocardium using a porous polysaccharide-based scaffold: a quantitative comparison with endocardial injection," *Tissue Engineering A*, vol. 18, no. 1-2, pp. 35–44, 2012.

[102] C. Ceccaldi, R. Bushkalova, C. Alfarano et al., "Evaluation of polyelectrolyte complex-based scaffolds for mesenchymal stem cell therapy in cardiac ischemia treatment," *Acta Biomaterialia*, vol. 10, no. 2, pp. 901–911, 2014.

[103] M. M. Lalu, L. McIntyre, C. Pugliese et al., "Safety of cell therapy with mesenchymal stromal cells (SafeCell): a systematic review and meta-analysis of clinical trials," *PLoS ONE*, vol. 7, no. 10, Article ID e47559, 2012.

[104] K. C. Rustad and G. C. Gurtner, "Mesenchymal stem cells home to sites of injury and inflammation," *Advances in Wound Care*, vol. 1, no. 4, pp. 147–152, 2012.

[105] M. Mohamadnejad, K. Alimoghaddam, M. Mohyeddin-Bonab et al., "Phase 1 trial of autologous bone marrow mesenchymal stem cell transplantation in patients with decompensated liver cirrhosis," *Archives of Iranian Medicine*, vol. 10, no. 4, pp. 459–466, 2007.

[106] P. Kharaziha, P. M. Hellström, B. Noorinayer et al., "Improvement of liver function in liver cirrhosis patients after autologous mesenchymal stem cell injection: a phase I-II clinical trial," *European Journal of Gastroenterology and Hepatology*, vol. 21, no. 10, pp. 1199–1205, 2009.

[107] Z. Zhang, H. Lin, M. Shi et al., "Human umbilical cord mesenchymal stem cells improve liver function and ascites in decompensated liver cirrhosis patients," *Journal of Gastroenterology and Hepatology*, vol. 27, supplement 2, pp. 112–120, 2012.

[108] P. S. Kamath, R. H. Wiesner, M. Malinchoc et al., "A model to predict survival in patients with end-stage liver disease," *Hepatology*, vol. 33, no. 2, pp. 464–470, 2001.

[109] J. E. Ware Jr. and C. D. Sherbourne, "The MOS 36-item short-form health survey (SF-36). I. Conceptual framework and item selection," *Medical Care*, vol. 30, no. 6, pp. 473–483, 1992.

[110] M. E. J. Reinders, J. W. de Fijter, H. Roelofs et al., "Autologous bone marrow-derived mesenchymal stromal cells for the treatment of allograft rejection after renal transplantation:Results of a phase I study," *Stem Cells Translational Medicine*, vol. 2, no. 2, pp. 107–111, 2013.

[111] J. M. Hare, J. H. Traverse, T. D. Henry et al., "A randomized, double-blind, placebo-controlled, dose-escalation study of intravenous adult human mesenchymal stem cells (prochymal) after acute myocardial infarction," *Journal of the American College of Cardiology*, vol. 54, no. 24, pp. 2277–2286, 2009.

[112] J. M. Hare, J. E. Fishman, G. Gerstenblith et al., "Comparison of allogeneic vs autologous bone marrow-derived mesenchymal stem cells delivered by transendocardial injection in patients with ischemic cardiomyopathy: the POSEIDON randomized trial," *JAMA*, vol. 308, no. 22, pp. 2369–2379, 2012.

[113] S. Saito, G. Matsumiya, T. Sakaguchi et al., "Cardiac fibrosis and cellular hypertrophy decrease the degree of reverse remodeling and improvement in cardiac function during left ventricular assist," *Journal of Heart and Lung Transplantation*, vol. 29, no. 6, pp. 672–679, 2010.

[114] J. J. Lataillade, C. Doucet, E. Bey et al., "New approach to radiation burn treatment by dosimetry-guided surgery combined with autologous mesenchymal stem cell therapy," *Regenerative Medicine*, vol. 2, no. 5, pp. 785–794, 2007.

[115] R. Rafii, M. M. Juarez, T. E. Albertson, and A. L. Chan, "A review of current and novel therapies for idiopathic pulmonary fibrosis," *Journal of Thoracic Disease*, vol. 5, no. 1, pp. 48–73, 2013.

[116] A. Salazar-Montes, L. Ruiz-Corro, A. López-Reyes, E. Castrejón-Gómez, and J. Armendáriz-Borunda, "Potent antioxidant role of Pirfenidone in experimental cirrhosis," *European Journal of Pharmacology*, vol. 595, no. 1–3, pp. 69–77, 2008.

[117] S. N. Iyer, G. Gurujeyalakshmi, and S. N. Giri, "Effects of pirfenidone on transforming growth factor-β gene expression at the transcriptional level in bleomycin hamster model of lung fibrosis," *Journal of Pharmacology and Experimental Therapeutics*, vol. 291, no. 1, pp. 367–373, 1999.

[118] S. Mirkovic, A. L. Seymour, A. Fenning et al., "Attenuation of cardiac fibrosis by pirfenidone and amiloride in DOCA-salt hypertensive rats," *British Journal of Pharmacology*, vol. 135, no. 4, pp. 961–968, 2002.

[119] K. Takakuta, A. Fujimori, T. Chikanishi et al., "Renoprotective properties of pirfenidone in subtotally nephrectomized rats," *European Journal of Pharmacology*, vol. 629, no. 1–3, pp. 118–124, 2010.

[120] A. di Sario, E. Bendia, G. Macarri et al., "The anti-fibrotic effect of pirfenidone in rat liver fibrosis is mediated by downregulation of procollagen α1(I), TIMP-1 and MMP-2," *Digestive and Liver Disease*, vol. 36, no. 11, pp. 744–751, 2004.

[121] N. L. Simone, B. P. Soule, L. Gerber et al., "Oral pirfenidone in patients with chronic fibrosis resulting from radiotherapy: a pilot study," *Radiation Oncology*, vol. 2, no. 1, article 19, 2007.

[122] *Pulmonary-Allergy Drugs Advisory Committee Complete Response on Pirfenidone*, Food and Drug Administration Center for Drug Evaluation and Research, March 2010, http://www.fda.gov/downloads/AdvisoryCommittees/CommitteesMeetingMaterials/Drugs/Pulmonary-AllergyDrugsAdvisoryCommittee/UCM206399.pdf.

[123] C. Jiang, H. Huang, J. Liu, Y. Wang, Z. Lu, and Z. Xu, "Adverse events of pirfenidone for the treatment of pulmonary fibrosis: a meta-analysis of randomized controlled trials," *PLoS ONE*, vol. 7, no. 10, Article ID e47024, 2012.

[124] J. Rosenbloom, F. A. Mendoza, and S. A. Jimenez, "Strategies for anti-fibrotic therapies," *Biochimica et Biophysica Acta: Molecular Basis of Disease*, vol. 1832, no. 7, pp. 1088–1103, 2013.

[125] M. A. Ngo, A. Muller, Y. Li et al., "Human mesenchymal stem cells express a myofibroblastic phenotype in vitro: comparison to human cardiac myofibroblasts," *Molecular and Cellular Biochemistry*, vol. 392, no. 1-2, pp. 187–204, 2014.

[126] L. Yang, N. Chang, X. Liu et al., "Bone marrow-derived mesenchymal stem cells differentiate to hepatic myofibroblasts by transforming growth factor-β1 via sphingosine kinase/sphingosine 1-phosphate (S1P)/S1P receptor axis," *The American Journal of Pathology*, vol. 181, no. 1, pp. 85–97, 2012.

[127] S. Chakraborty, P. Chopra, A. Hak, S. G. Dastidar, and A. Ray, "Hepatocyte growth factor is an attractive target for the treatment of pulmonary fibrosis," *Expert Opinion on Investigational Drugs*, vol. 22, no. 4, pp. 499–515, 2013.

Hindawi Publishing Corporation
Stem Cells International
Volume 2014, Article ID 939674, 7 pages
http://dx.doi.org/10.1155/2014/939674

Research Article

The Efficacy of Mesenchymal Stem Cell Transplantation in Caustic Esophagus Injury: An Experimental Study

Murat Kantarcioglu,[1] Bahadir Caliskan,[2] Hakan Demirci,[1] Ozgur Karacalioglu,[3] Murat Kekilli,[4] Zulfikar Polat,[1] Armagan Gunal,[5] Melih Akinci,[6] Cagri Uysal,[7] Sami Eksert,[1] Hasan Gurel,[1] Gurkan Celebi,[1] Ferit Avcu,[8] Ali Ugur Ural,[9] and Sait Bagci[1]

[1] *Department of Gastroenterology, Gulhane Military Medical Academy, Etlik, 06108 Ankara, Turkey*
[2] *Department of Pediatric Surgery, Gulhane Military Medical Academy, Etlik, 06108 Ankara, Turkey*
[3] *Department of Nuclear Medicine, Gulhane Military Medical Academy, Etlik, 06108 Ankara, Turkey*
[4] *Department of Gastroenterology, Hitit University, 19030 Corum, Turkey*
[5] *Department of Pathology, Gulhane Military Medical Academy, Etlik, 06108 Ankara, Turkey*
[6] *Department of General Surgery, Ankara Diskapi Training and Research Hospital, 06110 Ankara, Turkey*
[7] *Department of Plastic and Reconstructive Surgery, Faculty of Medicine, Baskent University, 06100 Ankara, Turkey*
[8] *Department of Hematology, Gulhane Military Medical Academy, Etlik, 06108 Ankara, Turkey*
[9] *Department of Hematology, Bayindir Hospital, 06250 Ankara, Turkey*

Correspondence should be addressed to Hakan Demirci; hakandemircigata@yahoo.com

Received 1 January 2014; Accepted 7 April 2014; Published 4 May 2014

Academic Editor: Katherine Athayde Teixeira de Carvalho

Introduction. Ingestion of corrosive substances may lead to stricture formation in esophagus as a late complication. Full thickness injury seems to exterminate tissue stem cells of esophagus. Mesenchymal stem cells (MSCs) can differentiate into specific cell lineages and have the capacity of homing in sites of injury. *Aim and Methods.* We aimed to investigate the efficacy of MSC transplantation, on prevention of esophageal damage and stricture formation after caustic esophagus injury in rats. 54 rats were allocated into four groups; 4 rats were sacrificed for MSC production. Group 1, untreated controls (*n*: 10). Group 2, membrane labeled MSCs-treated rats (*n*: 20). Group 3, biodistribution of fluorodeoxyglucose labeled MSCs via positron emission tomography (PET) imaging (*n*: 10). Group 4, sham operated (*n*: 10). Standard caustic esophageal burns were created and MSCs were transplanted 24 hours after. All rats were sacrificed at the 21st days. *Results.* PET scan images revealed the homing behavior of MSCs to the injury site. The histopathology damage score was not significantly different from controls. However, we demonstrated Dil labeled epithelial and muscle cells which were originating from transplanted MSCs. *Conclusion.* MSC transplantation after caustic esophageal injury may be a helpful treatment modality; however, probably repeated infusions are needed.

1. Introduction

Mostly in developing countries, caustic ingestion continues to be a significant medical problem in different age groups and is a leading cause of morbidity and mortality in the pediatric population [1]. Ingestion occurs as an intentional exposure in adults, while children's exposure is accidental. The basic pathophysiologic reaction of tissue subjected to caustic burn is the deposition, synthesis, and remodeling of extracellular matrix, and following full thickness injuries to the wall of esophagus, the normal esophageal tissue is replaced by connective tissue [2, 3].

Today, the aim of therapy in corrosive esophageal burn injuries is to prevent development of fibrosis, stricture formation, and perforation [4]. Several types of nonsurgical management such as steroid injections, antibiotics, esophageal dilatation, stent applications, and surgical treatment options are used for treatment [2, 4–6]; but current types of treatment are quiet ineffective for severe corrosive burns. In experimental models, some agents such as vitamins E and C,

heparin, mitomycin, penicillamine, caffeic acid, epidermal growth factor, interferon g, sphingosylphosphorylcholine, phenethyl ester, progesterone, and estradiol are found to be effective in different degrees [7–13]. However, they have not been applied as standard treatment protocols for corrosive esophageal burn injuries at present.

Mesenchymal stem cells (MSCs) constitute an alternative source of pluripotent stem cells. They have the capacity to differentiate into cells of mesodermal lineage, and also have a much broader differentiation potential. Cells with similar properties exist in a variety of other tissues, including adipose tissue, peripheral and umbilical cord blood, placenta, amniotic fluid, fetal tissues, synovial membrane, and deciduous teeth, but bone marrow MSCs are the best characterized ever [14–16]. MSCs also have the capacity to home in sites of injury [17]. Recent studies have shown that bone marrow-derived MSCs might play an important role in the repair processes of injured tissues [18, 19]. Okamoto et al. reported that bone marrow derived cells can repopulate esophagus epithelia in humans [20]. They also have shown that bone marrow derived cells are a potential source from which to repopulate esophagus epithelia especially during episodes of inflammation and regeneration, raising the possibility that clinical therapies to regenerate human esophagus epithelia with bone marrow cells may be possible [20]. Ringdén et al. reported that MSC transplantation plays a role in healing tissue toxicity in patients who had complications after allogeneic hematopoietic stem cell transplantation [21]. In addition, several groups have demonstrated the ability of MSCs to secrete angiogenic factors, such as vascular endothelial growth factor, hepatocyte growth factor, and interleukin-6 both in vitro [22, 23] and in vivo [24].

There has been no evidence about any effect of MSCs on improving any form of esophageal injury. The aim of this experimental study was to investigate the efficacy of allogeneic MSCs, which intravenously transplanted into a caustic esophagitis rat model. In this context we aimed to assess their homing behaviour towards the esophagus injury site and improve capacity on mucosal healing and reversing stricture formation.

2. Materials and Methods

This study was approved by the Experimental Ethical Committee of the Gulhane Military Medical Academy, Ankara, Turkey.

2.1. Study Groups. In order to have 90% power with 5% type I error level to detect a minimum clinically significant difference of 50%, 20 rats for treatment group and 10 rats for each left group (sham, control etc.) had to be recruited to the study.

The study was performed using 54 albino, male, 2-month-old Sprague-Dawley (SD) rats weighing between 250 and 300 g each. All the rats were obtained from our animal research center. Rats were kept in experimental animal production cages which were closed with plastic on bottom and sides and top with wire fence. Room temperature was maintained at 22°C. In order to simulate circadian rhythm, 12 h light and dark periods were carried out. The rats were fed by pellet type fabrication feed that is produced specially for experimental animals. Four rats were sacrificed and their tibias and femurs were excised. Their marrows were cultured to obtain MSCs. The remaining 50 animals were randomly allocated into 4 groups. Group 1 (control group), in which caustic esophageal burns were created but left without treatment (10 rats). Group 2 (MSCs-treated group), in which animals with caustic esophageal burns were transplanted, and membrane labelled allogeneic bone marrow derived 1×10^6 mesenchymal stem cells each one day after injury. They were sacrificed at 21st day and histopathologic analysis was performed to esophagus specimens (20 rats). Group 3 (MSCs tracked via PET imaging) in which stem cells were labeled with fluoro-D-glucose (FDG), and after transplantation, MSCs were tracked for 4 hours via positron emission tomography in order to observe their early homing behaviour (10 rats). Group 4 (sham-operated group), in which laparotomy was performed, esophagus was uninjured and untreated (10 rats).

2.2. Experimental Model. After 12 hours of fasting, each rat was anesthetized intraperitoneally with xylazine hydrochloride (15 mg/kg) and ketamine hydrochloride (100 mg/kg). A standardized esophageal caustic burn injury was produced using the method described by Gehanno and Guedon [25]. Applying sterile surgical techniques, a midline laparotomy was made and 2 cm of abdominal esophageal segment was isolated and tied with 2/0 silk sutures distally and proximally. A 24-F cannula was placed into the isolated segment through a gastric puncture. The esophageal injury was created by instilling 20% NaOH solution for 3 minutes until slight translucency of the esophageal wall and branching of the vessels were noted, and then the solution was aspirated. Subsequently, distilled water was used to irrigate the injured segment for a 60-second period. In the sham operated group, distal esophageal segments were instilled with 0.09% NaCl solution only. The laparotomy incision was closed and 10 mL of saline was administered subcutaneously in each animal. Rats were not allowed to feed for the next 24 h. All animals were kept in identical cages that provided food and water during the study period. On the 21st postoperative day, the rats were decapitated and distal 1.5 cm esophageal segments were harvested for histopathologic investigations. Specimens were placed in 10% buffered formaldehyde solution.

2.3. Bone Marrow (BM) Preparation and BM-Derived Rat MSC Generation. Briefly, 4 SD rats were sacrificed by decapitation and Bone Marrow (BM) was flushed with L-DMEM (Gibco Lab, Grand Island, NY) using a 23-gauge needle from femurs and tibias. The BM cells were then pelleted by centrifugation at 1000 rpm for 15 min. The BM cells were gently resuspended using an 18-gauge needle and filtered through a sterile nylon mesh. The viability was consistently >95% as determined by trypan blue exclusion. For the MSC generation, BM cells were plated in 25 cm^2 polystyrene flasks in L-DMEM supplemented with 10% fetal bovine serum (FBS) in 37°C with 5% CO^2 conditions (Gibco Lab, Grand

Table 1: Criteria for histopathologic evaluation.

Criteria	Score
Increase in submucosal collagen	
None	0
Mild (submucosal collagen at least twice the thickness of the muscularis mucosa)	1
Marked (submucosal collagen more than twice the thickness of the muscularis mucosa)	2
Damage to muscularis mucosa	
None	0
Present	1
Damage and collagen deposition in the muscularis propria	
None	0
Mild (collagen deposition around the smooth muscle fibers)	1
Marked (same as mild, with collagen deposition replacing some of the fibers)	2

Island, NY). Cells were allowed to adhere for 72 h followed by the removal of nonadherent cells and media changed every 3 to 4 days. Adherent cells were detached using trypsin-EDTA solution-B (EDTA 0.05%, Trypsin 0.25%, with Phenol Red, Biol. Ind., Israel) at 37°C for 10 min, and MSCs were expanded 3-4 times to achieve the desired cell numbers for use in vitro and in vivo experiments. MSCs were resuspended in DMEM and diluted to a final concentration of 1×10^6 cells/1 mL.

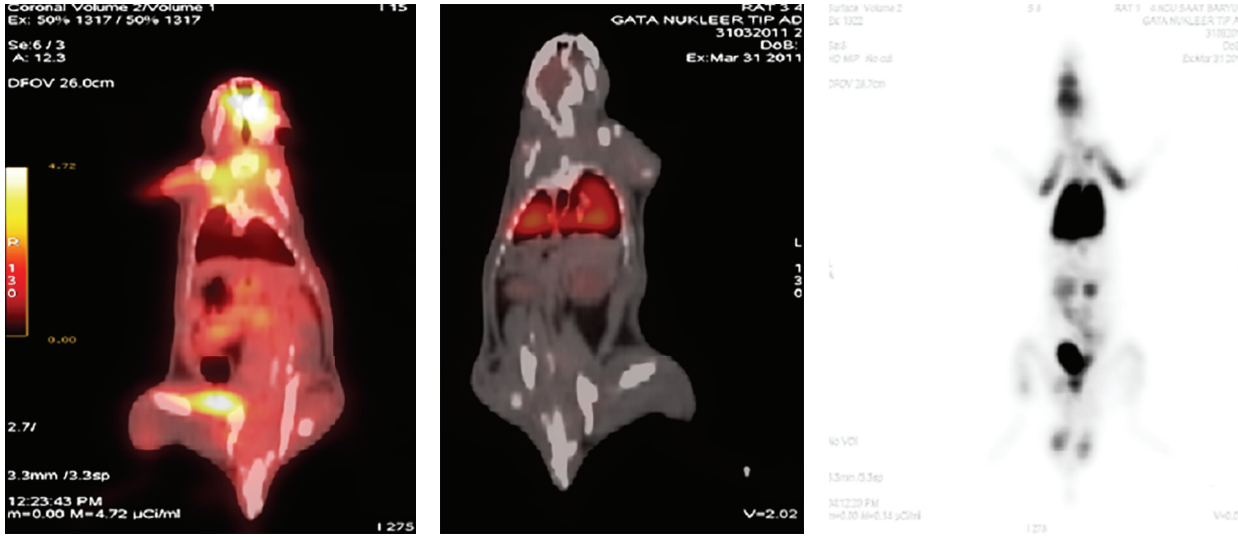
2.4. Dil Labeling of MSCs. In order to reveal the faith of the transplanted MSCs in injured tissue, for tracking procedure, the cells were labeled by 1,1-dioctadecyl-3,3,3,3-tetramethylindocarbocyanine (DiI); Vybrant CM-DiI cell-labeling solution (Molecular Probes Inc.). MSCs were suspended in DMEM with 1×10^6 cells in 1 mL and labeled with fluorescent DiI according to the manufacturer's recommendations [26]. Briefly, 5 μL of cell labeling solution was added per mL of cell suspension. After mixing well by gentle pipetting, cells were incubated for 20 minutes at 37°. Then the labeled suspension tubes were centrifuged at 1500 rpm for 5 minutes and supernatant was removed. Following labeling, the MSCs were incubated with beta-TCP in a culture medium. Positive DiI staining was confirmed, and for each recipient rat, 1×10^6 cells in 1 mL serum were injected via tail vein of the recipient rat.

2.5. PET Imaging. Stem cells were labeled with fluoro-D-glucose (FDG) following protocols similar to those described previously [27]. The activities of FDG, administered to the cell samples, were measured with a standard dose calibrator (Capintec CRC 10, Capintec Inc., Ramsey, NJ, USA). Cells were detached under mild conditions in 0.05% trypsin/0.02% EDTA (Biochrom, Berlin, Germany) for 5 minutes. The cell suspension was centrifuged at 800 g for 5 minutes and supernatant was removed. The cell pellet was resuspended in phosphate-buffered saline (PBS), and recentrifuged at 800 g for 5 minutes. For cell counting, the washed pellet was resuspended in 1 mL of PBS; the cell concentration was measured by microscopic examination of 10 μL portions of the suspension. For labelling, 1×10^6 cells were mixed with 2-deoxy-2-(18F) fluoro-D-glucose (FDG) at dose of 0.5 mCi/0.5 mL in plastic gamma tube and incubated for 45 minutes at 37°C in the benmari. At the end of the incubation period, the FDG-containing medium was removed and the MSCs were washed twice with PBS and they were resuspended with 0.3 mL saline.

The animals (which had been kept fasting for at least 4 h) were anesthetized with ketamine (15 mg/kg), and 37–47 MBq (1.0–1.3 mCi) and FDG labeled stem cells (1×10^6 cells labeled with ~16×10^6 MBq FDG/300 μL) were injected via intravenous tail vein. After 60 min of distribution, the rats underwent a whole body-imaging acquisition including 6 min for the 3-dimensional-mode emission scan and less than 10 seconds for the transmission scan in PET CT camera (GE Discovery 690, WI, USA). The images were reconstructed with optimized parameters. The FDG PET CT imaging of the animals was repeated to demonstrate the gastroesophageal junction 1 hour after the oral contrast (2 mL barium sulfate suspension) administration. The animals were sacrificed by decapitation method; gastroesophageal junctions were resected and reimaged by PET scanner.

2.6. Histopathologic Evaluation. All samples were detected by a blind gastrointestinal system pathologist. After 10% formalin fixation, esophageal resection samples were embedded paraffin blocks crossly. 5 μm thick sections were taken and then, stained with hematoxylin and eosin (HE) for general histologic evaluation and with Masson's trichrome for detecting collagen deposition to evaluate fibrosis. Firstly, with an automatized microscope-camera system (OlympusMicro DP-BSW Ver. 3.3, Olympus Co., Japan), the thickness of the esophageal wall (WT) and lumen diameter (LD) was measured, and the stenosis index (SI) was calculated to evaluate the degree of the stricture (SI = WT/LD). Secondly, tissue damage was scored on a scale based on 3 different categories described by Guven et al. (Table 1) [28].

The specimens were also examined by Nikon eclipse 80i microscope through Y-2EC filter (Wavelength; ex: 540–580 DM 595 BA 600–660) in order to visualize the differentiated DiI labeled MCSs.



(a) (b) (c)

Figure 1: PET/CT image demonstrated the altered biodistribution between FDG (a) and the labeled stem cells with FDG (b). Prominent accumulation of the labeled stem cells in the lung was demonstrated by PET/CT (B) and PET image (c).

2.7. Statistical Analysis. All statistical analyses were carried out using SPSS 15 (SPSS Inc., Chicago, IL, USA) statistical software for Windows. All data were presented as mean ± standard deviation. Differences in measured parameters among the three groups were analyzed by the Kruskal-Wallis test. Dual comparisons between groups that present significant values were evaluated with the Mann-Whitney U test. Differences were considered as significant when the P value was less than 0.05.

