

Firdos Alam Khan *Editor*

Stem Cell Production

Processes, Practices and Regulations

 Springer

Stem Cell Production

Firdos Alam Khan
Editor

Stem Cell Production

Processes, Practices and Regulations

 Springer

Editor

Firdos Alam Khan
Department of Stem Cell Biology
Institute for Research and Medical
Consultations, Imam Abdulrahman Bin
Faisal University
Dammam, Saudi Arabia

ISBN 978-981-16-7588-1

ISBN 978-981-16-7589-8 (eBook)

<https://doi.org/10.1007/978-981-16-7589-8>

© The Editor(s) (if applicable) and The Author(s), under exclusive license to Springer Nature Singapore Pte Ltd. 2022

This work is subject to copyright. All rights are solely and exclusively licensed by the Publisher, whether the whole or part of the material is concerned, specifically the rights of translation, reprinting, reuse of illustrations, recitation, broadcasting, reproduction on microfilms or in any other physical way, and transmission or information storage and retrieval, electronic adaptation, computer software, or by similar or dissimilar methodology now known or hereafter developed.

The use of general descriptive names, registered names, trademarks, service marks, etc. in this publication does not imply, even in the absence of a specific statement, that such names are exempt from the relevant protective laws and regulations and therefore free for general use.

The publisher, the authors, and the editors are safe to assume that the advice and information in this book are believed to be true and accurate at the date of publication. Neither the publisher nor the authors or the editors give a warranty, expressed or implied, with respect to the material contained herein or for any errors or omissions that may have been made. The publisher remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

This Springer imprint is published by the registered company Springer Nature Singapore Pte Ltd. The registered company address is: 152 Beach Road, #21-01/04 Gateway East, Singapore 189721, Singapore

Preface

The use of stem cells as regenerative medicine is one of the most sought-after technologies for cell-based therapy required by many patients who need cell transplantations. To be able to successfully use these stem cells in humans, these stem cells need to be produced by Good Manufacturing Practice (GMP), FDA and WHO recommended procedures and guidelines. The production of clinical grade stem cells needs GMP compliant facilities, protocols, production systems and quality checking mechanisms. Over the past few years, there has been tremendous increase in the demand of clinical grade stem cells and differentiated cells for cell therapy, and considering this aspect, there are not many books available that deal with the entire process of production of stem cells as per GMP guidelines. There is no single book available that comprehensively describes the entire process of stem cell production from raw materials to finished products. In this book, we have included all the topics that are important for stem cell production and provide a unique source of information for anyone who wants to learn and understand the entire process of production of stem cell-based products. This book will help stem cell therapy researchers, stem cell therapy professionals, and biotechnology companies to learn the entire process of stem cell production.

There are 11 chapters in the book which covers topics such as (1) stem cell therapy: significance and applications of stem cell products in tissue engineering and regenerative medicine, (2) Good Laboratory Practice (GLP) facility and production of stem cells: design, constructions and automation for clinical grade production of stem cells (facility, regulatory requirements and approvals process), (3) Current Good Manufacturing Practice (cGMP) facility and production of stem cells: design, constructions, automation and scalability for clinical grade stem cell productions (facility, regulatory requirements process), (4) Current Good Manufacturing Practice (cGMP) inspection, approval and certification: ISO certification, FDA inspections and cGMP certifications for stem cell productions, (5) stem cell bioreactors: design, structure and operation of stem cell bioreactors, (6) stem cell culture and expansion: role of culture, types of cells (stem cell, immune cell, primary cell), growth conditions (adherent vs. suspension), media nutrients, growth factors and growth phase cycle, (7) upscaling of clinical grade stem cell production: upstream processing (USP) and downstream processing (DSP) operations of cell expansion, harvesting, detachment, separation, washing and concentration steps, and regulatory

requirements, (8) characterizations of clinical grade stem cells: microscopic, cellular, molecular and functional characterizations of stem cells and their products for regulatory requirements and approval process (FDA), (9) stem cell safety and sterility testing: testing for stem cell sterility (microbial, viral or other contaminations) and stem cell product safety and regulatory requirements and approval process (FDA), (10) stem cells packaging, storage and transportation: formulations, packaging, transportation and storage of stem cell products and (11) stem cell clinical trials and stem cell market: current clinical trials (autologous and allogeneic transplantation), global market for stem cell products, and market value, cell therapy business and challenges of current stem cells production and business. Each chapter provides detailed and updated information on each topic supported with beautiful illustrations.

Dammam, Saudi Arabia

Firdos Alam Khan

Acknowledgements

I am grateful to the Almighty Allah for the blessings and guidance. I am thankful to Springer Publication for showing trust in me and providing me one more opportunity to edit this exciting book on stem cells. I appreciate the support of Dr. Bhavik Sawhney, Editor-Biomedicine, Springer Nature. I am thankful to all the members of the production team of Springer Nature for their support and cooperation.

I am grateful to all the authors and corresponding authors for their immense contributions and cooperation. I want to thank the entire management team of the Institute for Research & Medical Consultations (IRMC), Imam Abdulrahman Bin Faisal University, Dammam, Saudi Arabia, for their support, especially to Professor Ebtesam Al-Suhaimi, Dean, IRMC, Imam Abdulrahman Bin Faisal University, Dammam, Saudi Arabia, for her constant encouragement.

I am grateful to all my teachers and mentors, especially Professor Nishikant Subhedar and the Late Professor Obaid Siddiqi FRS, for their immense contributions to shaping my research career. I am also thankful to all my friends, well-wishers and colleagues for their support and cooperation.

I am grateful to my entire family members, especially to my father Late Nayeemuddin Khan and mother Late Sarwari Begum, my brothers (Aftab Alam Khan, Javed Alam Khan, Intekhab Alam Khan, Sarfaraz Alam Khan), my sisters (Late Sayeeda Khanum, Faheemida Khanum, Kahkashan Khanum, Ayesha Khanum), my wife Samina Khan, and my sons (Zuhayr Ahmad Khan, Zaid Ahmad Khan, and Zahid Ahmad Khan) and my daughter (Azraa Khan), my father-in-law (Abdul Qayyum Siddiqi) and mother-in-law (Uzma Siddiqi) for their support.

Enjoy reading!

Contents

1	Stem Cell Therapy: Significance and Applications of Stem Cell Products in Tissue Engineering and Regenerative Medicine	1
	Kholoud Al Ghamdi	
2	GLP Requirements of Stem Cells	23
	Hassan Ahmed Khan	
3	Current Good Manufacturing Practice (cGMP) Facility and Production of Stem Cell	37
	Wan Tai Seet, Mohd Asyraf Mat Afandi, Sharen Aini Shamsuddin, Yogeswaran Lokanathan, Min Hwei Ng, and Manira Maarof	
4	Global Regulatory Frameworks and Quality Standards for Stem Cells Therapy and Regenerative Medicines	69
	Sudhir Sawarkar and Asawari Bapat	
5	Stem Cell Bioreactors: Design, Structure, and Operation of Stem Cell Bioreactors	113
	Firdos Alam Khan	
6	Stem Cell Production: Processes, Practices, and Regulation	125
	Daniela Lisini, Simona Frigerio, Sara Nava, and Simona Pogliani	
7	Upscaling of Clinical Grade Stem Cell Production: Upstream Processing (USP) and Downstream Processing (DSP) Operations of Cell Expansion, Harvesting, Detachment, Separation, Washing and Concentration Steps, and the Regulatory Requirements	159
	Achim Cchitvsanzwhoh Satheka	
8	Characterization of Clinical-Grade Stem Cells: Microscopic, Cellular, Molecular, and Functional Characterization of Stem Cells and Their Products According to Regulatory Requirements for FDA Approval	185
	Tereza Souralova, Daniela Rehakova, Lenka Tesarova, Ales Hampl, and Irena Koutna	

9	Stem Cell Safety and Sterility Testing: A Promising Approach in Regenerative Medicine	205
	Pankhi Vatsa, Sadaf Jahan, Uzair Ahmad Ansari, Andleeb Khan, Shabir Ahmad Mir, Bader Alshehri, Ranjay Kumar Choudhary, and Arif Jamal Siddiqui	
10	Stem Cells Storage, Packaging, and Transportation	233
	Ali Raza Ishaq, Shouwen Chen, Ayesha Noor, Rabia Batool, He Pengui, and Min Xiong	
11	Stem Cell Clinical Trials and Stem Cell Market	257
	Arindam Mitra	

About the Editor

Firdos Alam Khan is a Professor and Chairman at the Department of Stem Cell Biology, Institute for Research and Medical Consultations (IRMC), Imam Abdulrahman Bin Faisal University, Dammam, Saudi Arabia. He has obtained his Ph.D. in Zoology with a specialization in Neuroscience from Nagpur University, India. Over the past 24 years, Professor Khan is involved in teaching various courses such as Cell Biology, Pharmacology, Business of Biotechnology, Biomedicine, Cell & Tissue Engineering, and Bioethics & IPR to undergraduate and postgraduate students. He was previously associated with the Manipal Academy of Higher Education, Dubai Campus, United Arab Emirates. He was a Professor and Chairperson, School of Life Sciences and served as Chairman of Research & Development Program. He has published more than 75 research articles in several peer-reviewed journals.



