eISBN: 978-1-68108-257-8
ISBN: 978-1-68108-258-5

eISSN: 2352-7633
ISSN: 2467-9593

Frontiers in Stem Cell and Regenerative Medicine Research Volume 3

Editors:
Atta-ur-Rahman, *FRS*
Shazia Anjum

Bentham e Books

Frontiers in Stem Cell and Regenerative Medicine Research

(Volume 3)

Edited By

Atta-ur-Rahman, *FRS*
Honorary Life Fellow, Kings College
University of Cambridge, Cambridge,UK

&

Shazia Anjum
Department of Chemistry, Cholistan Institute of Desert Studies, The Islamia University of Bahawalpur, Pakistan

Frontiers in Stem Cell and Regenerative Medicine Research

Volume # 3

Editor: Prof. Atta-ur-Rahman, *FRS* and Dr. Shazia Anjum

eISSN (Online): 2352-7633

ISSN (Print): 2467-9593

eISBN (Online): 978-1-68108-257-8

ISBN (Print): 978-1-68108-258-5

First published in 2017.

advertisements or ideas contained in the Work.

Limitation of Liability:

In no event will Bentham Science Publishers, its staff, editors and/or authors, be liable for any damages, including, without limitation, special, incidental and/or consequential damages and/or damages for lost data and/or profits arising out of (whether directly or indirectly) the use or inability to use the Work. The entire liability of Bentham Science Publishers shall be limited to the amount actually paid by you for the Work.

General:

1. Any dispute or claim arising out of or in connection with this License Agreement or the Work (including non-contractual disputes or claims) will be governed by and construed in accordance with the laws of the U.A.E. as applied in the Emirate of Dubai. Each party agrees that the courts of the Emirate of Dubai shall have exclusive jurisdiction to settle any dispute or claim arising out of or in connection with this License Agreement or the Work (including non-contractual disputes or claims).
2. Your rights under this License Agreement will automatically terminate without notice and without the need for a court order if at any point you breach any terms of this License Agreement. In no event will any delay or failure by Bentham Science Publishers in enforcing your compliance with this License Agreement constitute a waiver of any of its rights.
3. You acknowledge that you have read this License Agreement, and agree to be bound by its terms and conditions. To the extent that any other terms and conditions presented on any website of Bentham Science Publishers conflict with, or are inconsistent with, the terms and conditions set out in this License Agreement, you acknowledge that the terms and conditions set out in this License Agreement shall prevail.

Bentham Science Publishers Ltd.
Executive Suite Y - 2
PO Box 7917, Saif Zone
Sharjah, U.A.E.
Email: subscriptions@benthamscience.org

CONTENTS

PREFACE

Stem cell and regenerative medicine is a hot area of current research that is expected to have a profound impact on health and medicine. It is moving fast and often people are interested in finding out most contemporary discoveries. It has shown great potential to meet the worldwide organ shortage challenge by tissue regeneration and organ replacement.

The 3rd volume of *'Frontiers in Stem Cell and Regenerative Medicine Research'* contains reviews written by eminent specialists in major rapidly developing fields of stem cell and regenerative medicine research. LaRue and Kelly have presented the advantages of utilizing various types of stem cells to treat bone related diseases and difficult-to-heal fractures. Pacella *et al.* have reviewed the current status of stem cell therapy in neonatal brain injury, encompassing hypoxic-ischemic encephalopathy (HIE), perinatal stroke, and white matter injury. This holds promise for improving the methods of diagnosis, prevention and as well as the development of innovative treatments for such injuries.

Neurodegenerative, neurological, and psychiatric diseases (NNPDs) significantly contribute to disability and mortality. Zakian and Medvedev have focused on the cell models based on patient-specific induced pluripotent stem cell derived neurons and their use in the search for new drugs. The utility of genome engineering for the creation and study of cell models for NNPDs is discussed in detail.

Respiratory diseases represent a wide array of common diseases with great social and financial burden. Regenerative medicine could offer new tools for treating severe and progressive lung diseases as depicted in Alexander V. Averyanov's review. Patients suffering from esophageal defects and disease are commonly treated with surgical intervention, which can lead to a host of short and long term complications including the need for repeated surgery. Finck *et al.* have covered novel tissue engineering approaches for esophageal defects.

We hope that the readers will enjoy reading about the latest and exciting revolutions in prominent areas of stem cell and regenerative medicine research.

We are grateful to all the authors for their contributions. We are also thankful to the editorial staff of Bentham Science Publishers, particularly Dr. Faryal Sami, Mr. Shehzad Naqvi and Mr. Mahmood Alam for their constant support and great help.