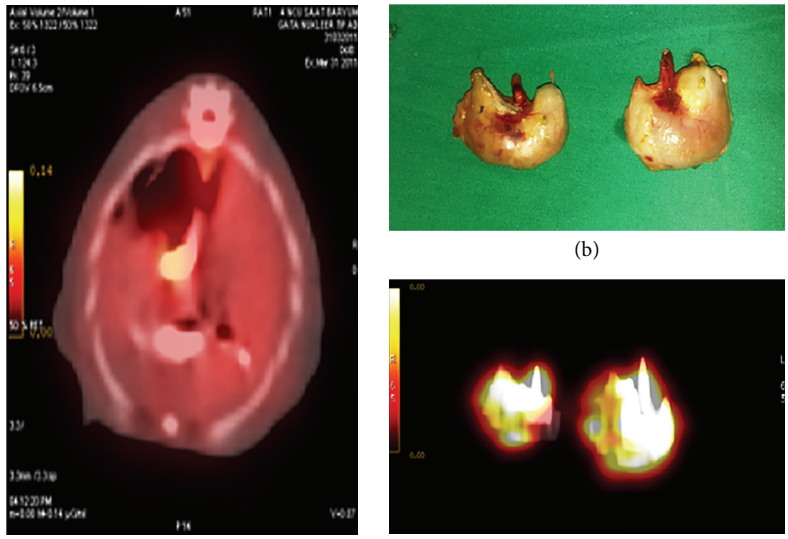
3. Results

All rats which stayed alive for 21 days were evaluated. 44 rats survived throughout the study (6 rats died; 4 from MSC treated group and 2 from control group). All animals in the sham-operated group survived during the study while two control and 4 MSCs treated rats died on different days during follow-up, after creating esophageal burns.

3.1. PET Imaging: FDG Labeled MSC Tracking. When the FDG alone was injected into the tail vein of the rats (n: 2), prominent FDG uptake was noticed in the central nervous system, muscles of extremities, in liver, and spleen. There was also prominent activity accumulation in the urinary system due to physiological excretion of the FDG (Figure 1(a)). On the other hand, 1 hour after the injection of the labeled stem cells into the tail vein of the rats (n: 8), prominent activity caused by labeled stem cells in the lung was detected (Figures 1(b) and 1(c)). Actually this finding can be expected when labeled cells with radiopharmaceuticals such as white blood cells labeled with Tc-99 m HMPAO were reinjected intravenously; homing of the labeled cells in the lung is a usual finding on the scan acquired at the first hour [29]. There was also prominent activity in the lower portion of the injured esophagus (Figures 2(a)–2(c)). Little activity in the urinary system and in the central nervous system was also noticed due to unbound FDG. Altered biodistribution between the labeled stem cells with FDG and the FDG alone revealed the success of the labeling process.

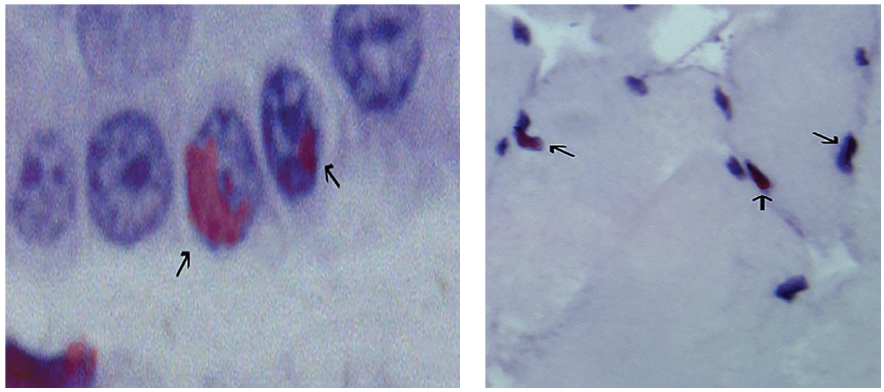
3.2. Morphologic and Histopathologic Evaluation. At the end of the study, the esophagus specimens of the sham-operated group rats were macroscopically normal and there was no adhesion (n: 10). In contrast, those of MSCs treated (n: 16) and control animals' (n: 10) showed considerable or complete esophageal obstruction, severe adhesion (possibly due to perforation), and residual chow in the esophageal lumen. There was no significant difference between SI values of MSC treated and control groups. This finding was supported with histopathologic evaluation. The SI in the treatment and control group was significantly higher than sham-operated groups ($P < 0.01$).

3.3. Histologic Tracking of DiI Labeled MSCs in Injury Site. The rats of Group 2 (n: 16) with caustic esophagitis which received Dil labeled MSCs treatment were sacrificed at the 21st day of caustic injury. The specimens were also examined by Nikon eclipse 80i microscope through Y-2EC filter (Wavelength; ex: 540–580 DM 595 BA 600–660). We noticed some esophagus epithelium (Figure 3(a)) and muscle cells (Figure 3(b)) stained positively for Dil which were originated from BM derived MSCs. The homing behaviour of MSCs that we have demonstrated by PET imaging, resulted in differentiation to epithelium and muscle cells. However, the number of differentiated cells was far apart from restoring the original architecture.



(a) (b) (c)

Figure 2: PET/CT image demonstrated the presence of the activity that can be interpreted as to be the activity of the labeled stem cells in the lower portion of the esophagus containing barium sulphate (a). Esophagogastric junction was resected (b) and imaged (c) by PET/CT to delineate the prominent activity in the lower portion of the injured esophagus.



(a) (b)

Figure 3: Epithelial differentiation of Dil labeled MSCs (a). Muscle cell differentiation of transplanted bone marrow derived Dil labeled MSCs (b).

4. Discussion

Ingestion of caustic materials is a major health problem with a high degree of morbidity [1]. Extensive damage to the esophagus can cause serious problems such as fibrosis and stricture formation [3]. Like many organs, esophagus was shown to have stromal proliferative "stem" cells by some illuminative studies carried out, regarding murine and human samples. Those mentioned cells seem to lie mostly in basement membrane of the esophagus [30]. The devastating effects of especially alkaline agents on esophagus wall by causing full thickness injury are well known. The destruction of esophageal stem cells during caustic injury and loss of regeneration capacity of the organ could easily be asserted for fibrotic healing process instead of renewal, after such a demolition. There is no valid algorithm accepted for the treatment of corrosive esophagitis [2, 3]. Esophageal stricture formation after caustic injury occurs at approximately 21 days and is completed from 28 to 42 days [31]. It is now widely accepted that the acute inflammatory response dominates the first week after injury, whereas the second week is the setting for fibroblastic proliferation and collagen formation, which represent the pathophysiological pathway of stricture formation [32, 33]. A successful management of corrosive injury involves prompt recognition and early treatment.

MSCs are a type of mesodermal-derived multipotent stem cells, found mainly in connective tissues, organs, and especially abundant in the bone marrow. Because of their multidirectional differentiation and self-replicating feature, bone marrow derived MSCs are considered the most potent for therapeutic application. Under specific induction conditions, bone marrow derived MSCs can differentiate into

multiple types of tissues, such as bone, fat, cartilage, muscle, tendon, ligament, neural, liver, myocardium, and endothelial cells in vivo or in vitro [15, 16, 34]. The multipotency of bone marrow derived MSCs can be well conserved even after multiple processes [35–38]. In recent years, it has been reported that allogeneic MSCs appear to suppress graft versus host disease (GvHD) and Crohn's disease as well as induce regenerative phenomena in the case of stroke, infarct, spinal cord injury, meniscus regeneration, tendinitis, acute renal failure, and heart disease in both human and animal models of disease [39].

Up to date, there has been no research related to the effect of MSCs transplantation on any form of esophageal disease. In this experimental study, we examined whether the MSCs transplantation treatment modality has a beneficial effect on caustic esophageal injury.

Stem cells which are more prone to differentiate to specialized cell types might have been used for this experimental procedure like pluripotent stromal cells, isolated from different organs. For example, Musina et al. isolated MSCs from menstrual bloods of volunteer women [40]. In the same context, Hida et al. demonstrated that those same cells are very capable of differentiation to cardiac precursor-like cells [41]. Same sort of cells originating from endometrium, with their high differentiation capacity to muscle cells, seems like a good candidate to restore esophagus wall and their efficacy may be sought in our experimental treatment model. Also another alternative for cell source may be the stem cells derived from human placenta and fetal membranes where they are obtained without any invasive procedures and in vitro expansion potential is significantly higher than BM derived MSCs [42]. However we have chosen BM derived MSCs with their well-known features.

Throughout the experimental process, we infused MSCs only once. However the pathology investigation results of esophagus specimens were far away from restoring the original esophagus architecture. Here we think a reasonable approach may be performing multiple infusions of MSCs in order to achieve a sustained regeneration. Transplantation of MSCs via direct injection into injury site seemed also a feasible way for this study. However, after 20% NaOH injury, affected esophagus wall sites were very fragile and prone to perforation. Therefore direct implantation of MSCs may have resulted in perforation and higher animal mortality rates.

Study results have shown that single dose MSCs transplantation did not improve the esophageal injury in rats; nevertheless, animal use to predict human response to treatment modalities is apparently a contentious issue [43]. Since rodent esophagus does not resemble the human's accurately, human tissue responses to MSC transplantation may be more satisfactory. On the other hand, this experimental study demonstrated intravenously injected MSCs' first step in circulation and their subsequent homing to injured tissues in the esophagus. Histopathology results confirmed both the homing behaviour and differentiation properties of transplanted MSCs. These results provide evidence for clinical application as a treatment modality for esophageal diseases in humans. Nevertheless, time course and dose dependent studies with different types of stem cells are necessary to determine the proper timing and dosage after the injury and effects on other organs and systems.

Conflict of Interests

All authors declare that they have no potential conflict of interests.

References

[1] A. L. de Jong, R. Macdonald, S. Ein, V. Forte, and A. Turner, "Corrosive esophagitis in children: a 30-year review," *International Journal of Pediatric Otorhinolaryngology*, vol. 57, no. 3, pp. 203–211, 2001.

[2] I. Karnak, F. C. Tanyel, N. Büyükpamukçu, and A. Hiçsönmez, "Combined use of steroid, antibiotics and early bougienage against stricture formation following caustic esophageal burns," *Journal of Cardiovascular Surgery*, vol. 40, no. 2, pp. 307–310, 1999.

[3] J. A. Haller Jr., H. G. Andrews, J. J. White et al., "Pathophysiology and management of acute corrosive burns of the esophagus: results of treatment in 285 children," *Journal of Pediatric Surgery*, vol. 6, no. 5, pp. 578–584, 1971.

[4] N. Corduk, U. Koltuksuz, N. Calli-Demirkan, S. Rota, G. Abban, and A. Sarioglu-Buke, "Effects of retinoic acid and zinc on the treatment of caustic esophageal burns," *Pediatric Surgery International*, vol. 26, no. 6, pp. 619–624, 2010.

[5] J. A. Fulton and R. S. Hoffman, "Steroids in second degree caustic burns of the esophagus: a systematic pooled analysis of fifty years of human data: 1956–2006," *Clinical Toxicology*, vol. 45, no. 4, pp. 402–408, 2007.

[6] N. K. Chokshi, Y. S. Guner, F. Ndiforchu, R. Mathis, C. E. Shin, and N. X. Nguyen, "Combined laparoscopic and thoracoscopic esophagectomy and gastric pull-up in a child," *Journal of Laparoendoscopic and Advanced Surgical Techniques*, vol. 19, supplement 1, pp. S197–S200, 2009.

[7] A. Bautista, R. Tojo, R. Varela, E. Estevez, A. Villanueva, and S. Cadranel, "Effects of prednisolone and dexamethasone on alkali burns of the esophagus in rabbit," *Journal of Pediatric Gastroenterology and Nutrition*, vol. 22, no. 3, pp. 275–283, 1996.

[8] M. Bingöl-Koloğlu, F. C. Tanyel, S. Müftüoğlu et al., "The preventive effect of heparin on stricture formation after caustic esophageal burns," *Journal of Pediatric Surgery*, vol. 34, no. 2, pp. 291–294, 1999.

[9] S. Demirbilek, F. Bernay, R. Rizalar, S. Baris, and N. Gurses, "Effects of estradiol and progesterone on the synthesis of collagen in corrosive esophageal burns in rats," *Journal of Pediatric Surgery*, vol. 29, no. 11, pp. 1425–1428, 1994.

[10] M. K. S. Heran, R. Baird, G. K. Blair, and E. D. Skarsgard, "Topical mitomycin-C for recalcitrant esophageal strictures: a novel endoscopic/fluoroscopic technique for safe endoluminal delivery," *Journal of Pediatric Surgery*, vol. 43, no. 5, pp. 815–818, 2008.

[11] U. Koltuksuz, H. M. Mutus, R. Kutlu et al., "Effects of caffeic acid phenethyl ester and epidermal growth factor on the development of caustic esophageal stricture in rats," *Journal of Pediatric Surgery*, vol. 36, no. 10, pp. 1504–1509, 2001.

[12] B. Berthet, J. Di Costanzo, C. Arnaud, R. Choux, and R. Assadourian, "Influence of epidermal growth factor and interferon γ on healing of oesophageal corrosive burns in the rat," *British Journal of Surgery*, vol. 81, no. 3, pp. 395–398, 1994.

[13] A. Yagmurlu, B. Aksu, M. Bingol-Kologlu et al., "A novel approach for preventing esophageal stricture formation: sphingosylphosphorylcholine-enhanced tissue remodeling," *Pediatric Surgery International*, vol. 20, no. 10, pp. 778–782, 2004.

[14] Y. Jiang, B. N. Jahagirdar, R. L. Reinhardt et al., "Pluripotency of mesenchymal stem cells derived from adult marrow," *Nature*, vol. 418, no. 6893, pp. 41–49, 2002.

[15] J. Oswald, S. Boxberger, B. Jørgensen et al., "Mesenchymal stem cells can be differentiated into endothelial cells in vitro," *Stem Cells*, vol. 22, no. 3, pp. 377–384, 2004.

[16] M. F. Pittenger, A. M. Mackay, S. C. Beck et al., "Multilineage potential of adult human mesenchymal stem cells," *Science*, vol. 284, no. 5411, pp. 143–147, 1999.

[17] A. Chapel, J. M. Bertho, M. Bensidhoum et al., "Mesenchymal stem cells home to injured tissues when co-infused with hematopoietic cells to treat a radiation-induced multi-organ failure syndrome," *Journal of Gene Medicine*, vol. 5, no. 12, pp. 1028–1038, 2003.

[18] D. Lu, Y. Li, L. Wang, J. Chen, A. Mahmood, and M. Chopp, "Intraarterial administration of marrow stromal cells in a rat model of traumatic brain injury," *Journal of Neurotrauma*, vol. 18, no. 8, pp. 813–819, 2001.

[19] D. Lu, A. Mahmood, L. Wang, Y. Li, M. Lu, and M. Chopp, "Adult bone marrow stromal cells administered intravenously to rats after traumatic brain injury migrate into brain and improve neurological outcome," *NeuroReport*, vol. 12, no. 3, pp. 559–563, 2001.

[20] R. Okamoto, T. Yajima, M. Yamazaki et al., "Damaged epithelia regenerated by bone marrow-derived cells in the human gastrointestinal tract," *Nature Medicine*, vol. 8, no. 9, pp. 1011–1017, 2002.

[21] O. Ringdén, M. Uzunel, B. Sundberg et al., "Tissue repair using allogeneic mesenchymal stem cells for hemorrhagic cystitis, pneumomediastinum and perforated colon," *Leukemia*, vol. 21, no. 11, pp. 2271–2276, 2007.

[22] K. Park, Y. Kim, J. Kim et al., "Trophic molecules derived from human mesenchymal stem cells enhance survival, function, and angiogenesis of isolated islets after transplantation," *Transplantation*, vol. 89, no. 5, pp. 509–517, 2010.

[23] V. Sordi, R. Melzi, A. Mercalli et al., "Mesenchymal cells appearing in pancreatic tissue culture are bone marrow-derived stem cells with the capacity to improve transplanted islet function," *Stem Cells*, vol. 28, pp. 140–151, 2010.

[24] T. Ito, S. Itakura, I. Todorov et al., "Mesenchymal stem cell and islet co-transplantation promotes graft revascularization and function," *Transplantation*, vol. 89, no. 12, pp. 1438–1445, 2010.

[25] P. Gehanno and C. Guedon, "Inhibition of experimental esophageal lye strictures by penicillamine," *Archives of Otolaryngology*, vol. 107, no. 3, pp. 145–147, 1981.

[26] http://tools.lifetechnologies.com/content/sfs/manuals/mp22885.pdf.

[27] W. J. Kang, H. J. Kang, H. S. Kim et al., "Tissue distribution of ^{18}F-FDG-labeled peripheral hematopoietic stem cells after intracoronary administration in patients with myocardial infarction," *Journal of Nuclear Medicine*, vol. 47, no. 8, pp. 1295–1301, 2006.

[28] A. Guven, S. Demirbag, B. Uysal et al., "Effect of 3-amino benzamide, a poly(adenosine diphosphate-ribose) polymerase inhibitor, in experimental caustic esophageal burn," *Journal of Pediatric Surgery*, vol. 43, no. 8, pp. 1474–1479, 2008.

[29] P. D. Robins, I. Salazar, L. A. Forstrom, B. P. Mullan, and J. C. Hung, "Biodistribution and radiation dosimetry of stabilized ^{99m}Tc-exametazime-labeled leukocytes in normal subjects," *Journal of Nuclear Medicine*, vol. 41, no. 5, pp. 934–940, 2000.

[30] D. Croagh, R. J. S. Thomas, W. A. Phillips, and P. Kaur, "Esophageal stem cells—a review of their identification and characterization," *Stem Cell Reviews*, vol. 4, no. 4, pp. 261–268, 2008.

[31] F. C. Rothstein, "Caustic injuries to the esophagus in children," *Pediatric Clinics of North America*, vol. 33, no. 3, pp. 665–674, 1986.

[32] N. L. Occleston, J. T. Daniels, R. W. Tarnuzzer et al., "Single exposures to antiproliferatives: long-term effects on ocular fibroblast wound-healing behavior," *Investigative Ophthalmology and Visual Science*, vol. 38, no. 10, pp. 1998–2007, 1997.

[33] P. T. Khaw, M. B. Sherwood, S. L. D. MacKay, M. J. Rossi, and G. Schultz, "Five-minute treatments with fluorouracil, floxuridine, and mitomycin have long-term effects on human Tenon's capsule fibroblasts," *Archives of Ophthalmology*, vol. 110, no. 8, pp. 1150–1154, 1992.

[34] A. Uccelli, L. Moretta, and V. Pistoia, "Mesenchymal stem cells in health and disease," *Nature Reviews Immunology*, vol. 8, no. 9, pp. 726–736, 2008.

[35] H. M. Lazarus, S. E. Haynesworth, S. L. Gerson, N. S. Rosenthal, and A. I. Caplan, "Ex vivo expansion and subsequent infusion of human bone marrow-derived stromal progenitor cells (mesenchymal progenitor cells): implications for therapeutic use," *Bone Marrow Transplantation*, vol. 16, no. 4, pp. 557–564, 1995.

[36] K. Le Blanc, I. Rasmusson, C. Götherström et al., "Mesenchymal stem cells inhibit the expression of CD25 (interleukin-2 receptor) and CD38 on phytohaemagglutinin-activated lymphocytes," *Scandinavian Journal of Immunology*, vol. 60, no. 3, pp. 307–315, 2004.

[37] I. Müller, M. Vaegler, C. Holzwarth et al., "Secretion of angiogenic proteins by human multipotent mesenchymal stromal cells and their clinical potential in the treatment of avascular osteonecrosis," *Leukemia*, vol. 22, no. 11, pp. 2054–2061, 2008.

[38] N. Noiseux, M. Gnecchi, M. Lopez-Ilasaca et al., "Mesenchymal stem cells overexpressing Akt dramatically repair infarcted myocardium and improve cardiac function despite infrequent cellular fusion or differentiation," *Molecular Therapy*, vol. 14, no. 6, pp. 840–850, 2006.

[39] R. E. Newman, D. Yoo, M. A. LeRoux, and A. Danilkovitch-Miagkova, "Treatment of inflammatory diseases with mesenchymal stem cells," *Inflammation and Allergy—Drug Targets*, vol. 8, no. 2, pp. 110–123, 2009.

[40] R. A. Musina, A. V. Belyavski, O. V. Tarusova, E. V. Solovyova, and G. T. Sukhikh, "Endometrial mesenchymal stem cells isolated from the menstrual blood," *Bulletin of Experimental Biology and Medicine*, vol. 145, no. 4, pp. 539–543, 2008.

[41] N. Hida, N. Nishiyama, S. Miyoshi et al., "Novel cardiac precursor-like cells from human menstrual blood-derived mesenchymal cells," *Stem Cells*, vol. 26, no. 7, pp. 1695–1704, 2008.

[42] C. M. Raynaud, M. Maleki, R. Lis et al., "Comprehensive characterization of mesenchymal stem cells from human placenta and fetal membrane and their response to osteoactivin stimulation," *Stem Cells International*, vol. 2012, Article ID 658356, 13 pages, 2012.

[43] N. Shanks, R. Greek, and J. Greek, "Are animal models predictive for humans?" *Philosophy, Ethics, and Humanities in Medicine*, vol. 4, no. 1, article 2, 2009.

Hindawi Publishing Corporation
Stem Cells International
Volume 2014, Article ID 276862, 6 pages
http://dx.doi.org/10.1155/2014/276862

Research Article

Enhanced Adipogenicity of Bone Marrow Mesenchymal Stem Cells in Aplastic Anemia

Naresh Kumar Tripathy, Saurabh Pratap Singh, and Soniya Nityanand

Stem Cell Research Facility, Department of Hematology, Sanjay Gandhi Post Graduate Institute of Medical Sciences, Raebareli Road, Lucknow 226014, India

Correspondence should be addressed to Soniya Nityanand; soniya_nityanand@yahoo.co.in

Received 18 February 2014; Accepted 3 April 2014; Published 30 April 2014

Academic Editor: Katherine Athayde Teixeira de Carvalho

Fatty bone marrow (BM) and defective hematopoiesis are a pathologic hallmark of aplastic anemia (AA). We have investigated adipogenic and osteogenic potential of BM mesenchymal stem cells (BM-MSC) in 10 AA patients (08 males and 02 females) with median age of 37 years (range: 06 to 79 years) and in the same number of age and sex matched controls. It was observed that BM-MSC of AA patients had a morphology, phenotype, and osteogenic differentiation potential similar to control subjects but adipocytes differentiated from AA BM-MSC had a higher density and larger size of lipid droplets and they expressed significantly higher levels of adiponectin and FABP4 genes and proteins as compared to control BM-MSC ($P < 0.01$ for both). Thus our data shows that AA BM-MSC have enhanced adipogenicity, which may have an important implication in the pathogenesis of the disease.

1. Introduction

Aplastic anemia (AA) is a bone marrow (BM) failure syndrome characterized by a fatty BM and peripheral pancytopenia. Defects in hematopoietic stem cells (HSC) as well in the BM stroma have been implicated in the pathogenesis of AA but the exact cause of the disease is still obscure [1]. We have previously shown in AA an increased apoptosis of BM cells [2], IL-8 levels [3], and expression of interferon-λ and tumor necrosis factor-α in BM T-cells as well as their increased levels in BM plasma [4]. These studies point towards the role of immune mechanisms and bone marrow microenvironment in the pathogenesis of the disease. Mesenchymal stem cells (MSC) are the key stem cells of the BM microenvironment that give rise to different stromal cell types including adipocytes, osteoblasts, endothelial cells, and stromal fibroblasts and maintain hematopoietic homeostasis in the marrow by cell-cell contact and by producing various hematopoietic cytokines and growth factors [5, 6]. The AA BM-MSC have been shown to have an abnormal gene expression profile [7] and abnormal immunological properties [8, 9] indicating a BM-MSC dysfunction in AA. It is also recently reported that adipocytes present in the BM suppress HSC maturation and differentiation and an imbalance between adipogenic and osteogenic differentiation of MSC may substantially influence hematopoiesis [10–12]. Thus, in order to further explore the role of BM-MSC in the disease, we have evaluated their adipogenic and osteogenic differentiation potential in patients with AA.

2. Materials and Methods

2.1. Subjects and Culture and Phenotypic Characterization of BM-MSC. Ten AA patients [12], 08 males and 02 females with median age of 37 years (range: 6 to 79 years), and the same number of age and sex matched controls were recruited in the study. After informed consent, 5 mL of BM was aspirated from the posterior superior iliac crest of each subject and BM-MSC were isolated, cultured and phenotypically characterized as per the standard protocol established in the lab [13]. The cells of 3rd passage were used in the experiments.

2.2. Adipogenic Differentiation. BM-MSC in 3rd passage were treated with adipogenic medium consisting of DMEM medium (Invitrogen) containing 10% FBS (Hyclone), 500 mM IBMX, 1 mM dexamethasone, 10 mg/mL insulin, and 100 mM indomethacin (adipogenesis kit, Chemicon). After 18 days, the cells were fixed and stained with oil red O stain to visualize the fat droplets in the cells.

2.3. Osteogenic Differentiation. BM-MSC in 3rd passage were treated with osteogenic medium consisting of DMEM medium (Gibco-Invitrogen) containing 10% FBS (Hyclone), 1 mM dexamethasone, 10 mg/mL glyceraldehyde 3-phosphate, and 0.1 mM ascorbic acid (osteogenesis kit, Chemicon). After 21 days, the cells were fixed with 4% paraformaldehyde and stained with alizarin red stain to visualize mineralization.