Stem Cell Therapy: Significance and Applications of Stem Cell Products in Tissue Engineering and Regenerative Medicine

1

Kholoud Al Ghamdi

Abstract

This chapter concentrated mainly on the use of stem cells and their products in tissue engineering and regenerative medicine. It showed the promising therapeutic role of stem cells and their products for many chronic debilitating diseases. Combining the suitable stem cells/products with the suitable biomaterial to engineer the therapy of choice for certain diseases that has no cure so far with no or minimum side effects is the ultimate goal of TERM. Researchers all over the world are working in their laboratories and producing results on daily basis regarding the latest in this multidisciplinary field that requires collaboration of basic scientists, physicians, biomedical and software engineers and members of the community to come up with a solution for most/if not all the diseases affecting humanity.

Keywords

Stem cell therapy · Applications of stem cell products · Tissue engineering · Regenerative medicine

1.1 Tissue Engineering and Regenerative Medicine (TERM)

Tissue engineering and regenerative medicine (TERM) refers to the attempt to create functional human tissues from cells in a laboratory. Its aims to regenerate and/or repair tissues that had failed due to disease, genetic errors, congenital abnormalities,

K. Al Ghamdi (✉)

Department of Physiology, College of Medicine, Imam Abdulrahman Bin Faisal University, Dammam, Saudi Arabia

e-mail: ksaalghamdi@iau.edu.sa

© The Author(s), under exclusive license to Springer Nature Singapore Pte Ltd. 2022

F. A. Khan (ed.), *Stem Cell Production*,
https://doi.org/10.1007/978-981-16-7589-8_1

1

and traumatic injuries. There are four important factors that are essential for the success of tissue engineering: (1) suitable cells from different cell sources, (2) suitable environment (scaffolds) to support these cells, (3) suitable biomolecules to help these cells to grow and differentiate, and (4) certain mechanical and physical forces to influence the development of the cells. The cells can be harvested directly from the target organs, developed from precursors or stem cells, or taken from cell lines grown in the laboratories. Supporting structures can be derived from donors' tissues or from natural or synthesized polymers. Biomolecules can be added directly or secreted from the cells residing on the scaffolds. These scaffolds may dissolve over time, or some may remain to provide support to the organ. Mechanical and physical stimuli of the engineered tissues regulate their function (gene expression and consequently protein formation) and their eventual differentiation and development.

In order for us to understand the mechanism upon which TERM functions, we need to understand the physiology of cell growth and development. Cells are the building blocks for tissues and tissues make organs. A group of cells make their own extracellular matrix (ECM) where many interactions and cell signalling occur. Through understanding how cells respond to and interact with these signals to form tissues and organs, researchers can manipulate or interfere with these processes to fix damaged cells or even create new ones.

While tissue engineering is basically formation of tissues through a successful combination of cells, scaffolds, and biomolecules, regenerative medicine is a broader term where tissue engineering is combined with other strategies such as gene therapy, cellular therapy, and immunomodulation to induce tissue regeneration (Lysaght and Cramer 2009; Lindroos et al. 2011; Salgado et al. 2013; Porada et al. 2016).

TERM uses basically three strategies for it to reach its goal of establishing a three-dimensional (3D) cellular complex that resembles functioning tissues and is able to repair or regenerate damaged ones. These three strategies are: (1) cell systems such as stem cell transplantation, (2) cell-seeded biomaterials to be implanted in the body to repair or regenerate damaged tissues, (3) biomaterials to be implanted in the body to undergo the process of tissue integration.

1.2 Biomaterial Scaffolds as Essential Factors for the Success of TERM

Work is still in progress to optimize the best suitable biomaterial scaffolds to be used in tissue engineering from stem cells. However, this field is promising and there are many studies that proved the importance of these scaffolds in the success of stem cell therapy (Willerth and Sakiyama-Elbert 2008). They can enhance the differentiation and survival of the stem cells seeded in them. Scaffolds ideally should provide both cell support as well as the cues (chemical, mechanical, and biological) that are needed for these cells to respond to the environmental stimuli. There are many factors affecting the selection of the suitable scaffolds. First, the source of the biomaterial that the scaffold is manufactured from. Polymer scaffolds (in the form

of hydrogels, fibrous meshes, or porous sponges) are the most used and are divided into synthetic and natural (Wong and Mooney 1997). Natural polymers, including polysaccharides and proteins, promote good cell adhesion and growth and are biocompatible compared to synthetic ones. However, synthetic polymers are much more thermally stable and possess better mechanical properties. Lately, it has been shown that composite scaffolds made of a combination of two or more different phases of biomaterials overcome the above disadvantages of both synthetic and natural polymers (Sionkowska and Planecka 2013). Second, the scaffolds should have the suitable mechanical properties (porous) that allows the cells embedded in it to exchange vital molecules easily. On the same time, scaffolds should be strong/stiff enough to bare handling in vitro and in vivo and to mimic the structure of the extracellular matrix (Hutmacher 2000). Third, the scaffold should be biocompatible providing the optimal environment for cell growth, differentiation, and migration with the least undesirable effects (Williams 2008). Fourth, the method of fabrication of these biomaterials also plays an important role in the success of cell therapy and should be selected carefully based on the desired purpose (Yarlagadda et al. 2005). They can support stem cells even after they are being transplanted for a long period. These methods of fabrication include different patterns and networks such as hydrogels, microcarriers, and three-dimensional (3D) bio-printable constructs. In conclusion, ideal biomaterial scaffolds both support embedded cells' growth, differentiation and infiltration and closely mimic the extracellular matrix, allowing the embedded cells to interact with their native environment (Wang et al. 2018; Skop et al. 2014; Lim et al. 2019).

1.3 Stem Cells and Their Products as Essential Factors for the Success of TERM

Three main classes of stem cells can be used for TERM as well as progenitor cells and adult tissue-derived cells. Stem cells include organ resident, lineage specific stem cells, culture-adapted pluripotent stem cells, and multipotent cells capable of mesodermal differentiation known as mesenchymal stem cells (MSCs). Stem cells are a major source for cells in TERM because of their indefinite proliferation and differentiation potentials (Mahla 2016).

Among these stem cells, MSCs derived from bone marrow, adipose tissue, blood, and amniotic fluid are the most widely used in TERM (Fontaine et al. 2016; Schäfer et al. 2016; Bertheuil et al. 2019). It has been reported that MSCs have been used in almost 100,000 patients and more than 1000 clinical trials (see [ClinicalTrials.gov](https://www.clinicaltrials.gov)) for many diverse conditions such as autoimmune diseases, musculoskeletal defects, and myocardial infarctions.

Induced pluripotent stem cells (iPSCs) are adult somatic stem cells that underwent genetic reprogramming to embryonic stem cell-like state. This process was first successful by Japanese scientists (Takahashi and Yamanaka 2006). These embryonic stem cell-like cells have the capability to self-renew and differentiate and the privilege of being ethically acceptable. Initially iPSCs were genetically

reprogrammed from multipotent adult stem cells by retrovirally introducing four key transcription factors (Oct3/4, Sox2, Klf4, and c-Myc). Nowadays, more safe methods to introduce these factors to somatic cells have been developed and iPSCs are considered one of the major cell sources in TERM (Omole and Fakoya 2018; Malik and Rao 2013).

MSCs are of particular interest in TERM. In addition to their anti-inflammatory and immunomodulatory characteristics mentioned above, they exhibit many other features that make them superior to other stem cells when it comes to cell transplantation (Nasef et al. 2008; Williams et al. 2011; Bernardo and Fibbe 2013). First, they can differentiate multilinearly to many cells such as adipocytes, hepatocytes, osteoblasts, neuronal cells, chondrocytes, and myoblasts (Han et al. 2019). In addition to their ability to differentiate, they can be directly isolated from almost all adult and perinatal tissues as well as from solid organs (Kern et al. 2006; Pittenger et al. 1999; Marquez-Curtis et al. 2015; Ullah et al. 2015). A third important feature of MSCs is that they are less immunogenic compared to iPSCs and are rarely rejected by the host after transplantation since they do not express significant histocompatibility complexes and immune triggering molecules (Zhou et al. 2011). When transplanted, MSCs are also able to find and return to their original “niche/home” through their ability to respond to migratory stimuli from damaged areas (Karp and Leng Teo 2009; Andreas et al. 2014).