2.4. Reverse-Transcription Polymerase Chain Reaction (RT-PCR). Expression of adiponectin, fatty acid binding protein 4 (FABP4) and osteopontin was done by RT-PCR. Total RNA of BM-MSC of AA patients and controls was extracted using RNeasy Mini RNA isolation kit (Invitrogen). One μg of total RNA was reverse transcribed into cDNA using random hexamers (Invitrogen). The gene primers (MWG Biotech, http://www.mwg-biotech.com/) used were as follows. Adiponectin: (forward) 5′-AAGGAGATCCAGGTCTTATTGG-3′ and (reverse) 5′ACCTTCAGCCCCGGGTAC-3′ (accession number: NM_004797.2); FABP4: (forward) 5′-CCTTTAAAAATACTG-AGATTTCCTTCA-3′ and (reverse) 5′- GGACACCCCCAT-CTAAGGTT-3′ (accession number: NM_001442.2); osteopontin: (forward) 5′-GGATCCCCAGATGCTGTGGCC-ACATG-3′ and (reverse) 5′-CTCGAGTTAATTGACCTC-AGAAGATGC-3′ (accession number: NM_001040058.1); and β-actin: (forward) 5′-GCTCGTCGTCGACAACGG-CTC-3′ and (reverse) 5′- CAAACATGATCTGGGTCAT-CTTCTC-3′ (accession number: BC016045). The amplicons were resolved on 2% agarose gel (Sigma-Aldrich) and pictures were acquired using gel documentation system (Alpha Imager, http://www.alphainnotech.com/).

2.5. Western Blotting. The BM-MSC treated with induction medium or untreated cells were homogenized in lysis buffer [10 mM Tris-Cl (pH 7.5), 50 mM NaCl, and 1% Triton-X-100 containing phenylmethylsulfonyl fluoride (1 mM) and protease inhibitor cocktail] and centrifuged at 12,000 xg for 15 min at 4°C and the supernatant was estimated for protein content. 100 μg protein of each sample was subjected to 6% SDS-PAGE and electrotransferred onto nitrocellulose membrane. The membranes were incubated with antibodies against adiponectin (Abcam), FABP4 (R&D Systems; http://www.rndsystems.com/), osteopontin (Abcam, http://www.abcam.com/), and β-actin (R&D Systems) followed by incubation with HRP-conjugated corresponding secondary antibodies. The signals were detected using an enhanced chemiluminescence detection system (Amersham Biosciences, http://www.gelifesciences.com/).

2.6. Statistical Analysis. The results were calculated as mean ± SD. The difference between control and aplastic anemia patients was evaluated by Student's *t*-test.

3. Results

3.1. Morphology and Phenotypes. The BM-MSC of patients with AA exhibited characteristic fibroblastoid morphology similar to those of controls. Flow cytometric analysis revealed that BM-MSC of AA patients and controls had similar expression of CD73 (96.77 ± 2.03% versus 94.68 ± 2.26%), CD90 (97.96% ± 3.34% versus 98.86 ± 2.64%), and CD105 (92.28 ± 3.88% versus 89.28% ± 3.62%) ($P > 0.5$, for all) and absence of expression of CD34, CD45, and CD14 (Figure 1).

3.2. Adipogenic Differentiation and Expression of Adipogenic Transcripts and Proteins. Oil red O staining of the adipocytes differentiated from AA BM-MSC had a higher density and larger size of lipid droplets, as compared to controls (Figure 2: (iA) and (iB)). The RT-PCR and Western blot analysis showed significantly higher expression of Adiponectin and FABP4 transcripts and proteins, respectively, in the adipocytes derived from AA BM-MSC than those of controls ($P < 0.01$, for all) (Figure 2: (ii) and (iii)).

3.3. Osteogenic Differentiation and Expression of Osteopontin Gene and Protein. The BM-MSC of patients with AA on treatment with osteogenic medium exhibited osteogenic differentiation similar to those of controls as shown by alizarin red staining (Figure 3: (iA) and (iB)). The RT-PCR and Western blot analysis showed no significant difference in the expression of osteopontin gene and protein, respectively, in osteocytes differentiated from BM-MSC of AA patients and those of controls ($P > 0.5$) (Figure 3: (ii) and (iii)).

4. Discussion

Our study shows that BM-MSC from patients with AA have morphology, phenotype, and osteogenic potential similar to those of controls but they exhibit an enhanced adipogenic potential as revealed by larger size and higher density of lipid droplets and a higher expression of adipogenic genes and proteins in adipocytes differentiated from AA patients as compared to controls.

The only one study available in the literature has demonstrated that AA BM-MSC have a normal phenotype and can be readily differentiated into adipocytes with increased expression of genes of adipocytokine signaling pathway including TRADD, PRKAB2, LEP, SLC2A1, and SOCS3 [7]. This study has also shown that AA BM-MSC are difficult to differentiate into osteoblasts but J. Li et al. have not studied the expression of the osteogenic genes or proteins in the differentiated cells. We have also observed a normal phenotype of BM-MSC

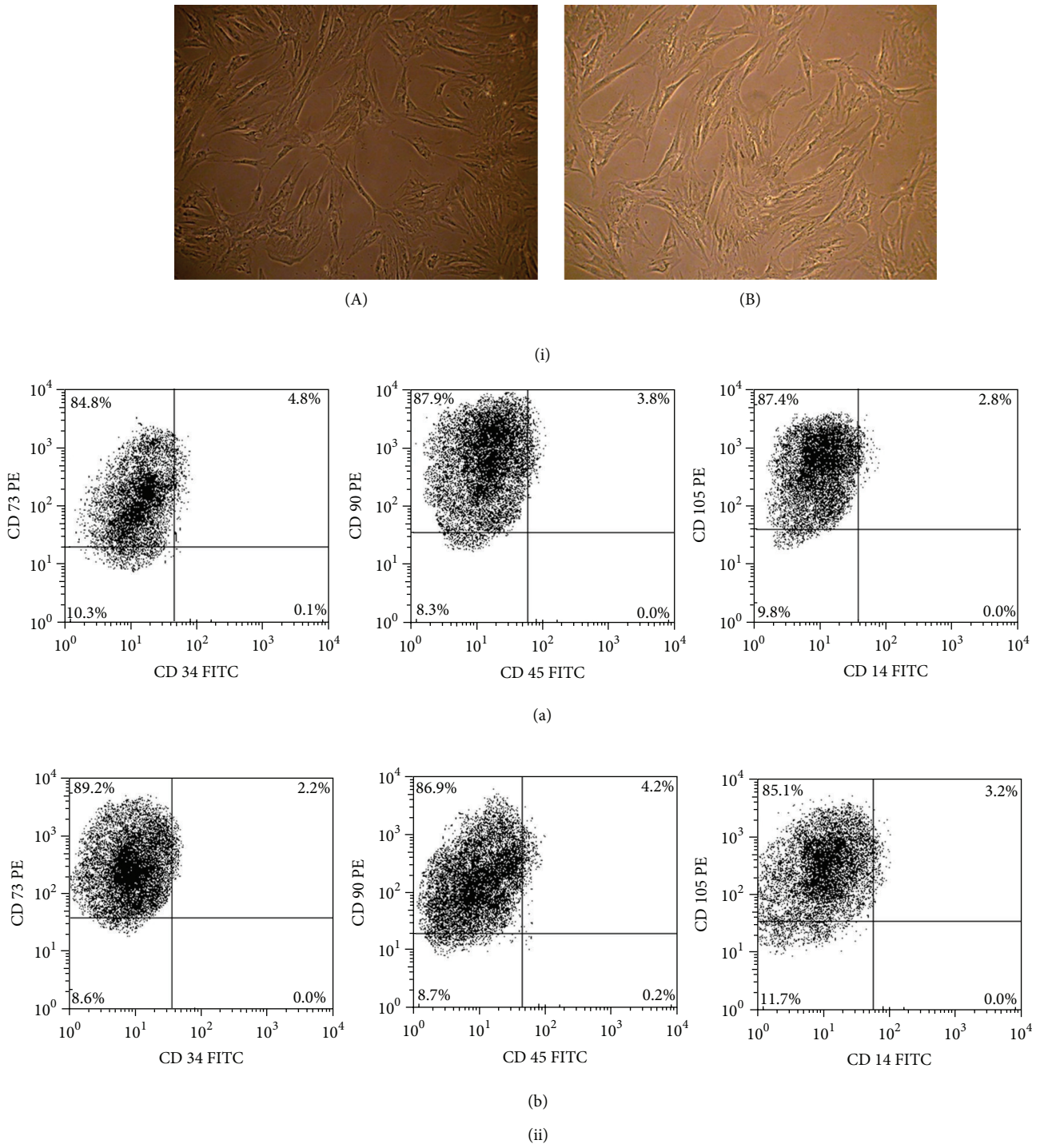


Figure 1: BM-MSC morphology and phenotype. (i) Fibroblastoid morphology of BM-MSC of (A) aplastic anemia patients and (B) control patients. (ii) Representative dot plots showing phenotype of BM-MSC of (a) aplastic anemia patients and (b) control patients.

from AA patients and demonstrated their enhanced adipogenicity by oil red staining of differentiated cells as well as by quantification of the adipogenic gene and proteins. However, we observed that expression of the osteopontin gene and proteins was similar to controls. Another study has reported that AA BM-MSC have a lower expression of GATA-2, which suppresses adipocytic differentiation, and a higher expression of peroxisome proliferator-activated receptor gamma that promotes adipocytic differentiation [14]. This study also lends support to our observation of enhanced adipogenic potential of AA BM-MSC.

Marrow adipocytes have long been viewed as space filler cells but some recent studies have shown that BM adipocytes

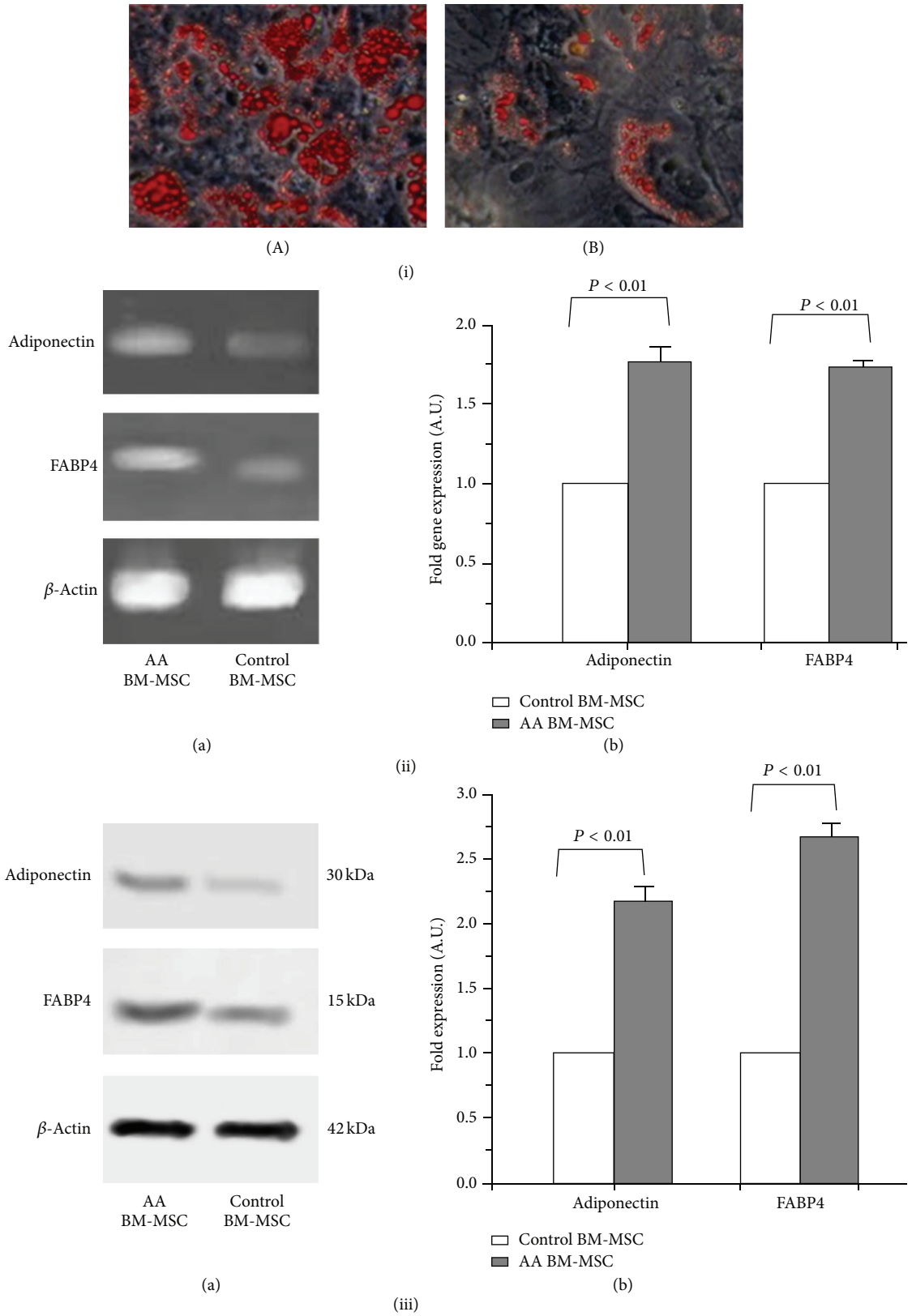


Figure 2: Adipogenic and osteogenic differentiation of BM-MSC of aplastic anemia patients. (i) Oil red O staining of adipocytes differentiated from BM-MSC of (A) aplastic anemia patients and (B) controls (bright field microscope view at 20x). (ii) Gene expression of adiponectin and FABP4 in adipocytes differentiated from BM-MSC of aplastic anemia patients and controls. (a) Representative gel pictures of RT-PCR. (b) Fold gene expression. (iii) Protein expression of adiponectin and FABP4 in adipocytes differentiated from BM-MSC of aplastic anemia patients and controls. (a) Representative Western-blot picture. (b) Fold expression of proteins.

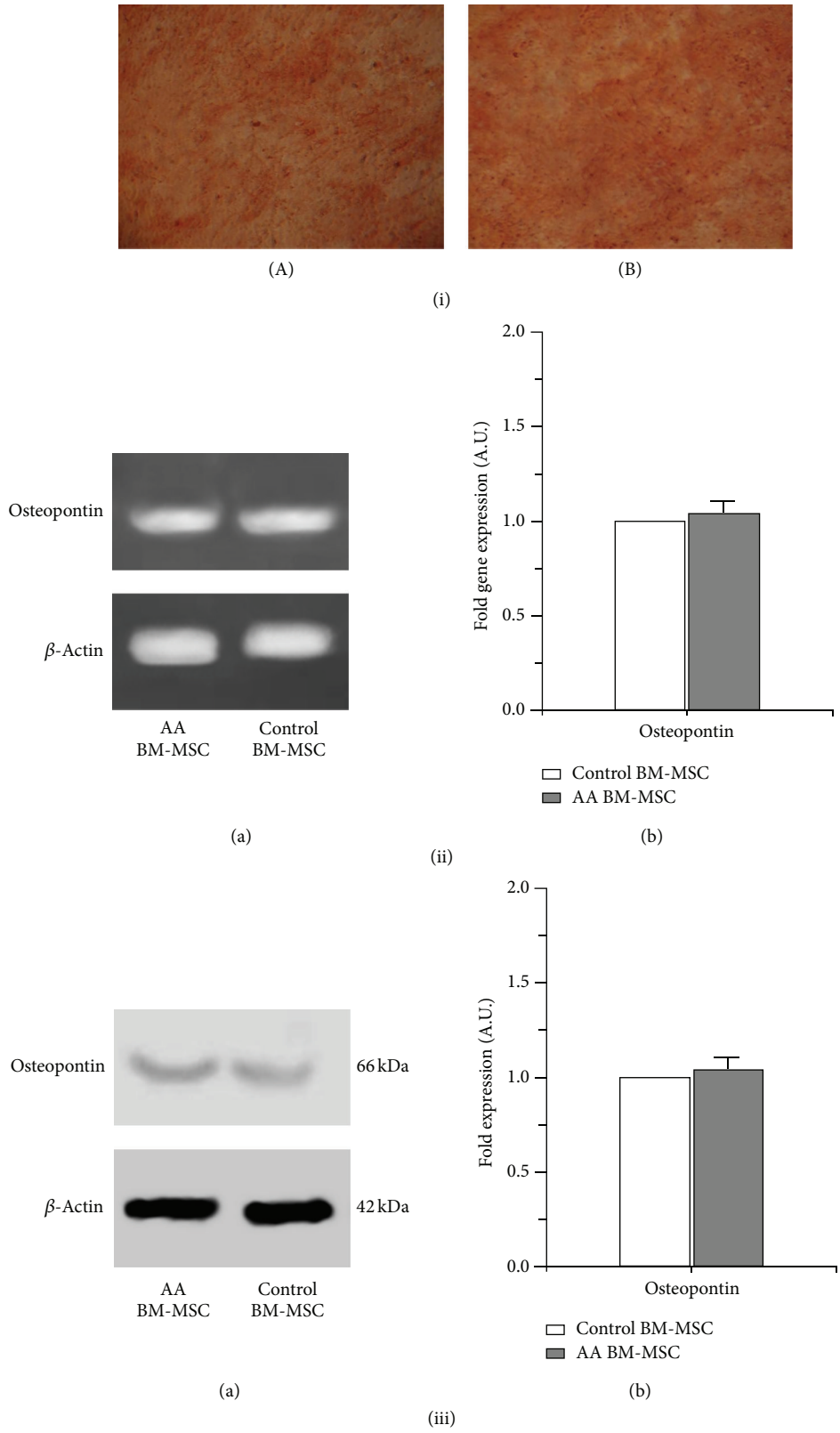


Figure 3: Osteogenic differentiation of BM-MSC of aplastic anemia patients. (i) Alizarin red staining of osteocytes differentiated from BM-MSC of (A) aplastic anemia patients (B) controls (bright field microscope view at 20x). (ii) Gene expression of osteopontin in osteocytes differentiated from BM-MSC of aplastic anemia patients and controls. (a) Representative gel pictures of RT-PCR. (b) Fold gene expression. (iii) Protein expression of osteopontin in osteocytes differentiated from BM-MSC of aplastic anemia patients and controls. (a) Representative Western-blot picture. (b) Fold expression of proteins.

are negative regulators of hematopoiesis and have a reciprocal relationship with osteoblasts that promote hematopoiesis [11, 14]. Moreover, it has also been reported that BM adipocytes produce neuropilin-1, adiponectin, and TNF-α and each of which has a suppressive effect on hematopoiesis [15]. We have shown that adipocytes differentiated from AA BM-MSC exhibit higher expression of adiponectin and FABP4 genes and proteins. Although the role of FABP4 in regulation of hematopoiesis is not known adiponectin is reported to potentially inhibit hematopoiesis. As BM adipocytes are a potent source of antihematopoietic cytokine TNF-α [15], the present study also supports our previous observation of increased levels of this cytokine in marrow plasma of patients with AA [4]. Thus the enhanced adipogenic potential of AA BM-MSC may contribute to the defective hematopoiesis in AA.

In summary, our study has demonstrated that BM-MSC of patients with AA possess an enhanced adipogenic potential which may have an important role in the pathogenesis of the disease. Further studies targeting molecular mechanisms involved in the hematopoietic inhibition by adipocytes and abnormalities in other biological properties of BM-MSC of AA patients would add new insights into the pathogenesis and treatment of the disease.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Authors' Contribution

Naresh Kumar Tripathy and Saurabh Pratap Singh have contributed equally to the work. The contribution of authors to this paper is as follows: Saurabh Pratap Singh performed the research work and analyzed the data, Naresh Kumar Tripathy designed the research study and has written the paper, and Soniya Nityanand designed the study, provided reagents, and tools and reviewed the paper.

Acknowledgment

This work was supported by an extramural Grant sanctioned by the Department of Biotechnology, Government of India, to Dr. Sonya Nityanand (BT/PR6519/MED/14/826/2005).

References

[1] N. S. Young, R. T. Calado, and P. Scheinberg, "Current concepts in the pathophysiology and treatment of aplastic anemia," *Blood*, vol. 108, no. 8, pp. 2509–2519, 2006.

[2] V. Vibhuti, N. K. Tripathy, and S. Nityanand, "Massive apoptosis of bone marrow cells in aplastic anaemia," *British Journal of Haematology*, vol. 117, no. 4, pp. 993–994, 2002.

[3] N. K. Tripathy, V. Vibhuti, and S. Nityanand, "Bone marrow and blood plasma levels of IL-8 in aplastic anemia and their relationship with disease severity," *American Journal of Hematology*, vol. 79, no. 3, pp. 240–242, 2005.

[4] S. Dubey, P. Shukla, and S. Nityanand, "Expression of interferon-γ and tumor necrosis factor-α in bone marrow T cells and their levels in bone marrow plasma in patients with aplastic anemia," *Annals of Hematology*, vol. 84, no. 9, pp. 572–577, 2005.

[5] M. K. Majumdar, M. A. Thiede, S. E. Haynesworth, S. P. Bruder, and S. L. Gerson, "Cutting edge communication: human marrow-derived mesenchymal stem cells (MSCs) express hematopoietic cytokines and support long-term hematopoiesis when differentiated toward stromal and osteogenic lineages," *Journal of Hematotherapy and Stem Cell Research*, vol. 9, no. 6, pp. 841–848, 2000.

[6] M. E. Bernardo, A. M. Cometa, and F. Locatelli, "Mesenchymal stromal cells: a novel and effective strategy for facilitating engraftment and accelerating hematopoietic recovery after transplantation?" *Bone Marrow Transplantation*, vol. 47, no. 3, pp. 323–329, 2012.

[7] J. Li, S. Yang, S. Lu et al., "Differential gene expression profile associated with the abnormality of bone marrow mesenchymal stem cells in aplastic anemia," *PLoS ONE*, vol. 7, no. 11, Article ID e47764, 2012.

[8] A. Bacigalupo, M. Valle, M. Podestà et al., "T-cell suppression mediated by mesenchymal stem cells is deficient in patients with severe aplastic anemia," *Experimental Hematology*, vol. 33, no. 7, pp. 819–827, 2005.

[9] J. P. Li, C. L. Zheng, and Z. C. Han, "Abnormal immunity and stem/progenitor cells in acquired aplastic anemia," *Critical Reviews in Oncology/Hematology*, vol. 75, no. 2, pp. 79–93, 2010.

[10] R. Sugimura and L. Li, "Shifting in balance between osteogenesis and adipogenesis substantially influences hematopoiesis," *Journal of Molecular Cell Biology*, vol. 2, no. 2, pp. 61–62, 2010.

[11] O. Naveiras, V. Nardi, P. L. Wenzel, P. V. Hauschka, F. Fahey, and G. Q. Daley, "Bone-marrow adipocytes as negative regulators of the haematopoietic microenvironment," *Nature*, vol. 460, no. 7252, pp. 259–263, 2009.

[12] B. M. Camitta, E. D. Thomas, and D. G. Nathan, "A prospective study of androgens and bone marrow transplantation for treatment of severe aplastic anemia," *Blood*, vol. 53, no. 3, pp. 504–514, 1979.

[13] S. P. Singh, N. K. Tripathy, and S. Nityanand, "Comparison of phenotypic markers and neural differentiation potential of MAPC and MSC," *World Journal of Stem Cells*, vol. 5, pp. 53–60, 2013.

[14] Y. Xu, Y. Takahashi, Y. Wang et al., "Downregulation of GATA-2 and over expression of adipogenic gene-PPARgamma in mesenchymal stem cells from patients with aplastic anemia," *Experimental Hematology*, vol. 37, no. 12, pp. 1393–1399, 2009.

[15] Z. Belaid-Choucair, Y. Lepelletier, G. Poncin et al., "Human bone marrow adipocytes block granulopoiesis through neuropilin-1-induced granulocyte colony-stimulating factor inhibition," *Stem Cells*, vol. 26, no. 6, pp. 1556–1564, 2008.

Hindawi Publishing Corporation
Stem Cells International
Volume 2014, Article ID 306573, 11 pages
http://dx.doi.org/10.1155/2014/306573

Review Article

Clinical Applications of Mesenchymal Stem Cells in Chronic Diseases

Andrea Farini, Clementina Sitzia, Silvia Erratico, Mirella Meregalli, and Yvan Torrente

Laboratorio di Cellule Staminali, Dipartimento di Fisiopatologia Medico-Chirurgica e dei Trapianti, Università degli Studi di Milano, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Centro Dino Ferrari, Via F. Sforza 35, 20122 Milano, Italy

Correspondence should be addressed to Yvan Torrente; yvan.torrente@unimi.it

Received 4 December 2013; Revised 14 April 2014; Accepted 15 April 2014; Published 30 April 2014

Academic Editor: Katherine Athayde Teixeira de Carvalho

Extraordinary progress in understanding several key features of stem cells has been made in the last ten years, including definition of the niche, and identification of signals regulating mobilization and homing as well as partial understanding of the mechanisms controlling self-renewal, commitment, and differentiation. This progress produced invaluable tools for the development of rational cell therapy protocols that have yielded positive results in preclinical models of genetic and acquired diseases and, in several cases, have entered clinical experimentation with positive outcome. Adult mesenchymal stem cells (MSCs) are nonhematopoietic cells with multilineage potential to differentiate into various tissues of mesodermal origin. They can be isolated from bone marrow and other tissues and have the capacity to extensively proliferate *in vitro*. Moreover, MSCs have also been shown to produce anti-inflammatory molecules which can modulate humoral and cellular immune responses. Considering their regenerative potential and immunoregulatory effect, MSC therapy is a promising tool in the treatment of degenerative, inflammatory, and autoimmune diseases. It is obvious that much work remains to be done to increase our knowledge of the mechanisms regulating development, homeostasis, and tissue repair and thus to provide new tools to implement the efficacy of cell therapy trials.