Historically, the first organ to be studied as a source of MSCs is the bone marrow and Arnold Caplan was the first researcher to grow MSCs from human tissues (Caplan 1991). Bone marrow-derived MSCs were cultured and differentiated to cells with osteogenic potentials (Haynesworth et al. 1992). Further studies demonstrated that the bone marrow was not the only source for MSCs, adipose tissue (Zuk et al. 2002; Rodriguez et al. 2005; Rodeheffer et al. 2008) and basically any vascularized tissue may have the potential to be a provider for the multipotent MSCs (Romanov et al. 2003; Mansilla et al. 2006; Zheng et al. 2007; Crisan et al. 2008). Of the above sources for MSCs, adipose tissue is evidenced to be more favourable since it is easy accessible, abundant, and less painful to extract. Adipose-derived mesenchymal stem cells (ADSCs) can be expanded for long periods in cultures without losing their differentiation capacity indicating high proliferative ability (Gimble et al. 2007; Mazini et al. 2019). ADSCs have been used in many pre-clinical and clinical trials as potential source for repairing and replacing damaged neuronal and bone cells for example (Mizuno 2010; Ruetze and Richter 2014; Im et al. 2013).

Due to the popularity of MSCs as a mean of treatment of certain diseases and the robust number of studies regarding their efficacy, the International Society for Cell and Gene Therapy (ISCT), formerly known as the International Society for Cellular Therapy, ought to put a certain definition for these cells to be biologically identified as MSCs in the laboratory for research purposes (Dominici et al. 2006). The following are the minimal criteria for identifying MSCs: Being plastic adherent fibroblastic cells with the potential to differentiate to osteogenic, chondrogenic, and adipogenic cells. Expressing the cell surface markers CD73, CD90, and

CD105, and not expressing haematopoietic and endothelial antigens (CD14 or CD11b, CD19 or CD79 α , CD34, CD45, HLA-DR).

Despite the popularity of MSCs and iPSCs in TERM, the exact mechanism for their cell-for-cell replacement is questionable. They recognized primarily as secretory and immunomodulatory agents (Sacchetti et al. 2007; Caplan 2017). It has been shown that their healing characteristics are mainly attributed to their paracrine action due to their ability to release extracellular vesicles (EVs) that play an important role in cell-to-cell communication and are directly involved in tissue regeneration (Barreca et al. 2020). Both MSCs and iPSCs exhibit the paracrine action through the release of EV. These vesicles act on neighbouring target cells by regulating their proliferation, viability, and survival. They also induce angiogenesis, downregulate proinflammatory cytokines, upregulate anti-inflammatory cytokines, and eventually reduce oxidative stress and further damage (Théry et al. 2018).

Extracellular vesicles are known to be released actively by almost all cell types in the body including stem cells, and they form an important mean for cell-to-cell communication via their secretory molecules along with cell adhesion molecules (CAMs) (Caby et al. 2005; Théry et al. 2006; Ratajczak et al. 2006; Turturici et al. 2014). They are classified based on their function and sizes to exosomes (<50 nm), membrane vesicles (50 nm–1 μ m) and apoptotic bodies (>1 μ m) (Colombo et al. 2014; György et al. 2011). EVs, based on their cell origin and internal and external stimuli, may contain proteins, nucleic acids, and bioactive lipids. They are coated by a lipid bilayer membrane that prevents them from being degraded by enzymes when released. However, many of these vesicles, when released, are broken down and some can reach their target cells and be engulfed through endocytosis, cell fusion or bound via ligand-receptor interaction after which they can exert their many actions on the target cells (Morel et al. 2004; Kupcova Skalnikova 2013; Candela et al. 2010).

1.4 Clinical Application of Stem Cells Therapy in TERM

1.4.1 Cardiovascular System

Stem cells and their products therapeutic potentials have been studied extensively in diseases of the cardiovascular system that are one of the main reasons for death worldwide. Shiba et al. (2012) showed that human ESCs-derived cardiomyocytes protected against arrhythmias and contracted synchronously with the guinea pig heart muscles. In addition, these ESCs-derived cardiomyocytes survived, matured, and enhanced the function of the infarcted heart in both mice (van Laake et al. 2007) and rats (Caspi et al. 2007; Laffamme et al. 2007).

iPSC is another source for cardiac cells. For example, both mouse (Ieda et al. 2010) and human (Fu et al. 2013; Wada et al. 2013) fibroblasts were reprogrammed into functioning induced cardiomyocytes-like cells (iCMs) in the presence of certain factors. Also, Menasché et al. (2008) injected autologous myoblasts along with coronary surgery in patients with ischemic cardiomyopathy. However, these cells

failed to improve the heart function and increased the number of early arrhythmias in the above patients. In another study from a homogenous sample conducted in one centre, Hagège et al. (2006) showed that skeletal myoblasts transplanted into a post myocardial infarction scar led to improvement of the heart function represented by increased left ventricular ejection fraction despite the arrhythmic risk that can be controlled through beta blockers.

Further, direct *in vivo* reprogramming of endogenous cardiac fibroblasts after injecting them with a reprogramming factor may convert them into iCMs internally. This shall decrease the tumorigenic risks of iPSCs and avoid cell transplantation in the future (Sadahiro et al. 2015).

Many studies lately showed that stem cells products released from extracellular vesicles (EVs) can overcome the pitfalls of cell-based therapy in cardiovascular diseases through inducing many cellular and molecular pathways (Pan et al. 2019; Arslan et al. 2013; Jiang et al. 2018). It has been shown that in some cases, MSCs products therapy is even of more effect compared to MSCs alone in reducing heart ischemia and enhancing cardiac repair (Lai et al. 2010; Shao et al. 2017). In addition, iPSC-EVs have demonstrated an important role in cardiovascular diseases protection (Feng et al. 2014). It has been shown *in vitro* that iPSCs products when infused to cardiomyocytes, can be protective against oxidative stress and can prevent cardiac cells apoptosis when transplanted *in vivo* (Wang et al. 2015). This is mainly attributed the iPSC-EVs miRNA content, precisely, CPCISX-9-derived EVs that have been shown to reduce fibrosis and promote angiogenesis in myocardial infarction (Xuan et al. 2019).

Scaffolds infused with stem cells and/or their products has been used in cardiovascular diseases lately. For example, iPSC-derived cardiomyocytes EVs were encapsulated in hydrogel patches. These engineered hydrogel patches slowly released their products after being implanted onto rats' hearts with myocardial infarction. Consequently, these rats showed improvement in their heart functions manifested both physiologically and anatomically (Liu et al. 2018). Furthermore, Wei et al. (2019) implanted heparinized electrospun polycaprolactone vascular grafts loaded with MSC-derived EVs into a segment of rat abdominal artery. These cell products loaded grafts were successful in enhancing the patency of the vessels in a rat model of hyperlipidemia by inhibiting thrombosis and calcifications.

1.4.2 Lungs

Repairing injured lung tissues by stem cells or their products has been an area of interest for many TERM scientists lately. It has been shown that MSCs derived from bone marrow, umbilical cord, and menstrual blood were effective in reducing fibrosis in many models of airway and lung injuries in rats and mice (Moodley et al. 2009; Kumamoto et al. 2009; Leblond et al. 2009; Xiang et al. 2017).

In addition to the direct effect of stem cell therapy on respiratory diseases, MSC-EVs have shown protective effects in various models of respiratory injuries. For example, MSC-EVs were successful in reducing hyperproliferating pathways in

the murine model of respiratory hypertension (Lee et al. 2012a, b, c). Also, Tang et al. (2017) showed that MSC-EVs, partially through their miRNA content, exerted an anti-inflammatory effect on a model of acute lung injury in mice. Potter et al. (2018) showed that although both MSCs and their products exert protective effects on the lungs, the mechanism of their molecular actions differs widely.

Extracellular vesicles from iPSC also showed important effects in lung diseases such as pulmonary fibrosis both in vitro and in vivo. iPSC-EVs containing antifibrotic hepatocyte growth factor increased the alveolar epithelial wound repair in vitro. It also attenuated fibrosis and collagen content in a model of acute lung injury in the rat after being administered intratracheally (Gazdhar et al. 2014).

1.4.3 Nerves

It is of urgent need to find cure to many chronic debilitating neurological disorders. TERM plays an important role in leading studies of such purpose (Lindvall and Kokaia 2006). Alzheimer's disease for example is a progressive neurological disorder characterized pathologically by deposition of amyloid beta plaques and neurofibrillary tangles made of tau proteins. Several studies have shown the effect of MSCs derived from bone marrow, umbilical cord, adipose tissue, or menstrual blood on improving both cognitive function and Alzheimer's disease pathology. These cells when injected intracerebrally in animal models of Alzheimer's disease, improved both functional (better spatial learning and memory) and neuropathological (fewer amyloid plaques and tau phosphorylation) aspects of the disease and this improvement is mostly attributed to their action on the microglial cells in the brain (Zhao et al. 2018; Lee and Landreth 2010; Lee et al. 2012a, b, c). Multiple sclerosis (MS) is another autoimmune neurological disorder characterized by relapses and remissions of its symptoms. Burt et al. (2009) reported that the use of autologous non-myeloablative haematopoietic stem cells in the relapsing remitting phase of MS was successful in reversing the neurological disability and sustained improvement. A third chronic disease of no cure so far is Parkinson's disease. It has been reported that after transplanting undifferentiated mouse ESCs into the striatum of rat model of Parkinson's disease, these cells differentiated into functioning dopaminergic neurons that restored the motor and behavioural functions in these rats (Bjorklund et al. 2002).