1. Introduction

Since the work of Friedenstein, that firstly described bone marrow- (BM-) derived stromal cells with the capacity of differentiation into bone [1], it was thought that nonhaematopoietic stem cell resided in the bone marrow, the so-called mesenchymal stem cells (MSCs) [2, 3]. The group of Caplan identified the MSCs from BM for the expression of the specific antigen markers CD105 and CD73 [4]. Pittenger defined the MSCs as multipotent stem cell with the ability to differentiate into adipose tissue, bone, and cartilage [5]. According to this multilineage differentiation potential, it was believed that MSCs mediated tissue and organ repair [6, 7]. However, further studies assessed that, following specific molecular cues, MSCs reached the site of injury and allowed the repair of tissues by means of the expression of different trophic factors [8–10]. In the last 20 years, MSCs were isolated from a wide range of tissues [11–14] and organs [15, 16]. Furthermore, it was demonstrated that under specific stimuli MSCs possessed an incredible capacity of transdifferentiation, developing in mesodermal (myocyte, osteocyte, endothelium, adipocyte, and cardiomyocyte), ectodermal (neuronal), and endodermal (hepatic, pancreatic, respiratory epithelium) lineages. In the presence of β-glycerol-phosphate, ascorbic acid-2-phosphate, dexamethasone, and fetal bovine serum, MSCs proliferated as osteoblasts. On the other side, when they were grown with a serum-free nutrient medium added with TFG-β or family-related molecules, MSCs proliferated as chondrocytes, expressing cartilage-specific extracellular matrix components [17]. Similarly, it could be possible to induce the formation of adipocytes by means of peroxisome proliferator-activated receptor-γ (PPAR-γ), fatty acid synthetase, and isobutylmethylxanthine while, on the contrary, IL-1 and TNF-α blocked MSCs-adipogenetic differentiation. As published by Barry and Murphy, MSCs differentiation into myoblasts was driven by

5-azacytidine and amphotericin B [17]. Recently, different works suggested that MSCs were strictly associated with vessels and possibly with pericytes, the perivascular cells that surround microvessels [18]. It was demonstrated that pericytes retained the ability to differentiate not only into osteoblasts, adipocytes, and fibroblasts but also into neural lineages if cultivated with bFGF [19] and into smooth muscle cells if stimulated with low concentration of oxygen [20]. It is well known that MSCs are able to express integrins, adhesion molecules, and chemokine receptors that regulate their capacity of migration and homing: CCR1, CCR4, CCR7, CCR10, and CXCR5 [4, 21]. Thanks to the expression of these molecules, MSCs can reach damaged tissues through endothelial cell layers and participate not only in tissue regeneration but also in BM microenvironment replenishment [22]. Stromal derived factor (SDF)-1 is associated with mobilization of stem cells into the periphery and homing to the site of injury [23, 24]: it was showed that in diverse tissue injures SDF-1 functions as a MSCs chemoattractant [25–28]. According to these evidences, MSCs were evaluated in several studies for their safety and efficacy of transplantation. Studies published by Gao and Herrera confirmed the ability of MSCs to engraft into various organs following transplantation (liver, bone, and lung) [29, 30], while the groups of Jackson and Orlic successfully used them in the preparation of infarcted myocardium [31–33]. Furthermore, MSCs were noted to enhance angiogenesis in the myocardium [34] and also to allow the reduction of myocardial fibrotic area, probably due to their capacity of increasing the capillary density [35]. Hofstetter and colleagues demonstrated that MSCs exert their role also indirectly, enhancing the expression of growth factors that allowed the regeneration of damaged tissues [36]. However, further studies are necessary to better identify (i) all the molecules other than chemokines and adhesion molecules that drive MSCs to the site of injury; (ii) growth media to obtain reproducible culture techniques and to enhance safety of expanded MSCs; (iii) host responses to allogenic MSC therapy.

2. MSCs Isolation

Citofluorimetric analysis performed on MSCs showed that they express CD44, CD73, CD90, and CD105 receptors while lacking hematopoietic stem cell markers such as CD14, CD31, CD33, CD34, and CD45. Due to the absence of specific mesenchymal cell markers and the heterogeneity of the MSC populations, the Mesenchymal and Tissue Stem Cell Committee of the International Society for Cellular Therapy (ISCT) established three minimal criteria that MSCs isolated from human bone marrow and other mesenchymal tissues must have *in vitro*: (i) adherence to plastic in standard culture conditions, (ii) display of a specific surface antigen expression pattern (CD73+ CD90+ CD105+ CD34− CD45− CD11b− CD14− CD19− CD79a− HLA-DR−), and (iii) multipotency, that is, differentiation potential along the osteogenic, chondrogenic, and adipogenic lineages [37]. The heterogeneity of the MSC population is demonstrated by *in vitro* differentiation assays, where most of the population showed a differentiation potential towards the classical three cell types.

3. Immunomodulatory Effects of MSCs

Several studies have demonstrated that MSCs can inhibit cytotoxic T cells and natural killer (NK) cells [38, 39] by means of different pathways. MSCs can exert their immunomodulatory functions by secreting suppressors of T-cell development (TGFβ and hepatocyte growth factor (HGF)) [40] and proliferation such as leukemia inhibitory factor (LIF) [41] and IFN-γ [42]. Furthermore, MSCs can induce the expression of TNF-α and IL-1 leading to unbalanced secretion of chemokines and inducible nitric oxide (iNOS) [43]. More interestingly, the works of Spaggiari et al. [44] and Poggi et al. [45] showed that MSCs isolated from BM are not recognized by NKs as they express human leukocyte antigen (HLA) class I molecules. This way, MSCs were seen as the most feasible population of stem cells for cell transplantation experiments. Otherwise, recent studies demonstrated that MSCs were efficiently lysed by the cytotoxic immune effectors [39, 46]. The work of Jewett et al. showed that IL-2 treated NKs recognized and destroyed MSCs while IFN-γ had the opposite effect [47]. As the IFN-γ is secreted by monocytes, the authors postulated that these cells not only served as protector of MSCs but also allowed the differentiation of stem cells by NFκB dependent and independent pathways [47]. Giuliani et al. showed that MSCs expressed functional Toll-like receptors (TLR) that promote their proliferation and cytokine secretion [48]. They also identified a molecule, called MICA, that formed a complex with other immunoregulatory proteins and together with TLRs ligands protected MSCs against NKs aggression [48].

4. MSCs and Chronic Diseases

4.1. MSCs and Musculoskeletal Diseases. As for the other tissues previously described, MSCs were isolated from human adult skeletal muscle [49, 50]. In addition, Gonçalves described the ability of MSCs to complement dystrophin deficiency [51], and Németh et al. showed that MSCs increased the survival rate of model animals by the modulation of macrophage activity [52]. For these reasons, these cells became feasible to therapeutic application for Duchenne muscular dystrophy (DMD). Different groups demonstrated that transplantation of MSCs into murine model of DMD replenished the host satellite cell compartment—allowing the expression of dystrophin and ameliorating the dystrophic phenotype—and also remained as a pool of quiescent satellite cells [53, 54]. Similarly, de Bari and coworkers isolated MSCs from human synovial membrane and injected them into mdx mice, showing MSCs persistence into host muscles up to six months [55]. Gang et al. demonstrated that MSCs derived from human umbilical cord blood (UCB-MSCs) differentiated into skeletal muscle and expressed MyoD and myogenin, muscle-specific transcription factors: transplanted into dystrophic mice, they allowed highly detectable expression of myosin [56].

The role that MSCs play in regulating inflammation is now clear. It is a "multistep" event as MSCs could exert their role as a negative controller/suppressor, by expressing SDF-1 and CCL2 [57], by inhibiting macrophage activation [58]

or by Th1, NK, and cytotoxic T-cell generation [39]. Alternatively, MSCs could act as positive controller/activator by enhancing the proliferation of Th2 cells and regulatory T cells (Treg) [59] and by the expression of immune suppressive cytokines and enzymes [60, 61]. Given these evidences, together with the fact that the only functional treatment for DMD is the corticosteroid therapy that regulates the inflammatory reactions, MSCs were widely used in dystrophic animal models. Firstly, MSCs were injected into the uterus of mdx mice at different days of pregnancy: the cells were observed to engraft in different muscles but their functionality was not altered [62, 63]. Adipose-derived MSCs (AD-MSCs) were transplanted into dystrophic mice and they homed to necrotic fibers. Moreover, AD-MSCs allowed the re-expression of dystrophin and muscular remodelling, even if at lower rate [64]. Furthermore, injection of MSCs was seen to inhibit the expression of creatine kinases whereas increasing the number of centrally nucleated myofibers [65]. According to these evidences, Kong et al. transplanted UCB-MSCs into animal model of limb girdle muscular dystrophies (LGMDs), characterized by predominant weakness and wasting of proximal muscles, but they did not obtain promising results [66]. Although the bones naturally restore without significant scarring, infections, trauma, and cancer could impair their functional restoration, causing several bone defects [67, 68]. Cell-based therapies need to isolate MSCs from the BM of the patient, to expand and enrich the cells and to seed them into the most suitable three-dimensional scaffold and/or matrix [69]. As an example, osteonecrosis is caused by femoral death due to poor blood supply [70]: three patients were treated with MSCs infusion with TCP-treated matrix and good results were obtained [71]. Similarly, Nöth et al. injected a preparation of MSCs into three patients and obtained encouraging results, as shown by radiographic and magnetic resonance imaging examination [72]. MSCs were also successfully used for spinal fusion disease [73], so that phase I clinical trials arose [74, 75]. Patients affected by severe osteogenesis imperfecta were injected systematically with purified allogenic MSCs: these cells were able to engraft into host bones, where they proliferated into osteoblasts and allowed an amelioration in the total bone mineral density [76, 77]. Although these encouraging studies were performed, the amount of MSCs recruited into the bones was too small in a clinical point of view. Alternatively, the group of Le Blanc treated a female fetus with multiple intrauterine fractures with allogenic fetal MSC [13].

4.2. MSCs and Cardiovascular Diseases. Starting from the evidences that MSCs not only secreted molecules that exerted important effects on cellular microenvironment [36] but also differentiated *in vitro* into cardiomyocytes [78, 79], these cells were extensively used for cardiovascular repair. Shake and Nagaya demonstrated that, following systemically injection into rodent models of these diseases, MSCs engrafted and partially repaired the infarcted myocardium [80, 81]. In particular, Nagaya and collaborators showed that transplanted MSCs increased capillary density and decreased the collagen volume fraction and the fibrosis in the myocardium of a rat suffering from dilated cardiomiopathy [82]. Furthermore, they also noted a significant ventricular functional recovery as previously demonstrated [83]. According to these promising evidences, Katritsis et al. treated 11 infarcted patients with autologous MSCs, together with endothelial progenitor's cells, and showed partial improvement of myocardial contractility. Unfortunately, they were not able to decipher the mechanisms responsible for these phenomena [84]. Similarly, several infarcted patients that were subjected to coronary intervention were transplanted with autologous MSCs that improved left ventricular function [85]. Takahashi and collaborators assessed that the molecules secreted by MSCs were able to protect the myocardium by preserving its contractile capacity; in particular, MSCs-derived cytokines inhibited the apoptosis of cardiomyocytes, allowing the formation of new vessels in damaged tissues [86].

4.3. MSCs and Liver Disease. Fulminant hepatic failure (FHF) is a severe disease characterized by massive hepatocellular death: the only treatment is liver transplantation that requires lifelong immunosuppression and high costs. Different works demonstrated that MSCs-secreted molecules not only allowed tissue repair of infarcted tissue [82] but furthermore prevented parenchymal cell loss [87]. This way, van Poll and colleagues reported that, following systemic injection of MSCs in a rat model for FHF, there was an amelioration of the pathological phenotype—so that liver injury biomarkers were not released—and, more interestingly, hepatocellular death was drastically reduced, while hepatocytes proliferation increased [88]. Concerning cirrhosis, four patients were injected with autologous MSCs in a phase I trial; they did not suffer from any side effects, thus improving their quality of life [89]. Similarly, 8 patients with end-stage liver disease were treated with MSCs and their condition ameliorated demonstrating that this treatment could be feasible and efficient for this kind of pathologies [90].

4.4. MSCs and Autoimmune Diseases. Since Riordan et al. suggested that in the bone marrow one of the most important functions of MSCs could be the protection of haematopoietic precursor from inflammatory damage [91], the use of MSCs as inhibitors of inflammation became conceptually appealing. This way, MSCs were used to block the development of chronic inflammatory processes that are typical of DMD (as described in detail in Section 4.1), autoimmune arthritis, diabetes, and lupus.

4.4.1. Rheumatoid Arthritis. Rheumatoid arthritis (RA) is characterized by chronic joint inflammation due to loss of immunologic self-tolerance. González and colleagues injected DBA/1 mice that suffered from collagen-induced arthritis, with MSCs derived from human adipose tissue, and evaluated the inflammatory response of treated animals [92]. They showed that following the injection of AD-MSCs the levels of inflammatory cytokines and chemokines were decreased as the expansion of antigen-specific Th1/Th17 cell. In contrast this treatment increased the production of IL-10. Together with its well-known function as an anti-inflammatory factor [93], recent findings demonstrated that

IL-10 is fundamental for the development of Tregs that control self-antigen–reactive T cells and induce peripheral tolerance *in vivo* [94]. Interestingly, they found that treated mice had an increase in the percentage of CD4+ CD25+ FoxP3+ Tregs and suggested that these cells could migrate to the joints, regulating the suppression of the self-reactive response [92]. It is known that type-II collagen (CII), one of the components of hyaline cartilage, acts as an autoantigen in RA. When CII and the other antigenic peptides are recognized by T cells, they cause the uncontrolled activation of immune system cells, leading to destruction of the joints typical of RA patients. Zheng et al. demonstrated that MSCs isolated from RA patients exerted immunosuppressive functions, by inhibiting T-cell proliferation, blocking the secretion of several proinflammatory cytokines, and allowing the expression of anti-inflammatory IL-10 [95]. They also obtained MSCs from chondrocytes and described that, following transplantation into RA joints, these cells not only suppressed the inflammation regulating the secretion of TGF-β 1 but also prevented joints destruction [95].

Similar to the previously described work of González et al. [92], experimental data from Augello [96] and Mao [97] confirmed the positive effects of MSCs transplantation into animal model of collagen-induced arthritis while others did not describe any amelioration [98, 99]. Schurgers et al. showed the discrepancy between the *in vitro* and *in vivo* immunosuppression ability of MSCs. *In vitro*, MSCs inhibited the proliferation of T cells by regulating the levels of IFN-γ whereas *in vivo* transplantation of these cells into CIA animal models did not affect the progression of the disease [100]. As an explanation, due to problems during intravenously injection, MSCs could not reach the spleen and lymph nodes, so that they did not exert their functions. As MSC treatment for this pathology was not efficacious, they also suggested to focus on Treg as in mice injected with these cells the pathological signs of arthritis were significantly ameliorated [101, 102]. Another work from Bouffi et al. demonstrated that MSCs elicited their immunosuppressive effect by means of a pathway regulated by the prostaglandine-2. Moreover, they showed that MSCs operated independently from Treg cell induction. Finally they suggested that the contradictory effect of MSCs transplantation could be related to the different age of the mice used in those studies [103].

4.4.2. Systemic Lupus Erythematosus. Systemic lupus erythematosus (SLE) is an autoimmune disease that can affect any part of the body [104]. Recent findings demonstrated several defects in the hematopoietic system of SLE patients, probably due to unbalanced expression of cytokines and other growth factors. Interestingly, it was found that bone marrow-derived CD34+ stem cells overexpressed different surface markers such as CD123 and CD166 that are closely related to T-cell development inflammation [105]. Accordingly, Kushida et al. showed that transplantation of hematopoietic stem cells prevented the onset of the disease in the most commonly studied mouse model of SLE, the MRL/lpr mice [106]. Sun and colleagues determined a possible role of BM-derived MSCs in the haematological disorder typical of SLE patients [107] and suggested that MSCs transplantation could be used to ameliorate the autoimmune progression of the disease [108]. In fact they found that MSCs inhibited T-lymphocytes and Th2 proliferation and B-cell production of autoantibody, so that the pathological signs of MRL/lpr mice were drastically diminished [108].

4.4.3. Type 1 Diabetes. Type 1 diabetes is an autoimmune disease mediated by the production of auto-antibody directed against the β-cells of the pancreas. As a consequence of the destruction of these cells, the quantity of insulin produced is not sufficient to control sugar blood level. Despite the exogenous administration of insulin, long-term consequences of hyperglycemia usually occur, including vascular degeneration, blindness, and kidney failure. Islet replacement is the best way to fully reproduce the physiological release of insulin; however, both the limited availability of transplantable organs and the need for immunosuppression have limited the application of this strategy [109]. Recently it has been suggested that MSCs can overcome these problems as they can be differentiated into glucose-responsive, insulin-producing cells and they possess immunomodulatory properties. It was hypothesized that resident pancreatic MSCs could be forced to adopt a pancreatic fate *in vitro*. Thus, Zulewski et al. reported the isolation of nestin-positive islet-derived progenitor cells from rat pancreatic islets and their ability to differentiate *in vitro* toward pancreatic endocrine phenotype [110]. Similarly Huang and Tang described the correction of hyperglycemia in diabetic NOD-SCID mice thanks to nestin-positive precursors derived from human fetal pancreas [111]. However, the results of these studies were controversial and partially inconclusive [112–115] so that MSCs from other tissues could be an alternative. Among them, bone marrow derived MSCs were shown to partially differentiate into endocrine pancreatic cells [116]; furthermore, *in vivo* maturation of these cells partially compensated their low differentiation efficiency *in vitro* [117]. An intriguing option comes from studies on umbilical cord blood-derived MSCs that demonstrated the expression of pancreatic development genes in these cells. Recently a population of UCB-derived cells was shown to behave like hES cells, recapitulating the same differentiation steps from early stages to β-cells [118]. In conclusion, before MSCs clinical application in diabetes further studies are needed to improve MSCs based protocols and above all to expand our knowledge on MSCs immunogenicity in a HLA-mismatched context.

4.5. MSCs and Neurodegenerative Diseases

4.5.1. Multiple Sclerosis. Multiple sclerosis is an important cause of neurological disability in young adults. Although it is a multifactorial disease, it is known that the presence of an aberrant immunoresponse leads to patches of damage throughout the brain and spinal cord. Autoreactive T cells cause myelin destruction and secondary oligodendrocyte and axonal damage [119]. Despite the efficacy of immunomodulatory or immunosuppressive drugs in controlling the number of relapses, no current therapy is effective to arrest the

progressive phase of the disease. The therapeutic potential of stem cell lies in enhancing myelin regeneration, through the replacement of lost oligodendrocytes, and therefore in preserving axons, thanks to the neurotrophic support [120]. However, the widespread distribution of lesions and the gray matter damage render the therapeutic efficacy of direct mesenchymal injection really low. Furthermore bone marrow-derived cells ability to make oligodendrocytes was low [121]. Nevertheless, bone marrow-derived MSCs have pronounced immune-modulating and immunosuppressive properties [122, 123] so as they were being tested in clinical trial for relapsing-remitting multiple sclerosis Mesenchymal Stem Cells for Multiple Sclerosis (MESEMS) (NCT01854957). Furthermore it was thought that mesenchymal stem cells promote self-repair by reducing scar formation, by stimulating the formation of new blood vessel, and by secreting growth and neuroprotective factors, such as superoxide dismutase-3 [124]. After intravenous injection, many cells entered the CNS and became widely distributed both in experimental models and in patients [125–127]: safety studies did not evidence adverse effect such as tumor formation, except for meningeal syndrome and some preliminary evidence of beneficial effects that were reported [128, 129].

4.5.2. Amyotrophic Lateral Sclerosis. Suzuki et al. isolated MSCs from muscles and genetically modified them to constitutively express glial-derived neurotrophic factor (GDNF). Then, they transplanted the engineered MSCs into rat model of amyotrophic lateral sclerosis (ALS), a neurodegenerative disease in which patients lose motor neurons and suffer from progressive and lethal paralysis. Interestingly, ALS rats ameliorated the pathological phenotype, increasing the number of neuromuscular connections [130].

4.5.3. Parkinson's Disease. Parkinson's disease (PD) is a neurodegenerative disorder characterized by the progressive loss of dopaminergic neurons (DA), especially in the pars compacta of the substantia nigra. The mesostriatal dopaminergic pathway projects in the striatum and their absence causes several motor complications, including rigidity, bradykinesia, and postural instability [131, 132]. DA agonists and Levodopa (l-dopa) are effective symptomatic therapy, but unfortunately, with long-term use, they become inefficient and patients develop significant side effects. Stem cell therapy, with the aim of replacing lost neurons, is the most promising strategy for this disease [133]. It has been demonstrated that MSCs cells can enhance the levels of tyrosine hydroxylase (TH) and dopamine levels after transplantation in PD animal models [134]. Furthermore it has been suggested that these cells contribute to neuroprotection by secreting trophic factors, like EGF, VEGF, NT3, FGF-2, HGF, and BDNF or through antiapoptotic signalling [135] without differentiation in neuronal phenotype [136]. For these reasons new strategies, involving the genetic modification of hMSCs, arose, as a tool to induce the secretion of specific factors or to increase the percentage of DA cell differentiation [137]. For example Barzilay et al. transduced adult-derived bone marrow MSCs with a lentivirus carrying LMX1a gene: these cells showed an expression profile similar to a developing mesodiencephalic neuron and allowed DA cell differentiation [138].

4.5.4. Alzheimer Disease. Alzheimer disease (AD) is the most common form of neurodegenerative dementia; affected patients suffer from progressive loss of memory and intellectual abilities. Major anatomopathological features of AD are represented by β-amyloid deposition and neurofibrillary tangles formation that ultimately end in cholinergic neurons degeneration. No treatment is currently able to stop the progression of AD [139]. Recently, different studies tried to ameliorate neuropathological deficits in animal model of Alzheimer's disease through stem cell therapy. In particular, Shin et al. focused on clearance of amyloid plaque and they demonstrated that MSCs could enhance the cell autophagy pathway increasing neuron survival both *in vitro* and *in vivo* [140]. Similarly Ma's group demonstrated that adipose-derived MSC, once transplanted in AD model mice, could modulate the inflammatory environment; in particular they caused an activation of the microglia that promoted the expression of alternative markers and Aβ-degrading enzymes, while decreasing expression levels of proinflammatory factors [141]. Following promising results from MSCs treatment for autoimmune diseases, it was thought to modulate the inflammatory environment of AD. In particular abnormalities of Tregs in cell number and/or function were observed [142] and it was shown that they could modulate microglial activation [143]. Yang et al. demonstrated that umbilical cord-derived MSCs activated Tregs *in vitro* and once transplanted in AD animal model, Tregs modulated microglia activation, increasing neuron survival [144].

5. Clinical Applications of MSCs

Before clinical applications of stem cells we need to understand their biological characteristics in order to obtain therapeutic effects. In case of MSCs, four properties are considered the most important to guarantee a clinical rescue: (i) the ability to home to the site of inflammation, following tissue injury, when injected intravenously; (ii) the ability to differentiate into various cell types; (iii) the ability to secrete multiple bioactive molecules capable of stimulating recovery of injured cells and of inhibiting inflammation; (iv) the lack of immunogenicity and the ability to perform immunemodulatory functions [87]. Moreover, the role of MSCs in therapeutic effects has still to be elucidated. MSCs have the capacity to migrate and to engraft in site of inflammation, after local or systemic administration. Various studies demonstrated that, under a variety of pathologic conditions, MSCs selectively home to sites of injury, indifferently from the tissue. Ortiz et al. showed that murine MSCs could home to lung in response to injury, adopt an epithelium-like phenotype, and reduce inflammation in lung tissue of mice challenged with bleomycin [145]. Liu et al. found that transplanted MSCs could migrate to injured muscle tissues in mdx mice [64]. Cell migration depends on many signals, including growth factors, interleukins, and chemokines, secreted by injured cells and immune cells [146]. Recently Yagi et al.

demonstrated that the migration of MSCs is under the control of many tyrosine kinase growth factor receptors like platelet-derived growth factor (PDGF) and insulin like growth factor 1 (IGF-1); in addition, several chemokines such as CCR2, CCR3, CCR4, or CCL5 ameliorate MSCs migration in *in vitro* migration assays [147].

The first clinical trial using culture-expanded MSCs was performed in 1995 and 15 patients were treated with autologous stem cells [148]. After the first one, a number of clinical trials have been conducted to test the feasibility and efficacy of MSCs therapy. From 2011, 206 clinical trials using MSCs were published on the public clinical trials database (http://www.clinicaltrials.gov/) showing a very wide range of therapeutic applications. Most of these trials are Phase I studies, Phase II, and a combination of Phase I/II studies. Only a small number of these trials are in Phase III or Phase II /III. Most of the trials reported lack of adverse effects in the medium timing, although few of them showed mild and transient peri-injection effects: in general, MSCs seem to be well tolerated [87]. Very promising results were obtained by the injection of autologous and allogenic MSCs in patients suffering from osteogenesis imperfecta [76] while *in vitro* expanded MSCs were used to treat severe [149] and treatment-resistant [150, 151] GVHD patients. In addition, many completed clinical trials have demonstrated the efficacy of MSC infusion for diseases including acute myocardial ischemia (AMI), stroke, amyotrophic lateral sclerosis (ALS), and muscular dystrophies.

6. Conclusions

MSCs have many characteristics required for an optimal cell source for cell-replacement therapies, as they are easy to isolate, and retain the ability to expand over a long period of time without serious technical problem. MSCs are linearly restricted; however, there is evidence that MSCs *in vitro* can also express property of ectodermal cells [12]. One requisite of the stem cell-based therapeutic approach is to replace damaged cells. For example, in PD, many studies have focused on examining whether cells replacement therapy could be used. Although transplanted MSCs showed a low cell replacement potential, they improve the environment through the release of neuroprotective factors and they can be engineered to ensure specific expression and secretion. Moreover, MSCs promote "bystander" immunomodulation, as they can release soluble molecules and express immunorelevant receptors that are able to modify the inflammatory environment. However, many questions have to be answered both from preclinical and clinical studies using MSCs before MSCs can be used in wider clinical practice. First of all the safety: now, after MSC administration, few adverse effects have been described in terms of immediate and late effects. The relatively small number of treated patients does not permit to draw definitive conclusions on the safety of MSCs. Unfortunately, MSCs have been reported to promote tumor growth [152] and metastases [153]. Prockop et al. described that MSCs cultured with the clinical cell-therapy protocols commonly used showed potential tumorigenic transformation [154]. Chen et al. found that MSCs could aggravate arthritis in collagen-induced arthritis model by at least upregulating secretion of IL-6, which favors Th17 differentiation [155]. Secondly, quality control: *in vitro* cell amplification needs bacteriological tests (mainly in liquid medium) to face contaminations. In addition, viability and phenotype tests, oncogenic tests, and endotoxin assay should also be included. For each disease type and severity, optimal timing of MSCs administration, cell dose, and schedule of administration need to be decided. Third, clinical grade production: clinical application of MSC requires a large number of cells, so *in vitro* expansion of MSC is necessary. Studies have suggested that continuous passaging of MSCs could lead to cell transformation. Bernardo et al. found that MSCs expansion *in vitro* can be safety until passage 25 [156]. Fourth, clinical transition: it is obvious that much work remains to be done to increase our knowledge of the mechanisms regulating development, homeostasis, and tissue repair and thus provide new tools to implement the efficacy of cell therapy trials. Additionally, there is an urgent requirement to address transplantation related issues, such as engraftment, angiogenesis, tissue remodeling, and modulation of the immune response. Currently, more randomized, controlled, multicenter clinical trials are needed to find the optimal conditions for MSC therapy. In general, we think that successful cell therapy necessitates continuous interaction among biologists, clinicians, and patient working groups in the context of different tissues and diseases.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Authors' Contribution

Farini Andrea and Sitzia Clementina contributed equally to this paper.

Acknowledgments

This work was supported by Associazione La Nostra Famiglia Fondo DMD Gli Amici di Emanuele, Associazione Amici del Centro Dino Ferrari, and EU's 7th Framework Programme Optistem 223098, Ministry of Health RF-2009-1547384.

References

[1] A. J. Friedenstein, J. F. Gorskaja, and N. N. Kulagina, "Fibroblast precursors in normal and irradiated mouse hematopoietic organs," *Experimental Hematology*, vol. 4, no. 5, pp. 267–274, 1976.

[2] A. I. Caplan, "Mesenchymal stem cells," *Journal of Orthopaedic Research*, vol. 9, no. 5, pp. 641–650, 1991.

[3] M. Owen, "Marrow stromal stem cells," *Journal of Cell Science. Supplement*, vol. 10, pp. 63–76, 1988.

[4] S. E. Haynesworth, M. A. Baber, and A. I. Caplan, "Cell surface antigens on human marrow-derived mesenchymal cells are detected by monoclonal antibodies," *Bone*, vol. 13, no. 1, pp. 69–80, 1992.

[5] M. F. Pittenger, A. M. Mackay, S. C. Beck et al., "Multilineage potential of adult human mesenchymal stem cells," *Science*, vol. 284, no. 5411, pp. 143–147, 1999.

[6] A. Mahmood, D. Lu, M. Lu et al., "Treatment of traumatic brain injury in adult rats with intravenous administration of human bone marrow stromal cells," *Neurosurgery*, vol. 53, no. 3, pp. 697–703, 2003.

[7] J. M. Murphy, D. J. Fink, E. B. Hunziker, and F. P. Barry, "Stem cell therapy in a caprine model of osteoarthritis," *Arthritis and Rheumatism*, vol. 48, no. 12, pp. 3464–3474, 2003.

[8] G. J. Block, S. Ohkouchi, F. Fung et al., "Multipotent stromal cells are activated to reduce apoptosis in part by upregulation and secretion of stanniocalcin-1," *Stem Cells*, vol. 27, no. 3, pp. 670–681, 2009.

[9] L. Chen, E. E. Tredget, P. Y. G. Wu, Y. Wu, and Y. Wu, "Paracrine factors of mesenchymal stem cells recruit macrophages and endothelial lineage cells and enhance wound healing," *PLoS ONE*, vol. 3, no. 4, Article ID e1886, 2008.

[10] J. M. Karp and G. S. L. Teo, "Mesenchymal stem cell homing: the devil is in the details," *Cell Stem Cell*, vol. 4, no. 3, pp. 206–216, 2009.

[11] A. Alhadlaq and J. J. Mao, "Mesenchymal stem cells: isolation and therapeutics," *Stem Cells and Development*, vol. 13, no. 4, pp. 436–448, 2004.

[12] N. B. Nardi and L. da Silva Meirelles, "Mesenchymal stem cells: isolation, in vitro expansion and characterization," *Handbook of Experimental Pharmacology*, no. 174, pp. 249–282, 2006.

[13] K. le Blanc and M. F. Pittenger, "Mesenchymal stem cells: progress toward promise," *Cytotherapy*, vol. 7, no. 1, pp. 36–45, 2005.

[14] M. Miura, S. Gronthos, M. Zhao et al., "SHED: stem cells from human exfoliated deciduous teeth," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 100, no. 10, pp. 5807–5812, 2003.

[15] M. J. Hoogduijn, M. J. Crop, A. M. A. Peeters et al., "Human heart, spleen, and perirenal fat-derived mesenchymal stem cells have immunomodulatory capacities," *Stem Cells and Development*, vol. 16, no. 4, pp. 597–604, 2007.

[16] C. S. Sondergaard, C. J. Hodonsky, L. Khait et al., "Human thymus mesenchymal stromal cells augment force production in self-organized cardiac tissue," *Annals of Thoracic Surgery*, vol. 90, no. 3, pp. 796–803, 2010.

[17] F. P. Barry and J. M. Murphy, "Mesenchymal stem cells: clinical applications and biological characterization," *International Journal of Biochemistry and Cell Biology*, vol. 36, no. 4, pp. 568–584, 2004.

[18] A. I. Caplan, "Why are MSCs therapeutic? New data: new insight," *The Journal of Pathology*, vol. 217, no. 2, pp. 318–324, 2009.

[19] P. Dore-Duffy, A. Katychev, X. Wang, and E. Van Buren, "CNS microvascular pericytes exhibit multipotential stem cell activity," *Journal of Cerebral Blood Flow and Metabolism*, vol. 26, no. 5, pp. 613–624, 2006.

[20] B. Meyrick and L. Reid, "The effect of continued hypoxia on rat pulmonary arterial circulation. An ultrastructural study," *Laboratory Investigation*, vol. 38, no. 2, pp. 188–200, 1978.

[21] M. K. Majumdar, M. A. Thiede, J. D. Mosca, M. Moorman, and S. L. Gerson, "Phenotypic and functional comparison of cultures of marrow-derived mesenchymal stem cells (MSCs) and stromal cells," *Journal of Cellular Physiology*, vol. 176, no. 1, pp. 57–66, 1998.

[22] G. Chamberlain, J. Fox, B. Ashton, and J. Middleton, "Concise review: mesenchymal stem cells: their phenotype, differentiation capacity, immunological features, and potential for homing," *Stem Cells*, vol. 25, no. 11, pp. 2739–2749, 2007.

[23] M. S. Penn, "Importance of the SDF-1: CXCR4 axis in myocardial repair," *Circulation Research*, vol. 104, no. 10, pp. 1133–1135, 2009.

[24] B. Schönemeier, S. Schulz, V. Hoellt, and R. Stumm, "Enhanced expression of the CXCl12/SDF-1 chemokine receptor CXCR7 after cerebral ischemia in the rat brain," *Journal of Neuroimmunology*, vol. 198, no. 1-2, pp. 39–45, 2008.

[25] B. T. G. Tan, M. M. G. Lee, and R. Ruan, "Bone marrow-derived cells that home to acoustic deafened cochlea preserved their hematopoietic identity," *Journal of Comparative Neurology*, vol. 509, no. 2, pp. 167–179, 2008.

[26] S. Avniel, Z. Arik, A. Maly et al., "Involvement of the CXCL12/CXCR4 pathway in the recovery of skin following burns," *Journal of Investigative Dermatology*, vol. 126, no. 2, pp. 468–476, 2006.

[27] A. Fox, J. Smythe, N. Fisher et al., "Mobilization of endothelial progenitor cells into the circulation in burned patients," *British Journal of Surgery*, vol. 95, no. 2, pp. 244–251, 2008.

[28] T. Kitaori, H. Ito, E. M. Schwarz et al., "Stromal cell-derived factor 1/CXCR4 signaling is critical for the recruitment of mesenchymal stem cells to the fracture site during skeletal repair in a mouse model," *Arthritis and Rheumatism*, vol. 60, no. 3, pp. 813–823, 2009.

[29] J. Gao, J. E. Dennis, R. F. Muzic, M. Lundberg, and A. I. Caplan, "The dynamic in vivo distribution of bone marrow-derived mesenchymal stem cells after infusion," *Cells Tissues Organs*, vol. 169, no. 1, pp. 12–20, 2001.

[30] M. B. Herrera, B. Bussolati, S. Bruno, V. Fonsato, G. M. Romanazzi, and G. Camussi, "Mesenchymal stem cells contribute to the renal repair of acute tubular epithelial injury," *International Journal of Molecular Medicine*, vol. 14, no. 6, pp. 1035–1041, 2004.

[31] K. A. Jackson, S. M. Majka, H. Wang et al., "Regeneration of ischemic cardiac muscle and vascular endothelium by adult stem cells," *The Journal of Clinical Investigation*, vol. 107, no. 11, pp. 1395–1402, 2001.

[32] D. Orlic, J. Kajstura, S. Chimenti et al., "Bone marrow cells regenerate infarcted myocardium," *Nature*, vol. 410, no. 6829, pp. 701–705, 2001.

[33] D. Orlic, J. Kajstura, S. Chimenti et al., "Mobilized bone marrow cells repair the infarcted heart, improving function and survival," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 98, no. 18, pp. 10344–10349, 2001.

[34] S. Gojo, N. Gojo, Y. Takeda et al., "In vivo cardiovasculogenesis by direct injection of isolated adult mesenchymal stem cells," *Experimental Cell Research*, vol. 288, no. 1, pp. 51–59, 2003.

[35] D. Zhang, F. Zhang, Y. Zhang et al., "Combining erythropoietin infusion with intramyocardial delivery of bone marrow cells is more effective for cardiac repair," *Transplant International*, vol. 20, no. 2, pp. 174–183, 2007.

[36] C. P. Hofstetter, E. J. Schwarz, D. Hess et al., "Marrow stromal cells form guiding strands in the injured spinal cord and promote recovery," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 99, no. 4, pp. 2199–2204, 2002.

[37] J. A. Kode, S. Mukherjee, M. V. Joglekar, and A. A. Hardikar, "Mesenchymal stem cells: immunobiology and role in immunomodulation and tissue regeneration," *Cytotherapy*, vol. 11, no. 4, pp. 377–391, 2009.

[38] W. E. Fibbe, A. J. Nauta, and H. Roelofs, "Modulation of immune responses by mesenchymal stem cells," *Annals of the New York Academy of Sciences*, vol. 1106, pp. 272–278, 2007.

[39] Z. Selmani, A. Naji, I. Zidi et al., "Human leukocyte antigen-G5 secretion by human mesenchymal stem cells is required to suppress T lymphocyte and natural killer function and to induce $CD4^+CD25^{high}FOXP3^+$ regulatory T cells," *Stem Cells*, vol. 26, no. 1, pp. 212–222, 2008.

[40] Z.-G. Zhao, W.-M. Li, Z.-C. Chen, Y. You, and P. Zou, "Immunosuppressive properties of mesenchymal stem cells derived from bone marrow of patients with chronic myeloid leukemia," *Immunological Investigations*, vol. 37, no. 7, pp. 726–739, 2008.

[41] A. Nasef, C. Mazurier, S. Bouchet et al., "Leukemia inhibitory factor: role in human mesenchymal stem cells mediated immunosuppression," *Cellular Immunology*, vol. 253, no. 1-2, pp. 16–22, 2008.

[42] H. Sheng, Y. Wang, Y. Jin et al., "A critical role of IFNγ in priming MSC-mediated suppression of T cell proliferation through up-regulation of B7-H1," *Cell Research*, vol. 18, no. 8, pp. 846–857, 2008.

[43] G. Ren, L. Zhang, X. Zhao et al., "Mesenchymal stem cell-mediated immunosuppression occurs via concerted action of chemokines and nitric oxide," *Cell Stem Cell*, vol. 2, no. 2, pp. 141–150, 2008.

[44] G. M. Spaggiari, A. Capobianco, S. Becchetti, M. C. Mingari, and L. Moretta, "Mesenchymal stem cell-natural killer cell interactions: evidence that activated NK cells are capable of killing MSCs, whereas MSCs can inhibit IL-2-induced NK-cell proliferation," *Blood*, vol. 107, no. 4, pp. 1484–1490, 2006.

[45] A. Poggi, C. Prevosto, A.-M. Massaro et al., "Interaction between human NK cells and bone marrow stromal cells induces NK cell triggering: role of NKp30 and NKG2D receptors," *The Journal of Immunology*, vol. 175, no. 10, pp. 6352–6360, 2005.

[46] J. Stagg, "Immune regulation by mesenchymal stem cells: two sides to the coin," *Tissue Antigens*, vol. 69, no. 1, pp. 1–9, 2007.

[47] A. Jewett, A. Arasteh, H.-C. Tseng et al., "Strategies to rescue mesenchymal stem cells (MSCs) and dental pulp stem cells (DPSCs) from NK cell mediated cytotoxicity," *PloS ONE*, vol. 5, no. 3, Article ID e9874, 2010.

[48] M. Giuliani, A. Bennaceur-Griscelli, A. Nanbakhsh et al., "TLR ligands stimulation protects MSC from NK killing," *Stem Cells*, vol. 32, no. 1, pp. 290–300, 2014.

[49] B. Zheng, B. Cao, M. Crisan et al., "Prospective identification of myogenic endothelial cells in human skeletal muscle," *Nature Biotechnology*, vol. 25, pp. 1025–1034, 2007.

[50] L. J. Nesti, W. M. Jackson, R. M. Shanti et al., "Differentiation potential of multipotent progenitor cells derived from war-traumatized muscle tissue," *Journal of Bone and Joint Surgery A*, vol. 90, no. 11, pp. 2390–2398, 2008.

[51] M. A. F. V. Gonçalves, A. A. F. de Vries, M. Holkers et al., "Human mesenchymal stem cells ectopically expressing full-length dystrophin can complement Duchenne muscular dystrophy myotubes by cell fusion," *Human Molecular Genetics*, vol. 15, no. 2, pp. 213–221, 2006.

[52] K. Németh, A. Leelahavanichkul, P. S. T. Yuen et al., "Bone marrow stromal cells attenuate sepsis via prostaglandin E 2-dependent reprogramming of host macrophages to increase their interleukin-10 production," *Nature Medicine*, vol. 15, no. 1, pp. 42–49, 2009.

[53] A. Asakura, P. Seale, A. Girgis-Gabardo, and M. A. Rudnicki, "Myogenic specification of side population cells in skeletal muscle," *The Journal of Cell Biology*, vol. 159, no. 1, pp. 123–134, 2002.

[54] Z. Qu-Petersen, B. Deasy, R. Jankowski et al., "Identification of a novel population of muscle stem cells in mice: potential for muscle regeneration," *The Journal of Cell Biology*, vol. 157, no. 5, pp. 851–864, 2002.

[55] C. de Bari, F. Dell'Accio, F. Vandenabeele, J. R. Vermeesch, J.-M. Raymackers, and F. P. Luyten, "Skeletal muscle repair by adult human mesenchymal stem cells from synovial membrane," *The Journal of Cell Biology*, vol. 160, no. 6, pp. 909–918, 2003.

[56] E. J. Gang, J. A. Jeong, S. H. Hong et al., "Skeletal myogenic differentiation of mesenchymal stem cells isolated from human umbilical cord blood," *Stem Cells*, vol. 22, no. 4, pp. 617–624, 2004.

[57] M. Rafei, P. M. Campeau, A. Aguilar-Mahecha et al., "Mesenchymal stromal cells ameliorate experimental autoimmune encephalomyelitis by inhibiting CD4 Th17 T cells in a CC chemokine ligand 2-dependent manner," *The Journal of Immunology*, vol. 182, no. 10, pp. 5994–6002, 2009.

[58] Y.-W. Yang, H. Bai, C.-B. Wang, M. Lin, and L.-Q. Wu, "Experimental study on influence of bone marrow mesenchymal stem cells on activation and function of mouse peritoneal macrophages," *Zhonghua Xue Ye Xue Za Zhi*, vol. 29, no. 8, pp. 540–543, 2008.

[59] F. Casiraghi, N. Azzollini, P. Cassis et al., "Pretransplant infusion of mesenchymal stem cells prolongs the survival of a semiallogeneic heart transplant through the generation of regulatory T cells," *The Journal of Immunology*, vol. 181, no. 6, pp. 3933–3946, 2008.

[60] D. Campioni, R. Rizzo, M. Stignani et al., "A decreased positivity for CD90 on human mesenchymal stromal cells (MSCs) is associated with a loss of immunosuppressive activity by MSCs," *Cytometry B: Clinical Cytometry*, vol. 76, no. 3, pp. 225–230, 2009.

[61] B. J. Jones, G. Brooke, K. Atkinson, and S. J. McTaggart, "Immunosuppression by placental indoleamine 2,3-dioxygenase: a role for mesenchymal stem cells," *Placenta*, vol. 28, no. 11-12, pp. 1174–1181, 2007.

[62] J. Chan, S. N. Waddington, K. O'Donoghue et al., "Widespread distribution and muscle differentiation of human fetal mesenchymal stem cells after intrauterine transplantation in dystrophic mdx mouse," *Stem Cells*, vol. 25, no. 4, pp. 875–884, 2007.

[63] T. C. MacKenzie, G. P. Kobinger, N. A. Kootstra et al., "Efficient transduction of liver and muscle after in utero injection of lentiviral vectors with different pseudotypes," *Molecular Therapy*, vol. 6, no. 3, pp. 349–358, 2002.

[64] Y. Liu, X. Yan, Z. Sun et al., "Flk-1^+ adipose-derived mesenchymal stem cells differentiate into skeletal muscle satellite cells and ameliorate muscular dystrophy in MDX Mice," *Stem Cells and Development*, vol. 16, no. 5, pp. 695–706, 2007.

[65] S.-W. Feng, X.-L. Lu, Z.-S. Liu et al., "Dynamic distribution of bone marrow-derived mesenchymal stromal cells and change of pathology after infusing into mdx mice," *Cytotherapy*, vol. 10, no. 3, pp. 254–264, 2008.

[66] K. Y. Kong, J. Ren, M. Kraus, S. P. Finklestein, and R. H. Brown Jr., "Human umbilical cord blood cells differentiate into muscle in sjl muscular dystrophy mice," *Stem Cells*, vol. 22, no. 6, pp. 981–993, 2004.

[67] J. Rauh, F. Milan, K.-P. Günther, and M. Stiehler, "Bioreactor systems for bone tissue engineering," *Tissue Engineering B: Reviews*, vol. 17, no. 4, pp. 263–280, 2011.

[68] R. S. Tuan, "Role of adult stem/progenitor cells in osseointegration and implant loosening," *The International Journal of Oral & Maxillofacial Implants*, vol. 26, supplement, pp. 50–69, 2011.

[69] P. Bianco and P. G. Robey, "Stem cells in tissue engineering," *Nature*, vol. 414, no. 6859, pp. 118–121, 2001.

[70] R. Cancedda, G. Bianchi, A. Derubeis, and R. Quarto, "Cell therapy for bone disease: a review of current status," *Stem Cells*, vol. 21, no. 5, pp. 610–619, 2003.

[71] K. Kawate, H. Yajima, H. Ohgushi et al., "Tissue-engineered approach for the treatment of steroid-induced osteonecrosis of the femoral head: transplantation of autologous mesenchymal stem cells cultured with beta-tricalcium phosphate ceramics and free vascularized fibula," *Artificial Organs*, vol. 30, no. 12, pp. 960–962, 2006.

[72] U. Nöth, J. Reichert, S. Reppenhagen et al., "Cell based therapy for the treatment of femoral head necrosis," *Orthopade*, vol. 36, no. 5, pp. 466–471, 2007.

[73] S. G. Pneumaticos, G. K. Triantafyllopoulos, S. Chatziioannou, E. K. Basdra, and A. G. Papavassiliou, "Biomolecular strategies of bone augmentation in spinal surgery," *Trends in Molecular Medicine*, vol. 17, no. 4, pp. 215–222, 2011.

[74] D. Neen, D. Noyes, M. Shaw, S. Gwilym, N. Fairlie, and N. Birch, "Healos and bone marrow aspirate used for lumbar spine fusion: a case controlled study comparing healos with autograft," *Spine*, vol. 31, no. 18, pp. E636–E640, 2006.

[75] P. Zhang, Y.-K. Gan, J. Tang et al., "Clinical study of lumbar fusion by hybrid construct of stem cells technique and biodegradable material," *Zhonghua Wai Ke Za Zhi*, vol. 46, no. 7, pp. 493–496, 2008.

[76] E. M. Horwitz, P. L. Gordon, W. K. K. Koo et al., "Isolated allogeneic bone marrow-derived mesenchymal cells engraft and stimulate growth in children with osteogenesis imperfecta: implications for cell therapy of bone," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 99, no. 13, pp. 8932–8937, 2002.

[77] E. M. Horwitz, D. J. Prockop, L. A. Fitzpatrick et al., "Transplantability and therapeutic effects of bone marrow-derived mesenchymal cells in children with osteogenesis imperfecta," *Nature Medicine*, vol. 5, no. 3, pp. 309–313, 1999.

[78] S. Makino, K. Fukuda, S. Miyoshi et al., "Cardiomyocytes can be generated from marrow stromal cells in vitro," *The Journal of Clinical Investigation*, vol. 103, no. 5, pp. 697–705, 1999.

[79] W. Xu, X. Zhang, H. Qian et al., "Mesenchymal stem cells from adult human bone marrow differentiate into a cardiomyocyte phenotype in vitro," *Experimental Biology and Medicine*, vol. 229, no. 7, pp. 623–631, 2004.

[80] N. Nagaya, T. Fujii, T. Iwase et al., "Intravenous administration of mesenchymal stem cells improves cardiac function in rats with acute myocardial infarction through angiogenesis and myogenesis," *American Journal of Physiology—Heart and Circulatory Physiology*, vol. 287, no. 6, pp. H2670–H2676, 2004.

[81] J. G. Shake, P. J. Gruber, W. A. Baumgartner et al., "Mesenchymal stem cell implantation in a swine myocardial infarct model: engraftment and functional effects," *The Annals of Thoracic Surgery*, vol. 73, no. 6, pp. 1919–1926, 2002.

[82] N. Nagaya, K. Kangawa, T. Itoh et al., "Transplantation of mesenchymal stem cells improves cardiac function in a rat model of dilated cardiomyopathy," *Circulation*, vol. 112, no. 8, pp. 1128–1135, 2005.

[83] K. C. Wollert, G. P. Meyer, J. Lotz et al., "Intracoronary autologous bone-marrow cell transfer after myocardial infarction: the BOOST randomised controlled clinical trial," *The Lancet*, vol. 364, no. 9429, pp. 141–148, 2004.

[84] D. G. Katritsis, P. A. Sotiropoulou, E. Karvouni et al., "Transcoronary transplantation of autologous mesenchymal stem cells and endothelial progenitors into infarcted human myocardium," *Catheterization and Cardiovascular Interventions*, vol. 65, no. 3, pp. 321–329, 2005.

[85] S.-L. Chen, W.-W. Fang, F. Ye et al., "Effect on left ventricular function of intracoronary transplantation of autologous bone marrow mesenchymal stem cell in patients with acute myocardial infarction," *The American Journal of Cardiology*, vol. 94, no. 1, pp. 92–95, 2004.