In addition to the cellular therapeutic effects of stem cells, their paracrine effects have been studied extensively lately in neurological diseases. Stem cell products are of particular importance in neurological disorders compared to stem cells themselves. MSC-EVs can cross the blood-brain barrier because of their lipid structure, hence they are able to reach to many targets in the brain. They are also able to last longer in the target tissues since they are less immunogenic and less detectable by the host immune system (Phinney and Pittenger 2017). Stroke is one of the most common neurological diseases that has low full recovery rates. In a rat model of stroke, Moon et al. (2019) compared rats treated with MSC-EVs to those treated with MSCs. MSC-EVs treated rats showed behavioural improvement. EVs were

concentrated in the target area and was less found in unwanted areas (lung and liver). EVs also contained the specific needed factors for angiogenesis and neurogenesis, concluding that MSC-EVs can be of superiority regarding safety and effectiveness when compared to cellular treatment for stroke.

Traumatic brain injury (TBI) is a common cause of morbidity and mortality worldwide, and there is an urgent need to develop treatments that prevent or decrease the progression of a secondary injury in TBI. It has been reported that MSC-EVs play an important role in reducing both apoptosis and cognitive impairment and promoting cortical vascular epithelial growth factor expression in rodents' models of TBI (Chuang et al. 2012; Kim et al. 2016). Furthermore, Patel et al. (2018) studied the effects of human adipose-derived stem cells (hASCs)-EVs on the prognosis of a rat model of TBI. EVs succeeded in upregulating anti-inflammatory molecules and downregulating proinflammatory molecules at the genomic level as well as in improving motor behaviours. Furthermore, Tsuji et al. (2019) group in Japan is currently preparing for their first human trial study involving the use of iPSC-derived neural precursor cells in the treatment of spinal cord injuries (SCIs). Also, the usefulness of MSC-EVs in peripheral nerve injury has been studied and a group of researchers found that these vesicles secreted from gingiva-derived MSCs promoted nerve growth and regeneration through partially repairing Schwann cells of injured mice sciatic nerves (Mao et al. 2019).

Genetically engineered scaffolds to treat neurological disorders especially SCIs gained attention lately. For example, a neurotrophin-3-containing chitosan-based scaffold, developed by Li et al. (2009), improved sensory and motor recovery in a rat model of SCI. This scaffold provided suitable environment to facilitate the activation of endogenous neural stem cells and decrease inflammation post injury (Duan et al. 2015). In another study of a rat model of SCI, Zeng et al. (2011) seeded 3D gelatin scaffolds with MSCs derived from bone marrow and transplanted them into a transected rat spinal cord. Few weeks later, the results showed that these scaffolds were biocompatible, and the seeded cells were able to reduce cavity formation, induce angiogenesis, and decrease inflammation. In another study, fibronectin secreted from MSC-derived neuron like cells engineered in gelatin sponge scaffolds succeeded at early stages in promoting neurite elongation of neural differentiating MSCs *in vitro*, concluding the potential future use of fibronectin in the treatment of SCIs (Zeng et al. 2016). In conclusion, the combination of scaffolds and stem cell products boasts their therapeutic effects in tackling debilitating neurological disorders.

1.4.4 Bone

Therapeutic combination of engineered biomaterials along with stem cell products to enhance bone growth and treat bone disorders has been experimented widely lately and gained much of attention. For example, a study by Xie et al. (2017) embedded bone marrow-derived MSC-EVs to decalcified bone matrix. These vesicles showed prior pro-angiogenic and pro-bone regeneration potentials *in vitro*. The modified EV

scaffolds were then evaluated in a bone formation model in mice, and they managed to enhance bone formation through promoting vascularization in the graft. Another study combined hADSC-EVs with poly lactic-co-glycolic acid scaffolds to produce a novel cell-free tissue engineered bone. The above engineered scaffolds enhanced bone restoration significantly in critical-sized calvarial defects in the mouse (Li et al. 2018). In addition, Kikuchi et al. (2018) showed that neural crest-like cells (NCLCs) derived from iPSCs were able to differentiate in vitro to dental MSCs that eventually gave rise to odontoblasts and dental pulp cells. These iPSC-NCLC-MSCs when transplanted into critical-sized calvarial defects in mice were able to differentiate into osteoblasts leading to bone regeneration without formation of any tumours. Ren et al. (2012) genetically modified hADSCs decreasing their expression of MHC I protein, hence decreasing their immunogenicity, and constructed tissue engineered bone that succeeded in repairing bone defects in pigs. This data shows promising results of the safe usage of genetically modified allogenic bone grafts as compared to autologous ones.

1.4.5 Cartilage

Repairing damaged cartilage is still not yet fully accomplished. However, engineered cartilage constructs containing suitable cells or cell products show promising therapeutic results. Park et al. (2017) reported the results of the first human clinical trial investigating the efficacy and safety of Cartistem (a composite of allogeneic human umbilical cord blood-derived MSCs and hyaluronic acid hydrogel) on osteoarthritic patients. Twelve weeks after the application of Cartistem to the lesion site, there were signs of cartilage repair seen arthroscopically. In addition, patient's pain scores on walking were decreased and no teratogenicity were observed 7 years after the trial. In addition, Li et al. (2017) seeded biodegradable scaffolds with bone marrow-derived MSCs forming engineered cartilage. This engineered cartilage was then wrapped with chondrocyte sheets and implanted into nude mice. It then showed typical cartilaginous features and succeeded in regenerating subcutaneous cartilage. Another study showed that after seeding ear-shaped biodegradable scaffold with both autologous microtia chondrocytes (25%) and bone marrow-derived MSCs (75%) and implanting the scaffold into a nude mouse, a human ear-shaped cartilage tissue with proper elasticity was constructed (Zhang et al. 2014a, b). Park et al. (2019) reported that in comparison to chondrogenic differentiated MSCs, undifferentiated MSCs were more effective in repairing critical-sized osteochondral defects in the rat femur, supporting the use of undifferentiated MSCs in cellular therapy for cartilage repair.

1.4.6 Tendon/Ligaments

Anterior cruciate ligaments tears and Achilles tendon defects are common sports injuries affecting many young age groups worldwide. Engineered tissues targeting

the repair of tendons and ligaments are under trials. A group of researchers succeeded in engineering functional ligaments analogues by assembling bone marrow-derived MSCs sheets on a knitted poly (L-lactide) scaffold (Ouyang et al. 2005) that can be used for the purpose of anterior cruciate ligaments repairs. In addition, Chen et al. (2009) reported that human ESCs-derived MSCs regenerated tendon tissues both in vitro and in vivo (patellar tendon regeneration model in rats) by secreting human foetal tendon-specific matrix and certain differentiation factors, indicating great potential for the use of ESCs in tendon injuries repairs. Another study reported enhanced tendon regeneration effects of tissue engineered construct composed of knitted silk-collagen sponge scaffold seeded with human ESCs-derived MSCs when exposed to appropriate mechanical stimulation, both in vitro and in vivo (Chen et al. 2010). In addition to the use of ESCs for tendon regeneration, Liu et al. (2017) reported that uniparental parthenogenetic stem cells can spontaneously differentiate to parthenogenetic MSCs that can be differentiated to functional tenocytes after their exposure to mechanical stretch. These tenocytes can be embedded on the surface of poly (lactic-co-glycolic) acid scaffolds and used for tissue engineered tendon regeneration.

1.4.7 Liver

Liver failure is a debilitating disease that decreases the patients' life expectancy if not treated by liver transplantation. Scientists are trying to find a cure for those who are not able to receive liver transplants possibly through tissue engineering. Chen et al. (2017) investigated the therapeutic effect of human menstrual blood-derived MSC-EVs on a mouse model of fulminant hepatic failure. It was reported that EVs (exosomes) expressed cytokines that markedly improved liver function and inhibited hepatic cells apoptosis leading to overall hepatoprotective activity.

1.4.8 Urinary System

Diseases of the urinary system can affect a wide range of organs including kidneys, ureters, urinary bladder, and urethra. Cell therapy has been initiated in some urinary system organs disorders. For example, it has been reported that urine-derived stem cells, discovered by Bharadwaj et al. (2013), have proliferative and multipotent potentials and can be used as a cell source in urinary tract reconstruction (Zhang et al. 2014a). In addition, ADSCs have been used to improve voiding dysfunction in animal models of hyperactive or hypoactive urinary bladder of different aetiologies (Mousa et al. 2015). Also, Wu et al. (2019) reported that urine-derived stem cells exosomes played an important role in improving stress urinary incontinence in a rat model of the disease by enhancing both the activation of muscle stellate cells and the phosphorylation of extracellular-regulated protein kinases (ERK).

MSC-EVs showed a therapeutic effect subsiding acute and/or chronic kidney injury through various vesicles products that succeeded in reducing inflammation,

migration of macrophages, preventing cell death, promoting wound healing, and stimulating angiogenesis (Gatti et al. 2011; Bruno et al. 2017; Zou et al. 2016). Also, Lee et al. (2012a, b, c) reported that the intrarenal administration of iPSCs into a rat model of acute kidney injury, at a particular dose, attenuated tubular injury and improved renal function. Moreover, it has been recently shown the iPSC-EVs reduced cell death and inflammatory responses in an *in vivo* model of ischemia-reperfusion kidney injury. These vesicles also protected the mitochondria and reduced oxidative stress, suggesting that iPSCs can be a potential source of these protective vesicles in kidney disease (Collino et al. 2020).

1.4.9 Cornea

Corneal endothelium dysfunction is a leading cause of blindness worldwide and there is an urgent need to find a cure for this disease, especially with the scarce number of corneal donors and the high rejection rates. The major function of this single layered corneal endothelium is to prevent the corneal oedema. Recently, Yamashita et al. (2018) reported that human umbilical cord-derived MSCs were able to differentiate to corneal endothelial-like cells expressing major endothelial corneal markers. These corneal endothelial-like cells were able to maintain corneal thickness and transparency when transplanted into a rabbit model of keratopathy, suggesting the potential use of these cells in corneal endothelial disease. In addition, Wu et al. (2014) reconstructed highly proliferative tissue engineered lamellar cornea by embedding a combination of corneal epithelial cells and genetically modified ESCs into an acellular porcine corneal stroma and amniotic membrane. The transplantation of the engineered lamellar cornea into the rabbit accelerated wound healing and manifested epithelial barrier function, indicating that the above combination of cells could form a base for corneal tissue engineering.

1.4.10 Skin

Skin is exposed to many external environmental factors all the time. This makes it vulnerable to diseases. Stem cells and their products can be of therapeutic benefit in skin-related diseases. For example, Lataillade et al. (2007) combined surgical therapy with bone marrow-derived MSCs in a patient who was accidentally exposed to high dose of radiation. MSCs were infused at the site of necrotic ulcer/burn and successfully improved healing of the ulcer mainly through the secretion of anti-inflammatory mediators. In addition, it has been reported that bone marrow-derived MSCs, via their anti-inflammatory mediators' effects, reduced inflammation and promoted healing in a pig model of radiation-induced burn. They also promoted muscle growth and revascularization in severe cases where deeper muscular tissues were affected (Linard et al. 2018). Another important skin problem is UV light induced skin ageing (photoageing). Oh et al. (2018) studied the effect of iPSC-EVs on aged human dermal fibroblasts and showed that these vesicles (exosomes)

exerted a skin protective role against photoageing. Another important function of the skin is wound healing. However, this function is affected by the overall status of the body. For example, Shi et al. (2017) engineered a scaffold by seeding gingival MSCs onto a chitosan/silk hydrogel sponge and used it to cover a skin wound in a diabetic rat skin defect model. The above combination effectively speeded up skin wound healing (that was originally delayed as an effect of diabetes on these rats) via enhancing both angiogenesis and re-epithelialization. Since cutaneous wound healing can lead to scar formation, Liu et al. (2014) demonstrated a reducing post wound healing scar formation effect of bone marrow-derived MSCs and attributed it to the anti-inflammatory properties of these cells and their mediators. It was also found that the inflammatory regulatory abilities are surprisingly in parallel with the apoptosis of the above cells. Moreover, the effect of a combination of fibroblasts and ADSCs embedded in a 3D tissue engineered skin system on epidermal morphogenesis has been studied. Results showed that having a mixture of cells in the engineered skin improved its epidermal morphogenesis manifested by the extensive proliferation of keratinocytes over the mixture compared to the lower proliferation seen in single mesenchymal cell types (Lu et al. 2012).

1.5 Future Perspective of Stem Cells and Their Products in TERM

This chapter concentrated mainly on the use of stem cells and their products in tissue engineering and regenerative medicine. It showed the promising therapeutic role of stem cells and their products for many chronic debilitating diseases. Combining the suitable stem cells/products with the suitable biomaterial to engineer the therapy of choice for certain diseases that has no cure so far with no or minimum side effects is the ultimate goal of TERM. Researchers all over the world are working in their laboratories and producing results on daily basis regarding the latest in this multidisciplinary field that requires collaboration of basic scientists, physicians, biomedical and software engineers and members of the community to come up with a solution for most/if not all the diseases affecting humanity.

References

- Andreas K, Sittinger M, Ringe J (2014) Toward in situ tissue engineering: chemokine-guided stem cell recruitment. *Trends Biotechnol* 32(9):483–492. <https://doi.org/10.1016/j.tibtech.2014.06.008>
- Arslan F, Lai RC, Smeets MB, Akeroyd L, Choo A, Aguor EN, Timmers L, van Rijen HV, Doevendans PA, Pasterkamp G, Lim SK, de Kleijn DP (2013) Mesenchymal stem cell-derived exosomes increase ATP levels, decrease oxidative stress and activate PI3K/Akt pathway to enhance myocardial viability and prevent adverse remodeling after myocardial ischemia/reperfusion injury. *Stem Cell Res* 10(3):301–312. <https://doi.org/10.1016/j.scr.2013.01.002>

- Barreca MM, Cancemi P, Geraci F (2020) Mesenchymal and induced pluripotent stem cells-derived extracellular vesicles: the new frontier for regenerative medicine? *Cell* 9(5):1163. <https://doi.org/10.3390/cells9051163>
- Bernardo ME, Fibbe WE (2013) Mesenchymal stromal cells: sensors and switchers of inflammation. *Cell Stem Cell* 13(4):392–402. <https://doi.org/10.1016/j.stem.2013.09.006>
- Bertheuil N, Chaput B, Ménard C, Varin A, Laloze J, Watier E, Tarte K (2019) Adipose mesenchymal stromal cells: definition, immunomodulatory properties, mechanical isolation and interest for plastic surgery. *Ann Chir Plast Esthet* 64(1):1–10. <https://doi.org/10.1016/j.anplas.2018.07.005>
- Bharadwaj S, Liu G, Shi Y, Wu R, Yang B, He T, Fan Y, Lu X, Zhou X, Liu H, Atala A, Rohozinski J, Zhang Y (2013) Multipotential differentiation of human urine-derived stem cells: potential for therapeutic applications in urology. *Stem Cells* (Dayton, Ohio) 31(9):1840–1856. <https://doi.org/10.1002/stem.1424>
- Bjorklund LM, Sánchez-Pernaute R, Chung S, Andersson T, Chen IY, McNaught KS, Brownell AL, Jenkins BG, Wahlestedt C, Kim KS, Isacson O (2002) Embryonic stem cells develop into functional dopaminergic neurons after transplantation in a Parkinson rat model. *Proc Natl Acad Sci U S A* 99(4):2344–2349. <https://doi.org/10.1073/pnas.022438099>
- Bruno S, Tapparo M, Collino F, Chiabotto G, Deregibus MC, Soares Lindoso R, Neri F, Kholia S, Giunti S, Wen S, Quesenberry P, Camussi G (2017) Renal regenerative potential of different extracellular vesicle populations derived from bone marrow mesenchymal stromal cells. *Tissue Eng Part A* 23(21–22):1262–1273. <https://doi.org/10.1089/ten.TEA.2017.0069>
- Burt RK, Loh Y, Cohen B, Stefoski D, Balabanov R, Katsamakis G, Oyama Y, Russell EJ, Stern J, Muraro P, Rose J, Testori A, Bucha J, Jovanovic B, Milanetti F, Storek J, Voltarelli JC, Burns WH (2009) Autologous non-myeloablative haemopoietic stem cell transplantation in relapsing-remitting multiple sclerosis: a phase I/II study. *Lancet Neurol* 8(3):244–253. [https://doi.org/10.1016/S1474-4422\(09\)70017-1](https://doi.org/10.1016/S1474-4422(09)70017-1)
- Caby MP, Lankar D, Vincendeau-Scherrer C, Raposo G, Bonnerot C (2005) Exosomal-like vesicles are present in human blood plasma. *Int Immunol* 17(7):879–887. <https://doi.org/10.1093/intimm/dxh267>
- Candela ME, Geraci F, Turturici G, Taverna S, Albanese I, Sconzo G (2010) Membrane vesicles containing matrix metalloproteinase-9 and fibroblast growth factor-2 are released into the extracellular space from mouse mesoangioblast stem cells. *J Cell Physiol* 224:144–151. <https://doi.org/10.1002/jcp.22111>
- Caplan AI (1991) Mesenchymal stem cells. *J Orthop Res* 9(5):641–650. <https://doi.org/10.1002/jor.1100090504>
- Caplan AI (2017) Mesenchymal stem cells: time to change the name! *Stem Cells Transl Med* 6(6):1445–1451. <https://doi.org/10.1002/sctm.17-0051>
- Caspi O, Huber I, Kehat I, Habib M, Arbel G, Gepstein A, Yankelson L, Aronson D, Beyar R, Gepstein L (2007) Transplantation of human embryonic stem cell-derived cardiomyocytes improves myocardial performance in infarcted rat hearts. *J Am Coll Cardiol* 50(19):1884–1893. <https://doi.org/10.1016/j.jacc.2007.07.054>
- Chen X, Song XH, Yin Z, Zou XH, Wang LL, Hu H, Cao T, Zheng M, Ouyang HW (2009) Stepwise differentiation of human embryonic stem cells promotes tendon regeneration by secreting fetal tendon matrix and differentiation factors. *Stem Cells* (Dayton, Ohio) 27(6):1276–1287. <https://doi.org/10.1002/stem.61>
- Chen JL, Yin Z, Shen WL, Chen X, Heng BC, Zou XH, Ouyang HW (2010) Efficacy of hESC-MSCs in knitted silk-collagen scaffold for tendon tissue engineering and their roles. *Biomaterials* 31(36):9438–9451. <https://doi.org/10.1016/j.biomaterials.2010.08.011>
- Chen L, Xiang B, Wang X, Xiang C (2017) Exosomes derived from human menstrual blood-derived stem cells alleviate fulminant hepatic failure. *Stem Cell Res Ther* 8:9. <https://doi.org/10.1186/s13287-016-0453-456>