[86] M. Takahashi, T.-S. Li, R. Suzuki et al., "Cytokines produced by bone marrow cells can contribute to functional improvement of the infarcted heart by protecting cardiomyocytes from ischemic injury," *American Journal of Physiology—Heart and Circulatory Physiology*, vol. 291, no. 2, pp. H886–H893, 2006.

[87] S. Wang, X. Qu, and R. C. Zhao, "Clinical applications of mesenchymal stem cells," *Journal of Hematology & Oncology*, vol. 5, article 19, 2012.

[88] D. van Poll, B. Parekkadan, C. H. Cho et al., "Mesenchymal stem cell-derived molecules directly modulate hepatocellular death and regeneration in vitro and in vivo," *Hepatology*, vol. 47, no. 5, pp. 1634–1643, 2008.

[89] M. Mohamadnejad, K. Alimoghaddam, M. Mohyeddin-Bonab et al., "Phase 1 trial of autologous bone marrow mesenchymal stem cell transplantation in patients with decompensated liver cirrhosis," *Archives of Iranian Medicine*, vol. 10, no. 4, pp. 459–466, 2007.

[90] P. Kharaziha, P. M. Hellström, B. Noorinayer et al., "Improvement of liver function in liver cirrhosis patients after autologous mesenchymal stem cell injection: a phase I-II clinical trial," *European Journal of Gastroenterology and Hepatology*, vol. 21, no. 10, pp. 1199–1205, 2009.

[91] N. H. Riordan, K. Chan, A. M. Marleau, and T. E. Ichim, "Cord blood in regenerative medicine: do we need immune suppression?" *Journal of Translational Medicine*, vol. 5, article 8, 2007.

[92] M. A. González, E. González-Rey, L. Rico, D. Büscher, and M. Delgado, "Treatment of experimental arthritis by inducing immune tolerance with human adipose-derived mesenchymal stem cells," *Arthritis and Rheumatism*, vol. 60, no. 4, pp. 1006–1019, 2009.

[93] M. Walmsley, P. D. Katsikis, E. Abney et al., "Interleukin-10 inhibition of the progression of established collagen-induced arthritis," *Arthritis and Rheumatism*, vol. 39, no. 3, pp. 495–503, 1996.

[94] E. J. Wehrens, B. J. Prakken, and F. van Wijk, "T cells out of control—impaired immune regulation in the inflamed joint," *Nature Reviews Rheumatology*, vol. 9, pp. 34–42, 2013.

[95] Z. H. Zheng, X. Y. Li, J. Ding, J. F. Jia, and P. Zhu, "Allogeneic mesenchymal stem cell and mesenchymal stem cell-differentiated chondrocyte suppress the responses of type II collagen-reactive T cells in rheumatoid arthritis," *Rheumatology*, vol. 47, no. 1, pp. 22–30, 2008.

[96] A. Augello, R. Tasso, S. M. Negrini, R. Cancedda, and G. Pennesi, "Cell therapy using allogeneic bone marrow mesenchymal stem cells prevents tissue damage in collagen-induced arthritis," *Arthritis and Rheumatism*, vol. 56, no. 4, pp. 1175–1186, 2007.

[97] F. Mao, W.-R. Xu, H. Qian et al., "Immunosuppressive effects of mesenchymal stem cells in collagen-induced mouse arthritis," *Inflammation Research*, vol. 59, no. 3, pp. 219–225, 2010.

[98] J.-J. Choi, S.-A. Yoo, S.-J. Park et al., "Mesenchymal stem cells overexpressing interleukin-10 attenuate collagen-induced

arthritis in mice," *Clinical and Experimental Immunology*, vol. 153, no. 2, pp. 269–276, 2008.

[99] F. Djouad, V. Fritz, F. Apparailly et al., "Reversal of the immunosuppressive properties of mesenchymal stem cells by tumor necrosis factor α in collagen-induced arthritis," *Arthritis and Rheumatism*, vol. 52, no. 5, pp. 1595–1603, 2005.

[100] E. Schurgers, H. Kelchtermans, T. Mitera, L. Geboes, and P. Matthys, "Discrepancy between the in vitro and in vivo effects of murine mesenchymal stem cells on T-cell proliferation and collagen-induced arthritis," *Arthritis Research & Therapy*, vol. 12, no. 1, article R31, 2010.

[101] H. Kelchtermans, L. Geboes, T. Mitera, D. Huskens, G. Leclercq, and P. Matthys, "Activated $CD4^+CD25^+$ regulatory T cells inhibit osteoclastogenesis and collagen-induced arthritis," *Annals of the Rheumatic Diseases*, vol. 68, no. 5, pp. 744–750, 2009.

[102] M. E. Morgan, R. Flierman, L. M. van Duivenvoorde et al., "Effective treatment of collagen-induced arthritis by adoptive transfer of $CD25^+$ regulatory T cells," *Arthritis and Rheumatism*, vol. 52, no. 7, pp. 2212–2221, 2005.

[103] C. Bouffi, C. Bony, G. Courties, C. Jorgensen, and D. Noël, "IL-6-dependent PGE2 secretion by mesenchymal stem cells inhibits local inflammation in experimental arthritis," *PLoS ONE*, vol. 5, no. 12, Article ID e14247, 2010.

[104] A. T. Borchers, C. L. Keen, Y. Shoenfeld, and M. E. Gershwin, "Surviving the butterfly and the wolf: mortality trends in systemic lupus erythematosus," *Autoimmunity Reviews*, vol. 3, no. 6, pp. 423–453, 2004.

[105] L.-Y. Sun, K.-X. Zhou, X.-B. Feng et al., "Abnormal surface markers expression on bone marrow $CD34^+$ cells and correlation with disease activity in patients with systemic lupus erythematosus," *Clinical Rheumatology*, vol. 26, no. 12, pp. 2073–2079, 2007.

[106] T. Kushida, M. Inaba, H. Hisha et al., "Intra-bone marrow injection of allogeneic bone marrow cells: a powerful new strategy for treatment of intractable autoimmune diseases in MRL/lpr mice," *Blood*, vol. 97, no. 10, pp. 3292–3299, 2001.

[107] L. Y. Sun, H. Y. Zhang, X. B. Feng, Y. Y. Hou, L. W. Lu, and L. M. Fan, "Abnormality of bone marrow-derived mesenchymal stem cells in patients with systemic lupus erythematosus," *Lupus*, vol. 16, no. 2, pp. 121–128, 2007.

[108] K. Zhou, H. Zhang, O. Jin et al., "Transplantation of human bone marrow mesenchymal stem cell ameliorates the autoimmune pathogenesis in MRL/lpr mice," *Cellular and Molecular Immunology*, vol. 5, no. 6, pp. 417–424, 2008.

[109] L. Inverardi, N. S. Kenyon, and C. Ricordi, "Islet transplantation: immunological perspectives," *Current Opinion in Immunology*, vol. 15, no. 5, pp. 507–511, 2003.

[110] H. Zulewski, E. J. Abraham, M. J. Gerlach et al., "Multipotential nestin-positive stem cells isolated from adult pancreatic islets differentiate ex vivo into pancreatic endocrine, exocrine, and hepatic phenotypes," *Diabetes*, vol. 50, no. 3, pp. 521–533, 2001.

[111] H. Huang and X. Tang, "Phenotypic determination and characterization of nestin-positive precursors derived from human fetal pancreas," *Laboratory Investigation*, vol. 83, no. 4, pp. 539–547, 2003.

[112] F. Atouf, H. P. Cheol, K. Pechhold, M. Ta, Y. Choi, and N. L. Lumelsky, "No evidence for mouse pancreatic β-cell epithelial-mesenchymal transition in vitro," *Diabetes*, vol. 56, no. 3, pp. 699–702, 2007.

[113] L. G. Chase, F. Ulloa-Montoya, B. L. Kidder, and C. M. Verfaillie, "Islet-derived fibroblast-like cells are not derived via epithelial-mesenchymal transition from Pdx-1 or insulin-positive cells," *Diabetes*, vol. 56, no. 1, pp. 3–7, 2007.

[114] M. C. Gershengorn, A. A. Hardikar, C. Wei, E. Ceras-Raaka, B. Marcus-Samuels, and B. M. Raaka, "Epithelial-to-mesenchymal transition generates proliferative human islet precursor cells," *Science*, vol. 306, no. 5705, pp. 2261–2264, 2004.

[115] L. Ouziel-Yahalom, M. Zalzman, L. Anker-Kitai et al., "Expansion and redifferentiation of adult human pancreatic islet cells," *Biochemical and Biophysical Research Communications*, vol. 341, no. 2, pp. 291–298, 2006.

[116] A. Ianus, G. G. Holz, N. D. Theise, and M. A. Hussain, "In vivo derivation of glucose-competent pancreatic endocrine cells from bone marrow without evidence of cell fusion," *The Journal of Clinical Investigation*, vol. 111, no. 6, pp. 843–850, 2003.

[117] S. M. Phadnis, M. V. Joglekar, M. P. Dalvi et al., "Human bone marrow-derived mesenchymal cells differentiate and mature into endocrine pancreatic lineage in vivo," *Cytotherapy*, vol. 13, no. 3, pp. 279–293, 2011.

[118] K. R. Prabakar, J. Domínguez-Bendala, R. D. Molano et al., "Generation of glucose-responsive, insulin-producing cells from human umbilical cord blood-derived mesenchymal stem cells," *Cell Transplantation*, vol. 21, no. 6, pp. 1321–1339, 2011.

[119] Y. Blanco, A. Saiz, E. Carreras, and F. Graus, "Autologous haematopoietic-stem-cell transplantation for multiple sclerosis," *The Lancet Neurology*, vol. 4, no. 1, pp. 54–63, 2005.

[120] C. M. Rice, K. Kemp, A. Wilkins, and N. J. Scolding, "Cell therapy for multiple sclerosis: an evolving concept with implications for other neurodegenerative diseases," *The Lancet*, vol. 382, no. 9899, pp. 1204–1213, 2013.

[121] D. Karussis, C. Karageorgiou, A. Vaknin-Dembinsky et al., "Safety and immunological effects of mesenchymal stem cell transplantation in patients with multiple sclerosis and amyotrophic lateral sclerosis," *Archives of Neurology*, vol. 67, no. 10, pp. 1187–1194, 2010.

[122] D. J. Prockop and J. Youn Oh, "Mesenchymal stem/stromal cells (MSCs): role as guardians of inflammation," *Molecular Therapy*, vol. 20, no. 1, pp. 14–20, 2012.

[123] A. Uccelli, V. Pistoia, and L. Moretta, "Mesenchymal stem cells: a new strategy for immunosuppression?" *Trends in Immunology*, vol. 28, no. 5, pp. 219–226, 2007.

[124] K. Kemp, K. Hares, E. Mallam, K. J. Heesom, N. Scolding, and A. Wilkins, "Mesenchymal stem cell-secreted superoxide dismutase promotes cerebellar neuronal survival," *Journal of Neurochemistry*, vol. 114, no. 6, pp. 1569–1580, 2010.

[125] D. Gordon, G. Pavlovska, J. B. Uney, D. C. Wraith, and N. J. Scolding, "Human mesenchymal stem cells infiltrate the spinal cord, reduce demyelination, and localize to white matter lesions in experimental autoimmune encephalomyelitis," *Journal of Neuropathology and Experimental Neurology*, vol. 69, no. 11, pp. 1087–1095, 2010.

[126] M. Osaka, O. Honmou, T. Murakami et al., "Intravenous administration of mesenchymal stem cells derived from bone marrow after contusive spinal cord injury improves functional outcome," *Brain Research*, vol. 1343, pp. 226–235, 2010.

[127] J. Zhang, Y. Li, M. Lu et al., "Bone marrow stromal cells reduce axonal loss in experimental autoimmune encephalomyelitis mice," *Journal of Neuroscience Research*, vol. 84, no. 3, pp. 587–595, 2006.

[128] C. M. Rice, E. A. Mallam, A. L. Whone et al., "Safey and feasibility of autologous bone marrow cellular therapy in

relapsing-progressive multiple sclerosis," *Clinical Pharmacology and Therapeutics*, vol. 87, no. 6, pp. 679–685, 2010.

[129] B. Yamout, R. Hourani, H. Salti et al., "Bone marrow mesenchymal stem cell transplantation in patients with multiple sclerosis: a pilot study," *Journal of Neuroimmunology*, vol. 227, no. 1-2, pp. 185–189, 2010.

[130] M. Suzuki, J. McHugh, C. Tork et al., "Direct muscle delivery of GDNF with human mesenchymal stem cells improves motor neuron survival and function in a rat model of familial ALS," *Molecular Therapy*, vol. 16, no. 12, pp. 2002–2010, 2008.

[131] Y. Agid, "Parkinson's disease: pathophysiology," *The Lancet*, vol. 337, no. 8753, pp. 1321–1324, 1991.

[132] S. J. Kish, K. Shannak, and O. Hornykiewicz, "Uneven pattern of dopamine loss in the striatum of patients with idiopathic Parkinson's disease. Pathophysiologic and clinical implications," *The New England Journal of Medicine*, vol. 318, no. 14, pp. 876–880, 1988.

[133] C. F. P. Teixeira, S. R. Zamunér, J. P. Zuliani et al., "Neutrophils do not contribute to local tissue damage, but play a key role in skeletal muscle regeneration, in mice injected with Bothrops asper snake venom," *Muscle and Nerve*, vol. 28, no. 4, pp. 449–459, 2003.

[134] I. Kan, T. Ben-Zur, Y. Barhum et al., "Dopaminergic differentiation of human mesenchymal stem cells-Utilization of bioassay for tyrosine hydroxylase expression," *Neuroscience Letters*, vol. 419, no. 1, pp. 28–33, 2007.

[135] F. Wang, T. Yasuhara, T. Shingo et al., "Intravenous administration of mesenchymal stem cells exerts therapeutic effects on parkinsonian model of rats: focusing on neuroprotective effects of stromal cell-derived factor-1α," *BMC Neuroscience*, vol. 11, article 52, 2010.

[136] A. Wilkins, K. Kemp, M. Ginty, K. Hares, E. Mallam, and N. Scolding, "Human bone marrow-derived mesenchymal stem cells secrete brain-derived neurotrophic factor which promotes neuronal survival in vitro," *Stem Cell Research*, vol. 3, no. 1, pp. 63–70, 2009.

[137] T. C. Moloney, G. E. Rooney, F. P. Barry, L. Howard, and E. Dowd, "Potential of rat bone marrow-derived mesenchymal stem cells as vehicles for delivery of neurotrophins to the Parkinsonian rat brain," *Brain Research*, vol. 1359, pp. 33–43, 2010.

[138] R. Barzilay, T. Ben-Zur, S. Bulvik, E. Melamed, and D. Offen, "Lentiviral delivery of LMX1a enhances dopaminergic phenotype in differentiated human bone marrow mesenchymal stem cells," *Stem Cells and Development*, vol. 18, no. 4, pp. 591–601, 2009.

[139] C. V. Borlongan, "Recent preclinical evidence advancing cell therapy for Alzheimer's disease," *Experimental Neurology*, vol. 237, no. 1, pp. 142–146, 2012.

[140] J. Y. Shin, H. J. Park, H. N. Kim et al., "Mesenchymal stem cells enhance autophagy and increase beta-amyloid clearance in Alzheimer disease models," *Autophagy*, vol. 10, no. 1, pp. 32–44, 2014.

[141] T. Ma, K. Gong, Q. Ao et al., "Intracerebral transplantation of adipose-derived mesenchymal stem cells alternatively activates microglia and ameliorates neuropathological deficits in Alzheimer's disease mice," *Cell Transplantation*, vol. 22, supplement 1, pp. S113–S126, 2013.

[142] M. Saresella, E. Calabrese, I. Marventano et al., "PD1 negative and PD1 positive $CD4^+$ T regulatory cells in mild cognitive impairment and Alzheimer's disease," *Journal of Alzheimer's Disease*, vol. 21, no. 3, pp. 927–938, 2010.

[143] J. T. Walsh and J. Kipnis, "Regulatory T cells in CNS injury: the simple, the complex and the confused," *Trends in Molecular Medicine*, vol. 17, no. 10, pp. 541–547, 2011.

[144] H. Yang, H. Yang, Z. Xie, L. Wei, and J. Bi, "Systemic transplantation of human umbilical cord derived mesenchymal stem cells-educated T regulatory cells improved the impaired cognition in AbetaPPswe/PS1dE9 transgenic mice," *PLoS ONE*, vol. 8, Article ID e69129, 2013.

[145] L. A. Ortiz, F. Gambelli, C. McBride et al., "Mesenchymal stem cell engraftment in lung is enhanced in response to bleomycin exposure and ameliorates its fibrotic effects," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 100, no. 14, pp. 8407–8411, 2003.

[146] E. Spaeth, A. Klopp, J. Dembinski, M. Andreeff, and F. Marini, "Inflammation and tumor microenvironments: defining the migratory itinerary of mesenchymal stem cells," *Gene Therapy*, vol. 15, no. 10, pp. 730–738, 2008.

[147] T. Yagi, D. Ito, Y. Okada et al., "Modeling familial Alzheimer's disease with induced pluripotent stem cells," *Human Molecular Genetics*, vol. 20, no. 23, Article ID ddr394, pp. 4530–4539, 2011.

[148] H. M. Lazarus, S. E. Haynesworth, S. L. Gerson, N. S. Rosenthal, and A. I. Caplan, "Ex vivo expansion and subsequent infusion of human bone marrow-derived stromal progenitor cells (mesenchymal progenitor cells): implications for therapeutic use," *Bone Marrow Transplantation*, vol. 16, no. 4, pp. 557–564, 1995.

[149] K. le Blanc, I. Rasmusson, B. Sundberg et al., "Treatment of severe acute graft-versus-host disease with third party haploidentical mesenchymal stem cells," *The Lancet*, vol. 363, no. 9419, pp. 1439–1441, 2004.

[150] K. le Blanc, F. Frassoni, L. Ball et al., "Mesenchymal stem cells for treatment of steroid-resistant, severe, acute graft-versus-host disease: a phase II study," *The Lancet*, vol. 371, no. 9624, pp. 1579–1586, 2008.

[151] O. Ringdén, M. Uzunel, I. Rasmusson et al., "Mesenchymal stem cells for treatment of therapy-resistant graft-versus-host disease," *Transplantation*, vol. 81, no. 10, pp. 1390–1397, 2006.

[152] F. Djouad, P. Plence, C. Bony et al., "Immunosuppressive effect of mesenchymal stem cells favors tumor growth in allogeneic animals," *Blood*, vol. 102, no. 10, pp. 3837–3844, 2003.

[153] A. E. Karnoub, A. B. Dash, A. P. Vo et al., "Mesenchymal stem cells within tumour stroma promote breast cancer metastasis," *Nature*, vol. 449, no. 7162, pp. 557–563, 2007.

[154] D. J. Prockop, M. Brenner, W. E. Fibbe et al., "Defining the risks of mesenchymal stromal cell therapy," *Cytotherapy*, vol. 12, no. 5, pp. 576–578, 2010.

[155] B. Chen, J. Hu, L. Liao et al., "$Flk\text{-}1^+$ mesenchymal stem cells aggravate collagen-induced arthritis by up-regulating interleukin-6," *Clinical and Experimental Immunology*, vol. 159, no. 3, pp. 292–302, 2010.

[156] M. E. Bernardo, F. Locatelli, and W. E. Fibbe, "Mesenchymal stromal cells: a novel treatment modality for tissue repair," *Annals of the New York Academy of Sciences*, vol. 1176, pp. 101–117, 2009.

Hindawi Publishing Corporation
Stem Cells International
Volume 2014, Article ID 197154, 11 pages
http://dx.doi.org/10.1155/2014/197154

Research Article

Magnetic Nanoparticle Based Nonviral MicroRNA Delivery into Freshly Isolated CD105$^+$ hMSCs

Anna Schade, Paula Müller, Evgenya Delyagina, Natalia Voronina, Anna Skorska, Cornelia Lux, Gustav Steinhoff, and Robert David

Department of Cardiac Surgery, Reference and Translation Center for Cardiac Stem Cell Therapy (RTC), University of Rostock, Schillingallee 35, 18057 Rostock, Germany

Correspondence should be addressed to Robert David; davidrob@med.uni-rostock.de

Received 31 January 2014; Accepted 2 March 2014; Published 31 March 2014

Academic Editor: Katherine Athayde Teixeira de Carvalho

Genetic modifications of bone marrow derived human mesenchymal stem cells (hMSCs) using microRNAs (miRs) may be used to improve their therapeutic potential and enable innovative strategies in tissue regeneration. However, most of the studies use cultured hMSCs, although these can lose their stem cell characteristics during expansion. Therefore, we aimed to develop a nonviral miR carrier based on polyethylenimine (PEI) bound to magnetic nanoparticles (MNPs) for efficient miR delivery in freshly isolated hMSCs. MNP based transfection is preferable for genetic modifications *in vivo* due to improved selectivity, safety of delivery, and reduced side effects. Thus, in this study different miR/PEI and miR/PEI/MNP complex formulations were tested *in vitro* for uptake efficiency and cytotoxicity with respect to the influence of an external magnetic field. Afterwards, optimized magnetic complexes were selected and compared to commercially available magnetic vectors (Magnetofectamine, CombiMag). We found that all tested transfection reagents had high miR uptake rates (yielded over 60%) and no significant cytotoxic effects. Our work may become crucial for virus-free introduction of therapeutic miRs as well as other nucleic acids *in vivo*. Moreover, in the field of targeted stem cell therapy nucleic acid delivery prior to transplantation may allowfor initial cell modulation *in vitro*.

1. Introduction

Bone marrow derived human mesenchymal stem cells (hMSCs) have been shown to bear great potential for cell based therapeutic strategies. The ability of these cells to differentiate into various cell types and to secrete a large spectrum of antiapoptotic, angiogenic, and immunomodulatory factors offers the possibility to use them for tissue repair [1–4]. Furthermore, hMSCs are characterized by the expression of specific stem cell surface markers (e.g., CD29, CD44, CD73, and CD105) and the absence of hematopoietic markers (e.g., CD45, CD117) [1]. Moreover, it was shown that CD105 (endoglin) is a suitable surface marker for efficient purification of hMSCs from bone marrow [2]. Currently, several clinical trials, which involve hMSCs for the treatment of graft-versus-host disease, cartilage and meniscus repair, stroke, spinal cord injury, and Crohn's disease, are in progress [3]. Moreover, it has been recently shown that microRNA (miR) based genetic modifications of hMSCs before transplantation can significantly improve their therapeutic potential and survival rates [4–6]. Furthermore, miRs play an important role in stem cell regulation by influencing cell proliferation, differentiation, survival, apoptosis, and production of paracrine factors of hMSCs [4, 7, 8]. To date, several synthetic miRs are commercially available. However, miR delivery methods suitable for clinical applications will be crucial. Initially, viral carriers were widely used to transfer genetic material into target cells as they provide high transduction efficiency and long term gene expression. However, clinical applications of virus based gene carriers are limited as they may induce toxicity, immunogenicity, mutagenesis, and carcinogenesis [9, 10]. Thus, various nonviral methods were developed. Nonviral vectors have the benefit to be noninflammatory, noninfectious, and less toxic for efficient delivery of nucleic acids [9]. To date, numerous nonviral transfection carriers based on cationic lipids and cationic polymers are available

on the market (lipoplexes and polyplexes, resp.). Thereof, Lipofectamine and polyethylenimine (PEI) are the best investigated nonviral transfection reagents for efficient nucleic acid transfer [11–13]. However, the clinical applications of Lipofectamine and PEI are restricted due to sensitivity and safety issues. Moreover, nonviral carriers can be combined with magnetic nanoparticles in order to improve selectivity and safety of delivery as well as to decrease side effects [14]. In 2002, Scherer and coworkers invented a novel technique termed "magnetofection." This technique combines different well investigated gene delivery vectors (e.g., retrovirus, Lipofectamine, and PEI) with superparamagnetic iron oxide nanoparticles via salt-induced aggregation. The group showed that an externally applied magnetic field enhanced sedimentation of transfection complexes, thus improving transfection efficiency *in vitro* and *in vivo* [15]. In the last years, magnet based transfection (e.g., Magnetofectamine) has become a powerful tool for highly efficient and fast delivery of DNA [15, 16] as well as siRNA [17–19]. Our own group has developed a paramagnetic nonviral vector composed of nucleic acids condensed by biotinylated PEI and bound to streptavidin-coated iron oxide magnetic nanoparticles (MNPs) via biotin-streptavidin interactions. These MNP containing complexes, carrying therapeutic DNA, could be targeted by an external magnetic field to the site of interest *in vivo* [20]. Recently, we demonstrated that transfection with DNA/PEI/MNP complexes had a significantly higher transfection efficiency in cultivated hMSCs compared to DNA/PEI complexes even without the application of a magnetic field. We concluded a more rapid and efficient release of DNA from magnetic complexes compared to PEI polyplexes [21]. In contrast to DNA/PEI complexes, MNP containing complexes did not enter the nucleus due to strong biotin-streptavidin connections but released the DNA in the perinuclear region [14]. We have recently transferred this approach to transfection of hMSCs with miR, as the latter binds to its target mRNAs in the proximity of the nucleus. *In vitro*, we could demonstrate that miR/PEI/MNP complexes had a better long term silencing effect compared to mere miR/PEI polyplexes, which might be beneficial for clinical applications [22].