- Chuang TJ, Lin KC, Chio CC, Wang CC, Chang CP, Kuo JR (2012) Effects of secretome obtained from normoxia-preconditioned human mesenchymal stem cells in traumatic brain injury rats. *J Trauma Acute Care Surg* 73(5):1161–1167. <https://doi.org/10.1097/TA.0b013e318265d128>
- Collino F, Lopes JA, Tapparo M, Tortelote GG, Kasai-Brunswick TH, Lopes G, Almeida DB, Skovronova R, Wendt C, Miranda KR, Bussolati B, Vieyra A, Lindoso RS (2020) Extracellular vesicles derived from induced pluripotent stem cells promote renoprotection in acute kidney injury model. *Cell* 9(2):453. <https://doi.org/10.3390/cells9020453>
- Colombo M, Raposo G, Théry C (2014) Biogenesis, secretion, and intercellular interactions of exosomes and other extracellular vesicles. *Annu Rev Cell Dev Biol* 30:255–289. <https://doi.org/10.1146/annurev-cellbio-101512-122326>
- Crisan M, Yap S, Casteilla L, Chen CW, Corselli M, Park TS, Andriolo G, Sun B, Zheng B, Zhang L, Norotte C, Teng PN, Traas J, Schugar R, Deasy BM, Badylak S, Buhning HJ, Giacobino JP, Lazzari L, Huard J et al (2008) A perivascular origin for mesenchymal stem cells in multiple human organs. *Cell Stem Cell* 3(3):301–313. <https://doi.org/10.1016/j.stem.2008.07.003>
- Dominici M, Le Blanc K, Mueller I, Slaper-Cortenbach I, Marini F, Krause D, Deans R, Keating A, Prockop D J, Horwitz E (2006) Minimal criteria for defining multipotent mesenchymal stromal cells. The international society for cellular therapy position statement. *Cytotherapy* 8(4): 315–317. <https://doi.org/10.1080/14653240600855905>
- Duan H, Ge W, Zhang A, Xi Y, Chen Z, Luo D, Cheng Y, Fan KS, Horvath S, Sofroniew MV, Cheng L, Yang Z, Sun YE, Li X (2015) Transcriptome analyses reveal molecular mechanisms underlying functional recovery after spinal cord injury. *Proc Natl Acad Sci U S A* 112(43): 13360–13365. <https://doi.org/10.1073/pnas.1510176112>
- Feng Y, Huang W, Wani M, Yu X, Ashraf M (2014) Ischemic preconditioning potentiates the protective effect of stem cells through secretion of exosomes by targeting Mecp2 via miR-22. *PLoS One* 9(2):e88685. <https://doi.org/10.1371/journal.pone.0088685>
- Fontaine MJ, Shih H, Schäfer R, Pittenger MF (2016) Unraveling the mesenchymal stromal cells' paracrine immunomodulatory effects. *Transfus Med Rev* 30:37–43. <https://doi.org/10.1016/j.tmr.2015.11.004>
- Fu JD, Stone NR, Liu L, Spencer CI, Qian L, Hayashi Y, Delgado-Olguin P, Ding S, Bruneau BG, Srivastava D (2013) Direct reprogramming of human fibroblasts toward a cardiomyocyte-like state. *Stem Cell Rep* 1(3):235–247. <https://doi.org/10.1016/j.stemcr.2013.07.005>
- Gatti S, Bruno S, Deregibus MC, Sordi A, Cantaluppi V, Tetta C, Camussi G (2011) Microvesicles derived from human adult mesenchymal stem cells protect against ischaemia-reperfusion-induced acute and chronic kidney injury. *Nephrol Dial Transplant Official Publ Eur Dial Transplant Assoc Eur Renal Assoc* 26(5):1474–1483. <https://doi.org/10.1093/ndt/gfr015>
- Gazdhar A, Grad I, Tamò L, Gugger M, Feki A, Geiser T (2014) The secretome of induced pluripotent stem cells reduces lung fibrosis in part by hepatocyte growth factor. *Stem Cell Res Ther* 5(6):123. <https://doi.org/10.1186/scrt513>
- Gimble JM, Katz AJ, Bunnell BA (2007) Adipose-derived stem cells for regenerative medicine. *Circ Res* 100(9):1249–1260. <https://doi.org/10.1161/01.RES.0000265074.83288.09>
- György B, Szabó TG, Pásztói M, Pál Z, Misják P, Aradi B, László V, Pállinger E, Pap E, Kittel A, Nagy G, Falus A, Buzás EI (2011) Membrane vesicles, current state-of-the-art: emerging role of extracellular vesicles. *Cell Mol Life Sci CMLS* 68(16):2667–2688. <https://doi.org/10.1007/s00018-011-0689-3>
- Hagège AA, Marolleau JP, Vilquin JT, Alhérière A, Peyrard S, Duboc D, Abergel E, Messas E, Mousseaux E, Schwartz K, Desnos M, Menasché P (2006) Skeletal myoblast transplantation in ischemic heart failure: long-term follow-up of the first phase I cohort of patients. *Circulation* 114(1 Suppl):I108–I113. <https://doi.org/10.1161/CIRCULATIONAHA.105.000521>
- Han Y, Li X, Zhang Y, Han Y, Chang F, Ding J (2019) Mesenchymal stem cells for regenerative medicine. *Cell* 8(8):886. <https://doi.org/10.3390/cells8080886>

- Haynesworth SE, Goshima J, Goldberg VM, Caplan AI (1992) Characterization of cells with osteogenic potential from human marrow. *Bone* 13(1):81–88. [https://doi.org/10.1016/8756-3282\(92\)90364-3](https://doi.org/10.1016/8756-3282(92)90364-3)
- Hutmacher DW (2000) Scaffolds in tissue engineering bone and cartilage. *Biomaterials* 21(24): 2529–2543. [https://doi.org/10.1016/s0142-9612\(00\)00121-6](https://doi.org/10.1016/s0142-9612(00)00121-6)
- Ieda M, Fu JD, Delgado-Olguin P, Vedantham V, Hayashi Y, Bruneau BG, Srivastava D (2010) Direct reprogramming of fibroblasts into functional cardiomyocytes by defined factors. *Cell* 142(3):375–386. <https://doi.org/10.1016/j.cell.2010.07.002>
- Im W, Ban J, Lim J, Lee M, Lee ST, Chu K, Kim M (2013) Extracts of adipose derived stem cells slows progression in the R6/2 model of Huntington's disease. *PLoS One* 8(4):e59438. <https://doi.org/10.1371/journal.pone.0059438>
- Jiang W, Tan Y, Cai M, Zhao T, Mao F, Zhang X, Xu W, Yan Z, Qian H, Yan Y (2018) Human umbilical cord MSC-derived exosomes suppress the development of CCl4-induced liver injury through antioxidant effect. *Stem Cells Int* 2018:6079642. <https://doi.org/10.1155/2018/6079642>
- Karp JM, Leng Teo GS (2009) Mesenchymal stem cell homing: the devil is in the details. *Cell Stem Cell* 4(3):206–216. <https://doi.org/10.1016/j.stem.2009.02.001>
- Kern S, Eichler H, Stoeve J, Klüter H, Bieback K (2006) Comparative analysis of mesenchymal stem cells from bone marrow, umbilical cord blood, or adipose tissue. *Stem Cells* (Dayton, Ohio) 24(5):1294–1301. <https://doi.org/10.1634/stemcells.2005-0342>
- Kikuchi K, Masuda T, Fujiwara N, Kuji A, Miura H, Jung HS et al (2018) Craniofacial bone regeneration using iPS cell-derived neural crest like cells. *J Hard Tissue Biol* 27:1–10. <https://doi.org/10.2485/jhtb.27.1>
- Kim DK, Nishida H, An SY, Shetty AK, Bartosh TJ, Prockop DJ (2016) Chromatographically isolated CD63+CD81+ extracellular vesicles from mesenchymal stromal cells rescue cognitive impairments after TBI. *Proc Natl Acad Sci U S A* 113(1):170–175. <https://doi.org/10.1073/pnas.1522297113>
- Kumamoto M, Nishiwaki T, Matsuo N, Kimura H, Matsushima K (2009) Minimally cultured bone marrow mesenchymal stem cells ameliorate fibrotic lung injury. *Eur Respir J* 34(3):740–748. <https://doi.org/10.1183/09031936.00128508>
- Kupcova Skalnikova H (2013) Proteomic techniques for characterisation of mesenchymal stem cell secretome. *Biochimie* 95(12):2196–2211. <https://doi.org/10.1016/j.biochi.2013.07.015>
- Laffamme MA, Chen KY, Naumova AV, Muskheli V, Fugate JA, Dupras SK, Reinecke H, Xu C, Hassanipour M, Police S, O'Sullivan C, Collins L, Chen Y, Minami E, Gill EA, Ueno S, Yuan C, Gold J, Murry CE (2007) Cardiomyocytes derived from human embryonic stem cells in pro-survival factors enhance function of infarcted rat hearts. *Nat Biotechnol* 25(9):1015–1024. <https://doi.org/10.1038/nbt1327>
- Lai RC, Arslan F, Lee MM, Sze NS, Choo A, Chen TS, Salto-Tellez M, Timmers L, Lee CN, El Oakley RM, Pasterkamp G, de Kleijn DP, Lim SK (2010) Exosome secreted by MSC reduces myocardial ischemia/reperfusion injury. *Stem Cell Res* 4(3):214–222. <https://doi.org/10.1016/j.scr.2009.12.003>
- Lataillade JJ, Doucet C, Bey E, Carsin H, Huet C, Clairand I, Bottollier-Depois JF, Chapel A, Ernou I, Gourven M, Boutin L, Hayden A, Carcamo C, Buglova E, Joussemet M, de Revel T, Gourmelon P (2007) New approach to radiation burn treatment by dosimetry-guided surgery combined with autologous mesenchymal stem cell therapy. *Regen Med* 2(5):785–794. <https://doi.org/10.2217/17460751.2.5.785>
- Leblond AL, Naud P, Forest V, Gourden C, Sagan C, Romefort B, Mathieu E, Delorme B, Collin C, Pagès JC, Sensebé L, Pitard B, Lemarchand P (2009) Developing cell therapy techniques for respiratory disease: intratracheal delivery of genetically engineered stem cells in a murine model of airway injury. *Hum Gene Ther* 20(11):1329–1343. <https://doi.org/10.1089/hum.2009.035>
- Lee CY, Landreth GE (2010) The role of microglia in amyloid clearance from the AD brain. *J Neural Transm* (Vienna, Austria: 1996) 117(8):949–960. <https://doi.org/10.1007/s00702-010-0433-4>