Although freshly isolated hMSCs are more relevant to clinical use, the quantity of these cells is too low to reach the desired effect [23, 24]. Thus, for our studies, as well as for clinical trials, hMSCs so far were expanded *in vitro* [21, 22, 25]. However, *in vitro* expansion of primary hMSCs is a costly and time-consuming procedure. In addition, the cells likely also lose their differentiation potential [2] and dramatically decrease their homing ability [26]. Therefore, genetic modifications of freshly isolated cells may be crucial to overcome these barriers and enable their clinical applications without previous *in vitro* expansion despite their low numbers available.

In this study, we applied a magnetic nonviral carrier for efficient miR transfection in freshly isolated hMSCs and compared it to commercially available magnetic vectors (Magnetofectamine, CombiMag particles) regarding uptake efficiency and cytotoxicity. We demonstrate that our novel magnetic transfection system is not inferior to the latter with respect to miR delivery and cellular tolerability.

2. Material and Methods

2.1. Isolation of CD105$^+$ hMSCs. CD105$^+$ cells were freshly isolated from sternal bone marrow. The bone marrow aspirates were obtained from patients during coronary artery bypass grafting at the Cardiac Surgery Department of the University of Rostock as previously described [27]. All donors gave their written consent to use their bone marrow for research proposes according to the Declaration of Helsinki.

At first, mononuclear cells (MNCs) were isolated by density gradient centrifugation. Afterwards, the CD105$^+$ cell fraction was magnetically isolated using MACS technique according to the manufacturers' instructions (Miltenyi Biotec GmbH, Bergisch Gladbach, Germany). Briefly, 1×10^7 MNCs were incubated with 20 μL of CD105 MicroBeads (Miltenyi Biotec GmbH) for 30 minutes at 4°C. Next, suspension cells were washed with MACS buffer containing 2 mM EDTA (Gibco, Carlsbad, CA, USA), 0.5% bovine serum albumin (BSA, Sigma-Aldrich, St. Louis, MO, USA), and PBS. Subsequently, magnetically labeled cells were loaded onto a MS MACS column (Miltenyi Biotec GmbH) and placed in a magnetic field of a MiniMACS separator (Miltenyi Biotec GmbH). Afterwards, the positive CD105$^+$ cell fraction was suspended in Mesenchymal Stem Cell Growth Medium (MSCGM, Lonza, Walkersville, MD, USA) containing 100 U/mL penicillin (PAA, Coelbe, Germany) and 100 μg/mL streptomycin (PAA). Isolated CD105$^+$ cells were immediately used in further *in vitro* experiments or expanded in MSCGM (Lonza) at 37°C and 5% CO_2.

2.2. Immunophenotyping of CD105$^+$ hMSCs. Cell surface markers of freshly isolated and cultured CD105$^+$ hMSCs were fluorescently labeled with anti-human antibodies CD29-APC, CD44-PerCP-Cy5.5, CD45-V500, CD73-PE, CD117-PE-Cy7 (BD Biosciences, Heidelberg, Germany), and CD105-AlexaFluor488 (AbD Serotec, Kidlington, UK). Respective mouse isotype antibodies served as negative controls. 3×10^4 events were acquired using BD FACS LSRII flow cytometer (BD Biosciences) and analyzed with BD FACSDiva Software 6 (BD Biosciences).

2.3. Functional Differentiation Assay of CD105$^+$ hMSCs. Differentiation capacity of hMSCs was performed using the Human Mesenchymal Stem Cell Function Identification Kit (R&D Systems, Minneapolis, MN, USA) according to the manufacturers' protocol. After 20 days under differentiation conditions, fatty acid binding protein-4 (FABP-4) and osteocalcin for adipogenic and osteogenic differentiation were fluorescently labeled, respectively. Nuclei were counter stained with 4′,6-diamidino-2-phenylindol (DAPI, Invitrogen, Carlsbad, CA, USA). Samples were analyzed using ELYRA PS.1 LSM 780 microscope (Carl Zeiss, Jena, Germany) and ZEN2011 software (Carl Zeiss, Göttingen, Germany).

2.4. Preparation of Polyplex Based Transfection Complexes. For preparation of polyplex based transfection complexes (miR/PEI, miR/PEI/MNP, and miR/PEI/CombiMag complexes), Cy3 dye-Labeled Pre-miR Negative Control number 1 (Ambion, Austin, TX, USA) for monitoring uptake efficiency and cytotoxicity and Pre-miR miRNA Precursor Molecules Negative Control number 1 (Ambion) for testing complex formation were used. Branched polyethylenimine (MW = 25 kDa, Sigma-Aldrich) was biotinylated as described previously [22] and was stored in aliquots at 4.41 mM amine concentration at 4°C.

Initially, miR/PEI complexes with different molar ratios of PEI nitrogen and miR phosphate (N/P ratios) were prepared as previously described [28]. Briefly, miR and PEI were diluted in equal volumes of 5% glucose solution, mixed, and incubated for 30 minutes at room temperature.

In order to form miR/PEI/MNP complexes, Streptavidin Magnesphere Paramagnetic Particles (Promega, Madison, WI, USA) were sonicated and filtered using 450 nm Millix-HV PVDF syringe driven filter (Millipore, Tullagreen, Ireland). MNP filtrate was stored in aliquots at 4°C. Afterwards, 1 μg/mL or 2 μg/mL MNPs was added to miR/PEI complexes and incubated for 30 minutes at room temperature.

For miR/PEI/CombiMag complex formation, CombiMag reagent (OZ Biosciences, Marseille, France) was sonicated for 20 minutes. Afterwards, 0.025 μL CombiMag per 1 pmol miR (0.025 μL CombiMag/pmol) was mixed with miR/PEI complexes and incubated for 20 minutes at room temperature. All transfection complexes were freshly prepared before use.

2.5. Preparation of Lipoplex Based Transfection Complexes. For the formation of lipoplex based transfection complexes (miR/Magnetofectamine complexes), Cy3 dye-Labeled Pre-miR Negative Control number 1 (Ambion) was used for monitoring uptake efficiency and cytotoxicity.

At first, miR/Lipofectamine 2000 complexes were prepared. Therefore, miR and Lipofectamine 2000 transfection reagent (0.05 μL Lipofectamine 2000/pmol miR, Invitrogen) were diluted separately each in 25 μL of Opti-MEM I Reduced Serum Medium (Gibco) for 5 minutes at room temperature. Subsequently, miR and Lipofectamine 2000 solutions were mixed and incubated for 20 minutes at room temperature. For the formation of miR/Magnetofectamine complexes, CombiMag reagent (OZ Biosciences) was sonicated for 20 minutes. Afterwards, 0.025 μL CombiMag/pmol was mixed with miR/Lipofectamine 2000 complexes as described above. Complexes were incubated for 20 minutes at room temperature according to Magnetofectamine instructions (OZ Biosciences). All transfection complexes were freshly prepared before use.

2.6. Condensation Assay of Transfection Complexes. The condensation of miR by PEI was studied by gel electrophoresis. miR/PEI complexes were prepared as described above, mixed with loading dye (Fermentas GmbH, St. Leon-Rot, Germany), and loaded onto 2% agarose gel containing ethidium bromide. An electric field of 100 V was applied for 30 minutes and image was taken using TS imaging system (Biometra GmbH, Göttingen, Germany).

2.7. Transfection. For transfection experiments, 1×10^5 freshly isolated hMSCs per well were seeded in 48 well plates. Transfection complexes were prepared as described above and added drop by drop to the cells. Afterwards, cells were treated with and without the application of a magnetic field for 20 minutes using a Super Magnetic Plate (OZ Biosciences). Subsequently, cells were incubated for 24 hours at 37°C and 5% CO_2.

2.8. Evaluation of Uptake Efficiency and Cytotoxicity. For quantification of uptake efficiency, hMSCs were transfected with complexes containing Cy3 dye-Labeled Pre-miR Negative Control number 1 (Ambion) as described above for 24 hours. To investigate cytotoxicity, cells were stained with Near-IR LIVE/DEAD Fixable Dead Cell Stain Kit (Molecular Probes, Eugene, OR, USA). Moreover, cells were labeled with Alexa Fluor 488 mouse anti-human CD105 (clone SN6, AbD Serotec) and fixed with 4% PFA. 3×10^4 events were acquired using BD FACS LSRII flow cytometer (BD Biosciences) and analyzed with BD FACSDiva Software 6 (BD Biosciences). For determination of uptake efficiency, the number of living $CD105^+$ Cy3-stained ($Cy3^+$) cells in relation to the total cell number of living $CD105^+$ cells was measured. To evaluate complex cytotoxicity, the percentage of dead $CD105^+$ cells in relation to the total cell number of $CD105^+$ cells was recorded.

2.9. Statistical Analysis. For all experiments, statistical analyses were performed by Student's t-test using SigmaPlot 11.0 software (Systat Software GmbH, Erkrath, Germany). Relative expression data of CD marker expressions are shown as mean ± standard deviation (SD). All other values are presented as mean ± standard error of the mean (SEM). A P value < 0.05 was considered to be statistically significant.

3. Results and Discussion

3.1. Results

3.1.1. Characterization of $CD105^+$ hMSCs. $CD105^+$ hMSCs were isolated from bone marrow and immediately characterized by multilineage differentiation and specific surface marker expression before use in further experiments [29, 30]. To investigate the differentiation capacity of hMSCs, cells were cultivated in adipogenic and osteogenic differentiation medium, respectively, and examined by fluorescent microscopy. Figure 1 illustrates that cells were able to differentiate into both adipocytes (Figure 1(a)) and osteocytes (Figure 1(b)). FACS analyses of freshly isolated $CD105^+$ cells showed high expression of CD44 and CD105, a moderate expression of CD29 and CD45, and no expression of CD73 and CD117 (Figures 1(c) and 1(d)). Moreover, we compared the immunophenotype of freshly isolated cells with expanded $CD105^+$ cells. Cultivated $CD105^+$ hMSCs presented high expressions of stem cell markers CD29, CD44, CD73,

and CD105 but had downregulated expression of hematopoietic markers CD45 and CD117 (Figure 1(d)).

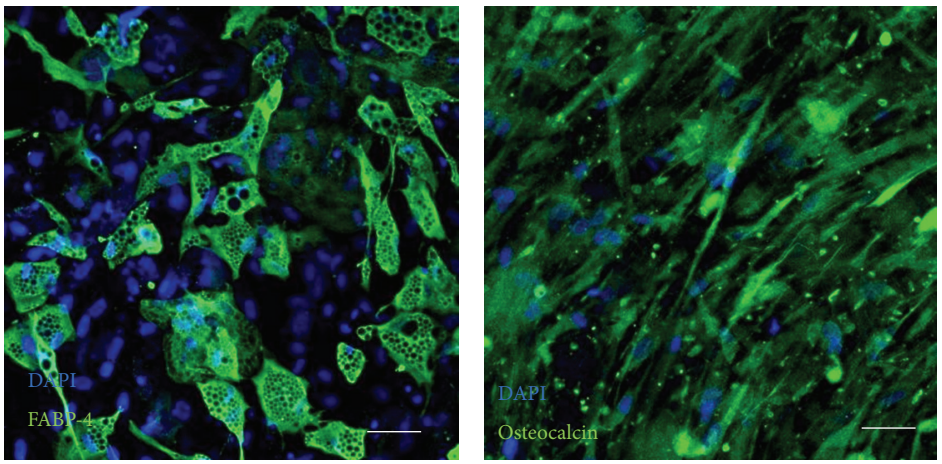
3.1.2. Characterization of Transfection Complexes. In order to examine condensation of miR by PEI, gel electrophoresis was performed. Thereby, different miR/PEI complexes with increasing amounts of PEI were investigated. Results demonstrated a big and sharp band for uncondensed miR that was used as a control (Figure 2(a)). At N/P ratios of 0.1, 0.25, and 0.5, miR has partly formed complexes with PEI. Due to the bigger size, miR/PEI complexes remained in the slots, while uncondensed miR migrated through the gel. However, the miR signal disappeared completely at N/P ratios greater than 1.

3.1.3. Transfection Optimization of miR/PEI Complexes. In order to optimize transfection of freshly isolated $CD105^+$ cells, polyplexes with different N/P ratios (N/P 2.5, N/P 10, and N/P 33) and miR amounts (5 pmol and 10 pmol) were tested using flow cytometry. Complexes with an N/P ratio of 10 combined with 5 pmol miR (56%) and an N/P ratio of 2.5 combined with 10 pmol miR (69%) showed the highest uptake efficiencies (Figure 2(d)). In order to increase uptake rates, higher N/P ratios (N/P 33) were investigated. However, an N/P ratio of 33 did not lead to further enhancement of uptake efficiencies. Likewise, potential cytotoxicity of the transfection complexes was investigated (Figure 2(e)). Thereby, untransfected cells were used as internal control (29% dead cells) which is reflecting cytotoxicity of the isolation procedure. Transfection complexes with an N/P ratio of 10 combined with 5 pmol miR led to moderately increased cell mortality (17%) compared to controls. Yet, polyplexes with an N/P ratio of 2.5 combined with 10 pmol miR showed no significant differences compared to controls (32% versus 29%). However, an increase in the N/P ratio (N/P 33) did lead to higher cytotoxicity in polyplexes with 5 pmol miR (57%) as well as with 10 pmol miR (55%). Therefore, with respect to the highest uptake rates and lowest cytotoxicity, polyplexes composed of an N/P ratio of 10 combined with 5 pmol miR and complexes with an N/P ratio of 2.5 combined with 10 pmol miR were considered to represent optimal compositions and were utilized in further experiments.

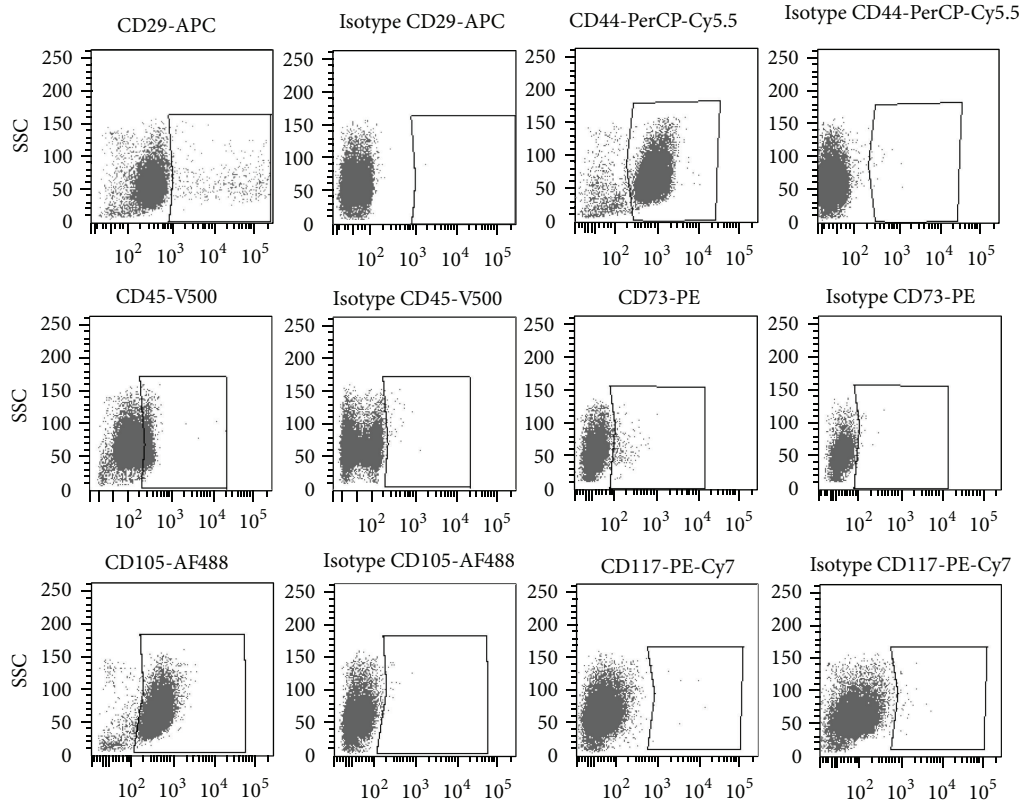
3.1.4. Transfection Optimization of miR/PEI/MNP Complexes. In order to increase selectivity of the carrier and safety for clinical applications, the two optimized miR/PEI complexes were combined with different MNP amounts (1 or 2 μg/mL MNPs). Therefore uptake rates (Figures 3(a) and 3(c)) and cytotoxicity (Figures 3(b) and 3(d)) of the different complex compositions as well as the influence of a magnetic field were investigated by flow cytometry. Magnetic polyplexes consisting of an N/P ratio of 10 combined with 5 pmol miR had similar uptake rates (up to 76%) compared to the corresponding miR/PEI complexes (81%) without the application of a magnetic field (Figure 3(a)). Moreover, cytotoxicity of these complexes was investigated. Magnetic polyplexes with 1 or 2 μg/mL MNPs (27% versus 24%) showed no cytotoxic effect when compared to miR/PEI complexes (30%, Figure 3(b)). Likewise, polyplexes consisting of an N/P ratio of 2.5 combined with 10 pmol miR coupled to MNPs were investigated. Uptake rates of magnetic complexes with 1 or 2 μg/mL MNPs (~65%), respectively, did not significantly differ compared to appropriate miR/PEI complexes (77%) in the absence of a magnetic field (Figure 3(c)). Furthermore, no significant differences in cytotoxicity were observed between the transfected cells (ranging from 15% to 18%) and the control (17%, Figure 3(d)). Additionally, the influence of a magnetic field was investigated. However, no significant improvement of uptake efficiency and reduction of cytotoxicity were observed when an external magnetic field was applied (Figure 3). For further experiments, two magnetic complexes were used: first an N/P ratio of 10 combined with 5 pmol miR bound to 1 μg/mL MNPs and second an N/P ratio of 2.5 combined with 10 pmol miR bound to 1 μg/mL MNPs.

3.1.5. Comparison of miR/PEI/MNP Complexes to Established Transfection Reagents. Optimized magnetic polyplexes were compared to commercially available magnetic vectors: Magnetofectamine and CombiMag particles. Therefore uptake efficiency (Figures 4(a) and 4(c)) and cytotoxicity (Figures 4(b) and 4(d)) of different transfection complexes were investigated using flow cytometry. miR/PEI/MNP complexes with an N/P ratio of 10 combined with 5 pmol miR bound to 1 μg/mL MNPs showed high uptake rates (68%, Figure 4(a)). Moreover, no significant differences in uptake efficiency were observed between miR/PEI/CombiMag (64%) and miR/Magnetofectamine (59%) complexes. Mortality between treated and control cells was comparable and ranged between 11% and 17% (Figure 4(b)). Moreover, uptake efficiency and cytotoxicity of miR/PEI/MNP complexes composed of an N/P ratio of 2.5 combined with 10 pmol miR bound to 1 μg/mL MNPs were investigated and compared to known transfection reagents. Uptake rates of miR/PEI/MNP complexes reached up to 79% and were compared to miR/PEI/CombiMag (56%) and miR/Magnetofectamine (75%, Figure 4(c)). Moreover, cell mortality of transfected cells (ranging from 9% to 14%) was not significantly different when compared to untreated control (9%, Figure 4(d)).

3.2. Discussion. Therapeutic potential of hMSCs can be improved by genetic modifications with distinct miRs [5, 6]. Our group has recently developed a magnetic nonviral transfection carrier consisting of biotinylated PEI bound to streptavidin-coated MNPs for highly efficient miR delivery (~75%) and applied it to cultivated hMSCs [22]. In general, hMSCs can be characterized by multilineage differentiation potential and the expression of specific surface markers (e.g., CD29, CD44, CD73, and CD105) [1]. Our previous studies have demonstrated that the purified $CD105^+$ fraction of expanded hMSCs had a beneficial effect in cardiac tissue regeneration [27]. However, the time-consuming, expensive, and potentially detrimental cell expansion process might be avoidable as it has previously been described by Aslan et al. They tested $CD105^+$ cells freshly isolated from bone marrow *in vitro* and *in vivo*. Importantly, these cells were indeed



(a) (b)



(c)

	CD29	CD44	CD45	CD73	CD105	CD117
Fresh hMSCs	4.8% ± 2.4	93.8% ± 1.3	23.8% ± 0.6	1.4% ± 0.9	93.0% ± 4.6	0.1% ± 0.1
Cultured hMSCs	99.6% ± 0.4	99.7% ± 0.3	0.5% ± 0.5	97.5% ± 3.3	98.5% ± 0.4	0.2% ± 0.2

(d)

Figure 1: Characterization of CD105$^+$ hMSCs. ((a), (b)) Functional differentiation capacity of freshly isolated CD105$^+$ cells was shown by immunostaining of FABP-4 (green) for adipocytes (a) and osteocalcin (green) for osteocytes (b) after 20 days in appropriate differentiation medium. Nuclei were counter stained with DAPI (blue). Scale bars = 50 μm. ((c), (d)) Immunophenotyping of freshly isolated ((c), (d)) and cultured CD105$^+$ hMSCs (d) was evaluated by flow cytometry after staining of specific cell surface markers. Corresponding isotype controls were used as negative controls (c). The relative expression values of CD marker expression are shown as mean ± SD; $n = 2$ (d).

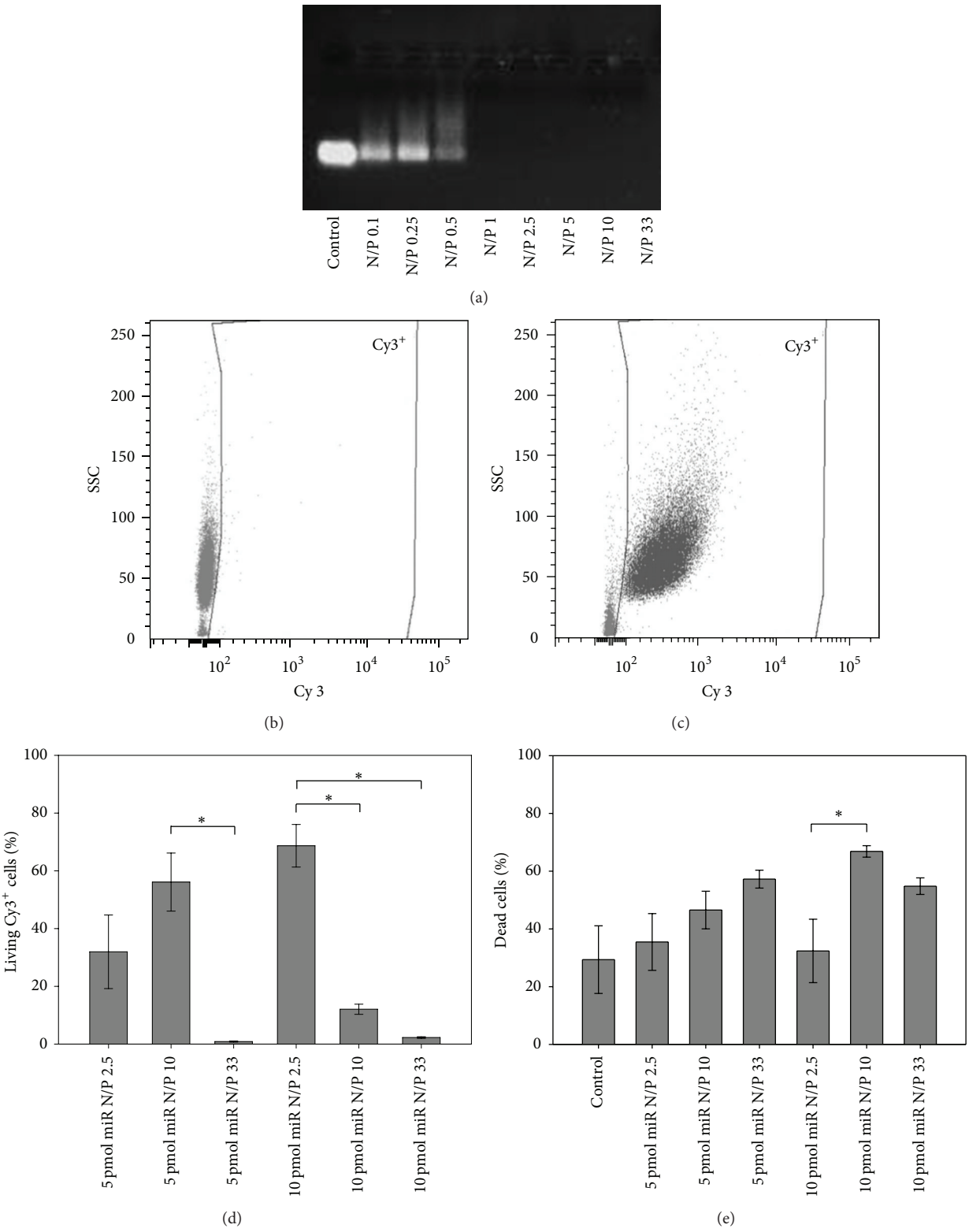


Figure 2: Characterization and transfection optimization of miR/PEI complexes. (a) Condensation of miR/PEI complexes composed of 20 pmol miR and different N/P ratios (ranging between 0.1 and 33) was analysed by gel electrophoresis. miR alone served as positive control. ((b), (c)) Gating strategy of FACS measurements. (b) Untransfected living CD105$^+$ cells were used as negative control. (c) Living CD105$^+$ cells transfected with Cy3-labeled complexes. ((d), (e)) Optimization of miR amounts and N/P ratios for transfection of freshly isolated hMSCs. hMSCs were transfected with Cy3-labeled miR/PEI complexes consisting of three different N/P ratios (N/P 2.5, N/P 10, and N/P 33) and two different miR amounts (5 and 10 pmol). 24 hours after transfection, uptake efficiency (d) and cytotoxicity (e) of complexes were analyzed by flow cytometry. Untransfected cells were used as control. Values are presented as mean ± SEM; $n = 3$; $* = P \leq 0.05$.

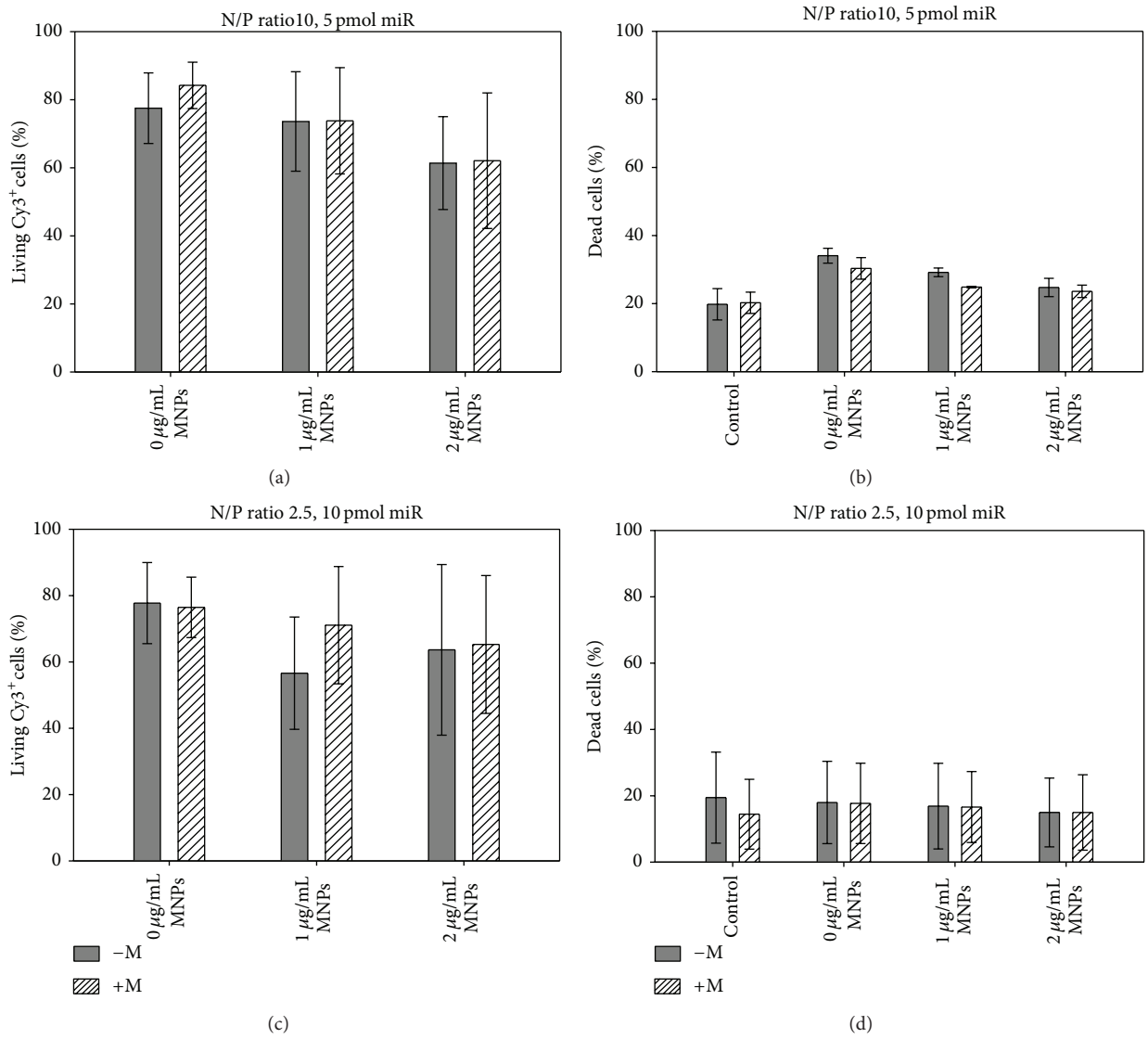


Figure 3: Transfection optimization of miR/PEI/MNP complexes. ((a)–(d)) Optimization of MNP amounts for transfection of freshly isolated hMSCs with (+M) and without (−M) the application of a magnetic field. hMSCs were transfected with Cy3-labeled miR/PEI and miR/PEI/MNP complexes composed of an N/P ratio of 10 combined with 5 pmol miR ((a), (b)) or an N/P ratio of 2.5 combined with 10 pmol miR ((c), (d)) and three different MNP amounts (0, 1, or 2 μg/mL MNPs). 24 hours after transfection, uptake efficiency ((a), (c)) and cytotoxicity ((b), (d)) of complexes were analyzed by flow cytometry. Untransfected cells were used as control. Values are presented as mean ± SEM; $n = 3$.

capable of giving rise to hMSCs bearing all typical properties after expansion [2]. Likewise in our study, freshly isolated CD105$^+$ cells showed a slightly altered immunophenotype when compared to hMSCs after expansion (Figures 1(c) and 1(d)). However, our freshly isolated CD105$^+$ cells also did adhere to plastic surfaces and showed a morphology and phenotype typical for hMSCs in expansion culture (Figure 1(d)). Moreover, we proofed the ability of these hMSCs derived from our freshly isolated CD105$^+$ cells to differentiate into adipocytes (Figure 1(a)) and osteocytes (Figure 1(b)) under appropriate culture conditions. This clearly confirmed their stem cell character. Therefore, our findings are in line with the report of Aslan et al. [2].

To optimize miR transfection of freshly isolated CD105$^+$ hMSCs, we initially tested miR/PEI complexes with different amounts of miR and PEI. In order to investigate the physicochemical properties of various miR/PEI complexes, condensation of miR by PEI was analyzed using gel electrophoresis (Figure 2(a)). Results demonstrated that, at N/P ratios from 1 to 33, no signals were observed. Therefore, we concluded that an N/P ratio 1 was sufficient to condense the whole amount of miR that is in correspondence with our previous findings [22]. Appropriate condensation of miR is essential for effective delivery into cells as it is protecting miR from early enzymatic degradation [11]. Furthermore, it prevents the activation of the innate immune system by double stranded RNA [31].

In order to establish efficient transfection complexes for miR delivery in freshly isolated CD105$^+$ hMSCs, miR/PEI complexes with different N/P ratios (2.5, 10, and 33) and miR

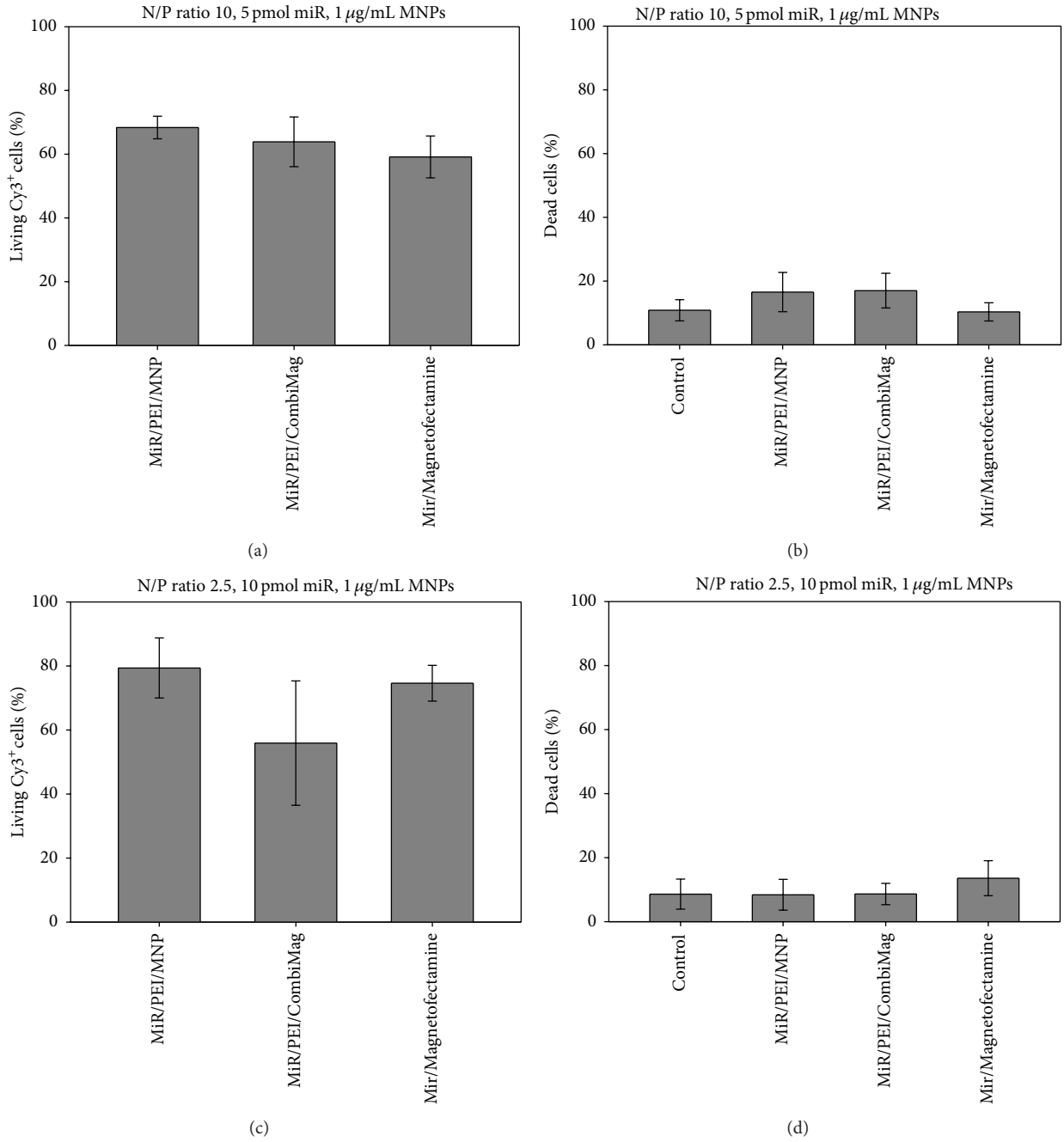


Figure 4: Comparison between optimized miR/PEI/MNP complexes and commercially available magnetic transfection reagents. Freshly isolated hMSCs were transfected with Cy3-labeled miR/PEI/MNP complexes composed of an N/P ratio of 10 combined with 5 pmol miR bound to 1 μg/mL MNPs ((a), (b)) or an N/P ratio of 2.5 combined with 10 pmol miR bound to 1 μg/mL MNPs ((c), (d)). For comparison with commercially available magnetic transfection complexes, appropriate amounts of Lipofectamine 2000 and CombiMag particles were used. 24 hours after transfection, uptake efficiency ((a), (c)) and cytotoxicity ((b), (d)) of complexes were determined by flow cytometry, while a magnetic field was applied. Untransfected cells were used as control. Values are presented as mean ± SEM; $n = 3$.

amounts (5 and 10 pmol) were tested (Figures 2(c) and 2(d)). Previous experiments showed that N/P ratios can significantly influence uptake efficiency of transfection complexes [32]; thus we selected 3 different N/P ratios for optimization experiments. Delyagina et al. showed that an N/P ratio of 2.5 showed the highest uptake efficiency of plasmid DNA in cultured hMSCs [21]. We have also demonstrated that miR was efficiently delivered using the same vector with N/P ratios of 10 and 33 [22]. Our current results demonstrated that miR/PEI complexes with 5 pmol provided the highest uptake efficiency at an N/P ratio of 10 (56%) for fresh hMSCs. In contrast to miR transfection in expanded hMSCs, we could further enhance miR uptake in freshly isolated hMSCs by increasing the miR amount to 10 pmol with an N/P ratio of 2.5 (69%). Due to the low N/P ratio, cytotoxicity could be decreased and was comparable to controls (32% versus

29%). The strategy of using low N/P ratios reduced the amount of used PEI that might be advantageous for genetic modifications. That is especially important for freshly isolated hMSCs as they might react very sensitive to potentially toxic reagents. Therefore, we decided to use miR/PEI complexes with an N/P ratio of 2.5 combined with 10 pmol miR for further experiments and compare it to complexes consisting of an N/P ratio of 10 combined with 5 pmol miR which were used in our previous work [22].

Furthermore, we combined optimized miR/PEI complexes with different MNP amounts to enable magnetic targeting of transfected cells (Figure 3). Based on previous work, miR/PEI/MNP complexes with 1 μg/mL or 2 μg/mL MNPs were investigated as they showed a significant increase in uptake efficiency compared to control (75% versus 50%) [22]. Our current investigations showed no significant differences in uptake rates and cytotoxicity of magnetic complexes with different compositions. The values of uptake efficiencies were comparable to those previously obtained for cultivated hMSCs [22] and were in the range from 56% to 79% (Figures 3(a) and 3(c)). Moreover, cell mortality was comparable to controls representing the basic level of cytotoxicity due to the isolation process (Figures 3(b) and 3(d)). Additionally, we investigated the influence of a magnetic field on miR transfection into freshly isolated cells. We observed no significant differences in uptake efficiency and cytotoxicity in cells transfected in the presence or absence of a magnetic field (Figure 3). These findings are in agreement with our previous data that showed efficient plasmid DNA transfection in hMSCs without the application of an external magnetic field [21]. This effect might be explained by the fact that a magnetic field has no influence on cellular uptake or intracellular transfection mechanism, as previously proposed [33].

Furthermore, we compared the performance of our miR/PEI/MNP vector and commercially available magnetic transfection reagents in freshly isolated CD105$^+$ cells (Figure 4). Therefore we chose magnetic complexes consisting of an N/P ratio of 10 combined with 5 pmol miR bound to 1 μg/mL MNPs as they showed good transfection values in both freshly isolated and cultured hMSCs [22]. Additionally, we selected complexes with an N/P ratio of 2.5 combined with 10 pmol miR bound to 1 μg/mL MNPs as lower amount of PEI and MNPs in their composition facilitates their application. Magnetofectamine is a combined transfection reagent which consists of Lipofectamine 2000 and CombiMag particles and can be used for delivery of different nucleic acids in various cell types in the presence of an external magnetic field [34]. Lipofectamine is the most effective and best investigated cationic lipid, which can interact spontaneously with nucleic acids through electrostatic interactions forming stable lipoplexes [13, 35]. CombiMag particles can also serve as transfection reagent when combined with cationic polymers or cationic lipids [34]. However, the combination of CombiMag particles with Lipofectamine 2000 is proposed as the most effective approach. [34]. We could demonstrate that our miR/PEI/MNP vector was able to reach uptake rates similar to Magnetofectamine and miR/PEI/CombiMag complexes (Figures 4(a) and 4(c)). Moreover, cytotoxicity of all transfection complexes was comparable to controls indicating no cytotoxic effect of the different nonviral complexes on freshly isolated hMSCs (Figures 4(b) and 4(d)).

It has previously been shown by our group that both nonviral and viral magnetic complexes carrying labeled or therapeutic plasmid DNA can be targeted *in vivo* [20, 36]. Yet, thereby, no transfection into stem cells was performed. Furthermore, miR as nucleic acid of interest has so far not been used for *in vivo* targeting. This shortcoming led us to transfer our approach to an efficient delivery of miR into freshly isolated CD105$^+$ hMSCs using three different magnetic carrier systems for the first time. All systems investigated yielded around 65% uptake of labeled miR combined with high cell viability. Therefore, we here introduce a novel carrier system which provides equal efficiency and cellular tolerance as commercially available magnetic transfection reagents.

Overall, the specific properties of the respective magnetic carriers for various applications may allow for direct nonviral delivery of therapeutic miRs as well as other nucleic acids *in vivo*. Moreover, introduction of these nucleic acids *in vitro* prior to cell transplantation may enable stem cell modulation for targeted stem cell therapy.

4. Conclusion

In this report, we successfully developed magnetic nonviral carriers for efficient miR transfection into freshly isolated CD105$^+$ hMSCs. These magnetic vectors were able to reach high uptake rates (68% versus 79%) with no significant cytotoxic effect. The performance of the novel miR carrier equaled that of commercially available magnetic transfection reagents. Magnetic nanoparticle based miR transfection may become important to optimize stem cells meant for transplantation, although further preclinical experiments are required.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

Authors' Contribution

Anna Schade and Paula Müller contributed equally to this work.

Acknowledgments

This work was supported by ESF Grant (UR11 041; ESF/IV-BM-B35-0010/12); the Reference and Translation Center for Cardiac Stem Cell Therapy (BMBF: FKZ 0312138A); and Land MV (FKZ V630-F-075-2010/183; V630-S-075-2010/185). Robert David is supported by the DFG (DA1296/2-1).

References

[1] M. Dominici, K. le Blanc, I. Mueller et al., "Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement," *Cytotherapy*, vol. 8, no. 4, pp. 315–317, 2006.

[2] H. Aslan, Y. Zilberman, L. Kandel et al., "Osteogenic differentiation of noncultured immunoisolated bone marrow-derived $CD105^+$ cells," *Stem Cells*, vol. 24, no. 7, pp. 1728–1737, 2006.

[3] A. I. Caplan, "Adult mesenchymal stem cells for tissue engineering versus regenerative medicine," *Journal of Cellular Physiology*, vol. 213, no. 2, pp. 341–347, 2007.

[4] Z. Wen, S. Zheng, C. Zhou, W. Yuan, J. Wang, and T. Wang, "Bone marrow mesenchymal stem cells for post-myocardial infarction cardiac repair: microRNAs as novel regulators," *Journal of Cellular and Molecular Medicine*, vol. 16, no. 4, pp. 657–671, 2012.

[5] J. J. Chen and S. H. Zhou, "Mesenchymal stem cells overexpressing MiR-126 enhance ischemic angiogenesis via the AKT/ERK-related pathway," *Cardiology Journal*, vol. 18, no. 6, pp. 675–681, 2011.

[6] F. Huang, X. Zhu, X. Q. Hu et al., "Mesenchymal stem cells modified with miR-126 release angiogenic factors and activate Notch ligand Delta-like-4, enhancing ischemic angiogenesis and cell survival," *International Journal of Molecular Medicine*, vol. 31, no. 2, pp. 484–492, 2013.

[7] M. Tome, P. Lopez-Romero, C. Albo et al., "miR-335 orchestrates cell proliferation, migration and differentiation in human mesenchymal stem cells," *Cell Death and Differentiation*, vol. 18, no. 6, pp. 985–995, 2011.

[8] P. Jakob and U. Landmesser, "Role of microRNAs in stem/progenitor cells and cardiovascular repair," *Cardiovascular Research*, vol. 93, no. 4, pp. 614–622, 2012.

[9] H. Boulaiz, J. A. Marchal, J. Prados, C. Melguizo, and A. Aranega, "Non-viral and viral vectors for gene therapy," *Cellular and Molecular Biology*, vol. 51, no. 1, pp. 3–22, 2005.

[10] F. H. Lin, C. H. Chen, W. T. Cheng, and T. F. Kuo, "Modified montmorillonite as vector for gene delivery," *Biomaterials*, vol. 27, no. 17, pp. 3333–3338, 2006.

[11] T. Bieber, W. Meissner, S. Kostin, A. Niemann, and H. P. Elsasser, "Intracellular route and transcriptional competence of polyethylenimine-DNA complexes," *Journal of Controlled Release*, vol. 82, no. 2-3, pp. 441–454, 2002.

[12] M. A. Mintzer and E. E. Simanek, "Nonviral vectors for gene delivery," *Chemical Reviews*, vol. 109, no. 2, pp. 259–302, 2009.

[13] S. Zhang, B. Zhao, H. Jiang, B. Wang, and B. Ma, "Cationic lipids and polymers mediated vectors for delivery of siRNA," *Journal of Controlled Release*, vol. 123, no. 1, pp. 1–10, 2007.

[14] E. Delyagina, W. Li, N. Ma, and G. Steinhoff, "Magnetic targeting strategies in gene delivery," *Nanomedicine*, vol. 6, no. 9, pp. 1593–1604, 2011.

[15] F. Scherer, M. Anton, U. Schillinger et al., "Magnetofection: enhancing and targeting gene delivery by magnetic force in vitro and in vivo," *Gene Therapy*, vol. 9, no. 2, pp. 102–109, 2002.

[16] H. H. Yiu, S. C. McBain, Z. A. Lethbridge, M. R. Lees, and J. Dobson, "Preparation and characterization of polyethylenimine-coated Fe_3O_4-MCM-48 nanocomposite particles as a novel agent for magnet-assisted transfection," *Journal of Biomedical Materials Research A*, vol. 92, no. 1, pp. 386–392, 2010.

[17] P. del Pino, A. Munoz-Javier, D. Vlaskou, P. Rivera Gil, C. Plank, and W. J. Parak, "Gene silencing mediated by magnetic lipospheres tagged with small interfering RNA," *Nano Letters*, vol. 10, no. 10, pp. 3914–3921, 2010.

[18] J. Lim, M. A. Clements, and J. Dobson, "Delivery of short interfering ribonucleic acid-complexed magnetic nanoparticles in an oscillating field occurs via caveolae-mediated endocytosis," *PLoS ONE*, vol. 7, no. 12, article e51350, 2012.

[19] C. Plank, O. Zelphati, and O. Mykhaylyk, "Magnetically enhanced nucleic acid delivery. Ten years of magnetofection-Progress and prospects," *Advanced Drug Delivery Reviews*, vol. 63, no. 14-15, pp. 1300–1331, 2011.

[20] W. Li, N. Ma, L.-L. Ong et al., "Enhanced thoracic gene delivery by magnetic nanobead-mediated vector," *The Journal of Gene Medicine*, vol. 10, no. 8, pp. 897–909, 2008.

[21] E. Delyagina, A. Schade, D. Scharfenberg et al., "Improved transfection in human mesenchymal stem cells: effective intracellular release of pDNA by magnetic polyplexes," *Nanomedicine*, 2013.

[22] A. Schade, E. Delyagina, D. Scharfenberg et al., "Innovative strategy for microRNA delivery in human mesenchymal stem cells via magnetic nanoparticles," *International Journal of Molecular Sciences*, vol. 14, no. 6, pp. 10710–10726, 2013.

[23] S. Bobis, D. Jarocha, and M. Majka, "Mesenchymal stem cells: characteristics and clinical applications," *Folia Histochemica et Cytobiologica*, vol. 44, no. 4, pp. 215–230, 2006.

[24] P. Mark, M. Kleinsorge, R. Gaebel et al., "Human mesenchymal stem cells display reduced expression of CD105 after culture in serum-free medium," *Stem Cells International*, vol. 2013, Article ID 698076, 8 pages, 2013.

[25] http://clinicaltrials.gov/.

[26] W. J. Rombouts and R. E. Ploemacher, "Primary murine MSC show highly efficient homing to the bone marrow but lose homing ability following culture," *Leukemia*, vol. 17, no. 1, pp. 160–170, 2003.

[27] R. Gaebel, D. Furlani, H. Sorg et al., "Cell origin of human mesenchymal stem cells determines a different healing performance in cardiac regeneration," *PLoS ONE*, vol. 6, no. 2, e15652, 2011.

[28] W. Wang, W. Li, L. Ou et al., "Polyethylenimine-mediated gene delivery into human bone marrow mesenchymal stem cells from patients," *Journal of Cellular and Molecular Medicine*, vol. 15, no. 9, pp. 1989–1998, 2011.

[29] Y. H. Choi, A. Kurtz, and C. Stamm, "Mesenchymal stem cells for cardiac cell therapy," *Human Gene Therapy*, vol. 22, no. 1, pp. 3–17, 2011.

[30] M. F. Pittenger and B. J. Martin, "Mesenchymal stem cells and their potential as cardiac therapeutics," *Circulation Research*, vol. 95, no. 1, pp. 9–20, 2004.

[31] A. Judge and I. MacLachlan, "Overcoming the innate immune response to small interfering RNA," *Human Gene Therapy*, vol. 19, no. 2, pp. 111–124, 2008.

[32] W. T. Godbey, K. K. Wu, and A. G. Mikos, "Poly(ethylenimine) and its role in gene delivery," *Journal of Controlled Release*, vol. 60, no. 2-3, pp. 149–160, 1999.

[33] S. Huth, J. Lausier, S. W. Gersting et al., "Insights into the mechanism of magnetofection using PEI-based magnetofectins

for gene transfer," *The Journal of Gene Medicine*, vol. 6, no. 8, pp. 923–936, 2004.

[34] http://www.ozbiosciences.com/.

[35] S. N. Uddin, "Cationic lipids used in non-viral gene delivery systems," *Biotechnology and Molecular Biology Review*, vol. 2, pp. 58–67, 2007.

[36] Y. Zhang, W. Li, L. Ou et al., "Targeted delivery of human VEGF gene via complexes of magnetic nanoparticle-adenoviral vectors enhanced cardiac regeneration," *PLoS ONE*, vol. 7, no. 7, article e39490.