- Lee C, Mitsialis SA, Aslam M, Vitali SH, Vergadi E, Konstantinou G, Sdrimas K, Fernandez-Gonzalez A, Kourembanas S (2012a) Exosomes mediate the cytoprotective action of mesenchymal stromal cells on hypoxia-induced pulmonary hypertension. *Circulation* 126(22):2601–2611. <https://doi.org/10.1161/CIRCULATIONAHA.112.114173>
- Lee PY, Chien Y, Chiou GY, Lin CH, Chiou CH, Tarng DC (2012b) Induced pluripotent stem cells without c-Myc attenuate acute kidney injury via downregulating the signaling of oxidative stress and inflammation in ischemia-reperfusion rats. *Cell Transplant* 21(12):2569–2585. <https://doi.org/10.3727/096368912X636902>
- Lee HJ, Lee JK, Lee H, Carter JE, Chang JW, Oh W, Yang YS, Suh JG, Lee BH, Jin HK, Bae JS (2012c) Human umbilical cord blood-derived mesenchymal stem cells improve neuropathology and cognitive impairment in an Alzheimer's disease mouse model through modulation of neuroinflammation. *Neurobiol Aging* 33(3):588–602. <https://doi.org/10.1016/j.neurobiolaging.2010.03.024>
- Li X, Yang Z, Zhang A, Wang T, Chen W (2009) Repair of thoracic spinal cord injury by chitosan tube implantation in adult rats. *Biomaterials* 30(6):1121–1132. <https://doi.org/10.1016/j.biomaterials.2008.10.063>
- Li D, Zhu L, Liu Y, Yin Z, Liu Y, Liu F, He A, Feng S, Zhang Y, Zhang Z, Zhang W, Liu W, Cao Y, Zhou G (2017) Stable subcutaneous cartilage regeneration of bone marrow stromal cells directed by chondrocyte sheet. *Acta Biomater* 54:321–332. <https://doi.org/10.1016/j.actbio.2017.03.031>
- Li W, Liu Y, Zhang P, Tang Y, Zhou M, Jiang W, Zhang X, Wu G, Zhou Y (2018) Tissue-engineered bone immobilized with human adipose stem cells-derived exosomes promotes bone regeneration. *ACS Appl Mater Interfaces* 10(6):5240–5254. <https://doi.org/10.1021/acsami.7b17620>
- Lim KS, Baptista M, Moon S, Woodfield TBF, Rnjak-Kovacina J (2019) Microchannels in development, survival, and vascularisation of tissue analogues for regenerative medicine. *Trends Biotechnol* 37(11):1189–1201. <https://doi.org/10.1016/j.tibtech.2019.04.004>
- Linard C, Brachet M, L'homme B, Strup-Perrot C, Busson E, Bonneau M, Lataillade JJ, Bey E, Benderitter M (2018) Long-term effectiveness of local BM-MSCs for skeletal muscle regeneration: a proof of concept obtained on a pig model of severe radiation burn. *Stem Cell Res Ther* 9(1):299. <https://doi.org/10.1186/s13287-018-1051-6>
- Lindroos B, Suuronen R, Miettinen S (2011) The potential of adipose stem cells in regenerative medicine. *Stem Cell Rev* 7:269–291. <https://doi.org/10.1007/s12015-010-9193-9197>
- Lindvall O, Kokaia Z (2006) Stem cells for the treatment of neurological disorders. *Nature* 441(7097):1094–1096. <https://doi.org/10.1038/nature04960>
- Liu S, Jiang L, Li H, Shi H, Luo H, Zhang Y, Yu C, Jin Y (2014) Mesenchymal stem cells prevent hypertrophic scar formation via inflammatory regulation when undergoing apoptosis. *J Invest Dermatol* 134(10):2648–2657. <https://doi.org/10.1038/jid.2014.169>
- Liu W, Yin L, Yan X, Cui J, Liu W, Rao Y, Sun M, Wei Q, Chen F (2017) Directing the differentiation of parthenogenetic stem cells into tenocytes for tissue-engineered tendon regeneration. *Stem Cells Transl Med* 6(1):196–208. <https://doi.org/10.5966/sctm.2015-0334>
- Liu B, Lee BW, Nakanishi K, Villasante A, Williamson R, Metz J, Kim J, Kanai M, Bi L, Brown K, Di Paolo G, Homma S, Sims PA, Topkara VK, Vunjak-Novakovic G (2018) Cardiac recovery via extended cell-free delivery of extracellular vesicles secreted by cardiomyocytes derived from induced pluripotent stem cells. *Nature Biomed Eng* 2(5):293–303. <https://doi.org/10.1038/s41551-018-0229-7>
- Lu W, Yu J, Zhang Y, Ji K, Zhou Y, Li Y, Deng Z, Jin Y (2012) Mixture of fibroblasts and adipose tissue-derived stem cells can improve epidermal morphogenesis of tissue-engineered skin. *Cells Tissues Organs* 195(3):197–206. <https://doi.org/10.1159/000324921>
- Lysaght MJ, Crager J (2009) Origins. *Tissue Eng Part A* 15:1449–1450. <https://doi.org/10.1089/ten.tea.2007.0412>
- Mahla RS (2016) Stem cells applications in regenerative medicine and disease therapeutics. *Int J Cell Biol* 2016:6940283. <https://doi.org/10.1155/2016/6940283>

- Malik N, Rao MS (2013) A review of the methods for human iPSC derivation. *Methods Mol Biol* (Clifton, N.J.) 997:23–33. https://doi.org/10.1007/978-1-62703-348-0_3
- Mansilla E, Marín GH, Drago H, Sturla F, Salas E, Gardiner C, Bossi S, Lamonega R, Guzmán A, Nuñez A, Gil MA, Piccinelli G, Ibar R, Soratti C (2006) Bloodstream cells phenotypically identical to human mesenchymal bone marrow stem cells circulate in large amounts under the influence of acute large skin damage: new evidence for their use in regenerative medicine. *Transplant Proc* 38(3):967–969. <https://doi.org/10.1016/j.transproceed.2006.02.053>
- Mao Q, Nguyen PD, Shanti RM, Shi S, Shakoori P, Zhang Q, Le AD (2019) Gingiva-derived mesenchymal stem cell-extracellular vesicles activate Schwann cell repair phenotype and promote nerve regeneration. *Tissue Eng Part A* 25(11–12):887–900. <https://doi.org/10.1089/ten.TEA.2018.0176>
- Marquez-Curtis LA, Janowska-Wieczorek A, McGann LE, Elliott JA (2015) Mesenchymal stromal cells derived from various tissues: biological, clinical and cryopreservation aspects. *Cryobiology* 71(2):181–197. <https://doi.org/10.1016/j.cryobiol.2015.07.003>
- Mazini L, Rochette L, Amine M, Malka G (2019) Regenerative capacity of adipose derived stem cells (ADSCs), comparison with mesenchymal stem cells (MSCs). *Int J Mol Sci* 20(10):2523. <https://doi.org/10.3390/ijms20102523>
- Menasché P, Alfieri O, Janssens S, McKenna W, Reichenspurner H, Trinquart L, Vilquin JT, Marolleau JP, Seymour B, Larghero J, Lake S, Chatellier G, Solomon S, Desnos M, Hagege AA (2008) The Myoblast Autologous Grafting in Ischemic Cardiomyopathy (MAGIC) trial: first randomized placebo-controlled study of myoblast transplantation. *Circulation* 117(9):1189–1200. <https://doi.org/10.1161/CIRCULATIONAHA.107.734103>
- Mizuno H (2010) Adipose-derived stem and stromal cells for cell-based therapy: current status of preclinical studies and clinical trials. *Curr Opin Mol Ther* 12(4):442–449
- Moodley Y, Atienza D, Manuelpillai U, Samuel CS, Tchongue J, Ilancheran S, Boyd R, Trounson A (2009) Human umbilical cord mesenchymal stem cells reduce fibrosis of bleomycin-induced lung injury. *Am J Pathol* 175(1):303–313. <https://doi.org/10.2353/ajpath.2009.080629>
- Moon GJ, Sung JH, Kim DH, Kim EH, Cho YH, Son JP, Cha JM, Bang OY (2019) Application of mesenchymal stem cell-derived extracellular vesicles for stroke: biodistribution and MicroRNA study. *Transl Stroke Res* 10(5):509–521. <https://doi.org/10.1007/s12975-018-0668-1>
- Morel O, Toti F, Hugel B, Freyssinet JM (2004) Cellular microparticles: a disseminated storage pool of bioactive vascular effectors. *Curr Opin Hematol* 11(3):156–164. <https://doi.org/10.1097/01.moh.0000131441.10020.87>
- Mousa NA, Abou-Taleb HA, Orabi H (2015) Stem cell applications for pathologies of the urinary bladder. *World J Stem Cells* 7(5):815–822. <https://doi.org/10.4252/wjsc.v7.i5.815>
- Nasef A, Ashammakhi N, Fouillard L (2008) Immunomodulatory effect of mesenchymal stromal cells: possible mechanisms. *Regen Med* 3(4):531–546. <https://doi.org/10.2217/17460751.3.4.531>
- Oh M, Lee J, Kim YJ, Rhee WJ, Park JH (2018) Exosomes derived from human induced pluripotent stem cells ameliorate the aging of skin fibroblasts. *Int J Mol Sci* 19(6):1715. <https://doi.org/10.3390/ijms19061715>
- Omole AE, Fakoya A (2018) Ten years of progress and promise of induced pluripotent stem cells: historical origins, characteristics, mechanisms, limitations, and potential applications. *PeerJ* 6:e4370. <https://doi.org/10.7717/peerj.4370>
- Ouyang HW, Toh SL, Goh J, Tay TE, Moe K (2005) Assembly of bone marrow stromal cell sheets with knitted poly (L-lactide) scaffold for engineering ligament analogs. *J Biomed Mater Res Part B Appl Biomater* 75B:264–271. <https://doi.org/10.1002/jbm.b.30281>
- Pan W, Zhu Y, Meng X, Zhang C, Yang Y, Bei Y (2019) Immunomodulation by exosomes in myocardial infarction. *J Cardiovasc Transl Res* 12(1):28–36. <https://doi.org/10.1007/s12265-018-9836-7>
- Park YB, Ha CW, Lee CH, Yoon YC, Park YG (2017) Cartilage regeneration in osteoarthritic patients by a composite of allogeneic umbilical cord blood-derived mesenchymal stem cells and hyaluronate hydrogel: results from a clinical trial for safety and proof-of-concept with 7 years of

- extended follow-up. *Stem Cells Transl Med* 6:613–621. <https://doi.org/10.5966/sctm.2016-2157>
- Park YB, Ha CW, Kim JA, Kim S, Park YG (2019) Comparison of undifferentiated versus chondrogenic predifferentiated mesenchymal stem cells derived from human umbilical cord blood for cartilage repair in a rat model. *Am J Sports Med* 47:451–461. <https://doi.org/10.1177/0363546518815151>
- Patel NA, Moss LD, Lee JY, Tajiri N, Acosta S, Hudson C, Parag S, Cooper DR, Borlongan CV, Bickford PC (2018) Long noncoding RNA MALAT1 in exosomes drives regenerative function and modulates inflammation-linked networks following traumatic brain injury. *J Neuroinflammation* 15(1):204. <https://doi.org/10.1186/s12974-018-1240-3>
- Phinney DG, Pittenger MF (2017) Concise review: MSC-derived exosomes for cell-free therapy. *Stem Cells (Dayton, Ohio)* 35(4):851–858. <https://doi.org/10.1002/stem.2575>
- Pittenger MF, Mackay AM, Beck SC, Jaiswal RK, Douglas R, Mosca JD, Moorman MA, Simonetti DW, Craig S, Marshak DR (1999) Multilineage potential of adult human mesenchymal stem cells. *Science (New York, NY)* 284(5411):143–147. <https://doi.org/10.1126/science.284.5411.143>
- Porada CD, Atala AJ, Almeida-Porada G (2016) The hematopoietic system in the context of regenerative medicine. *Methods* 99:44–61. <https://doi.org/10.1016/j.ymeth.2015.08.015>
- Potter DR, Miyazawa BY, Gibb SL, Deng X, Togaratti PP, Croze RH, Srivastava AK, Trivedi A, Matthay M, Holcomb JB, Schreiber MA, Pati S (2018) Mesenchymal stem cell-derived extracellular vesicles attenuate pulmonary vascular permeability and lung injury induced by hemorrhagic shock and trauma. *J Trauma Acute Care Surg* 84(2):245–256. <https://doi.org/10.1097/TA.0000000000001744>
- Ratajczak J, Wysoczynski M, Hayek F, Janowska-Wieczorek A, Ratajczak MZ (2006) Membrane-derived microvesicles: important and underappreciated mediators of cell-to-cell communication. *Leukemia* 20(9):1487–1495. <https://doi.org/10.1038/sj.leu.2404296>
- Ren ML, Peng W, Yang ZL, Sun XJ, Zhang SC, Wang ZG, Zhang B (2012) Allogeneic adipose-derived stem cells with low immunogenicity constructing tissue-engineered bone for repairing bone defects in pigs. *Cell Transplant* 21(12):2711–2721. <https://doi.org/10.3727/096368912X654966>
- Rodeheffer MS, Birsoy K, Friedman JM (2008) Identification of white adipocyte progenitor cells in vivo. *Cell* 135:240–249. <https://doi.org/10.1016/j.cell.2008.09.036>
- Rodriguez AM, Elabd C, Amri EZ, Ailhaud G, Dani C (2005) The human adipose tissue is a source of multipotent stem cells. *Biochimie* 87:125–128. <https://doi.org/10.1016/j.biochi.2004.11.007>
- Romanov YA, Svintsitskaya VA, Smirnov VN (2003) Searching for alternative sources of postnatal human mesenchymal stem cells: candidate MSC-like cells from umbilical cord. *Stem Cells* 21:105–110. <https://doi.org/10.1634/stemcells.21-1-105>
- Ruetze M, Richter W (2014) Adipose-derived stromal cells for osteoarticular repair: trophic function versus stem cell activity. *Expert Rev Mol Med* 16:e9. <https://doi.org/10.1017/erm.2014.9>
- Sacchetti B, Funari A, Michienzi S, Di Cesare S, Piersanti S, Saggio I, Tagliafico E, Ferrari S, Robey PG, Riminucci M, Bianco P (2007) Self-renewing osteoprogenitors in bone marrow sinusoids can organize a hematopoietic microenvironment. *Cell* 131(2):324–336. <https://doi.org/10.1016/j.cell.2007.08.025>
- Sadahiro T, Yamanaka S, Ieda M (2015) Direct cardiac reprogramming: progress and challenges in basic biology and clinical applications. *Circ Res* 116:1378–1391. <https://doi.org/10.1161/CIRCRESAHA.116.305374>
- Salgado AJ, Oliveira JM, Martins A, Teixeira FG, Silva NA, Neves NM, Sousa N, Reis RL (2013) Tissue engineering and regenerative medicine: past, present, and future. *Int Rev Neurobiol* 108:1–33. <https://doi.org/10.1016/B978-0-12-410499-0.00001-0>
- Schäfer R, Spohn G, Baer PC (2016) Mesenchymal stem/stromal cells in regenerative medicine: can preconditioning strategies improve therapeutic efficacy? *Transfus Med Hemother* 43:256–267. <https://doi.org/10.1159/000447458>