Prof. Atta-ur-Rahman, *FRS*
Honorary Life Fellow
Kings College
University of Cambridge
Cambridge
UK

&

Dr. Shazia Anjum
Department of Chemistry
Cholistan Institute of Desert Studies
The Islamia University of Bahawalpur
Pakistan

List of Contributors

Alexander V. Averyanov	Federal Research Clinical Center under Federal Medical Biological Agency of Russia, Moscow, Russia
Amanda C. LaRue	Research Services, Ralph H. Johnson Veterans Affairs Medical Center, USA Department of Pathology and Laboratory Medicine, Medical University of South Carolina, Charleston, South Carolina
Christine Finck	Connecticut Children's Medical Center Department of Surgery, 282 Washington Street Hartford, CT 06106, USA
Marisa J. Pacella	Department of Pediatrics, University of Florida, Gainesville, FL, USA
Martha Douglas-Escobar	Department of Pediatrics, University of California San Francisco, CA, USA
Michael D. Weiss	Department of Pediatrics, University of Florida, Gainesville, FL, USA McKnight Brain Institute, University of Florida, Gainesville, FL, USA
Ryan R. Kelly	Research Services, Ralph H. Johnson Veterans Affairs Medical Center, USA Department of Pathology and Laboratory Medicine, Medical University of South Carolina, Charleston, South Carolina
Sergey P. Medvedev	Federal Research Center Institute of Cytology and Genetics, Siberian Branch, Russian Academy of Sciences, Novosibirsk, Russia Institute of Chemical Biology and Fundamental Medicine, Siberian Branch, Russian Academy of Sciences, Novosibirsk, Russia State Research Institute of Circulation Pathology, Novosibirsk, Russia Novosibirsk State University, Novosibirsk, Russia
Suren M. Zakian	Federal Research Center Institute of Cytology and Genetics, Siberian Branch, Russian Academy of Sciences, Novosibirsk, Russia Institute of Chemical Biology and Fundamental Medicine, Siberian Branch, Russian Academy of Sciences, Novosibirsk, Russia State Research Institute of Circulation Pathology, Novosibirsk, Russia Novosibirsk State University, Novosibirsk, Russia
Todd Jensen	Connecticut Children's Medical Center Department of Surgery, 282 Washington Street Hartford, CT 06106, USA
Tong Zheng	McKnight Brain Institute, University of Florida, Gainesville, FL, USA
Wael Sayej	Connecticut Children's Medical Center Department of Surgery, 282 Washington Street Hartford, CT 06106, USA

CHAPTER 1

Stem Cell-Based Therapies for Bony Repair

Ryan R. Kelly[1,2] and **Amanda C. LaRue**[1,2,*]

[1] *Research Services, Ralph H. Johnson Veterans Affairs Medical Center, Charleston, South Carolina, USA*

[2] *Department of Pathology and Laboratory Medicine, Medical University of South Carolina, Charleston, South Carolina, USA*

Abstract: Normal bone remodeling requires a continuous supply of osteoblasts and osteoclasts from stem cells in the bone marrow. In bone-related diseases and fractures, this process becomes disrupted. There are a number of therapeutic strategies being employed that are attempting to repair diseased or damaged bone, including bone grafts, demineralized bone implantation, and recombinant protein treatments. However, likely more than these strategies, stem cells hold great potential to completely regenerate bone tissue. In this chapter, we provide a discussion on the physiology and pathophysiology of osteogenesis, as well as current therapies for bony defects, with specific emphasis placed on the advantages of utilizing various types of stem cells as therapeutic approaches to treat bone-related diseases and difficult-to-heal fractures.

Keywords: Bone graft, Bone-related disease, Bone remodeling, Fracture, Hematopoietic stem cell, Mesenchymal stem cell, Mobilization, Regeneration, Skeletal stem cell, Stem cell.

INTRODUCTION

Stem cells are required for maintenance and repair of bone. However, the molecular processes of stem cell differentiation for osteogenesis are still poorly understood. The bone marrow (BM) contains two types of stem cells, the mesenchymal stromal cell (MSC), thought to generate cells of the mesenchymal lineage, and the hematopoietic stem cell (HSC), thought to generate blood cells,

* **Corresponding author Amanda C. LaRue**: Research Services, Ralph H. Johnson VAMC, Charleston, South Carolina, USA; Tel: (843)-789-6713; Fax: (843) 876-5381; E-mail: laruerc@musc.edu

Atta-ur-Rahman & Shazia Anjum (Eds.)

osteoclasts, and mast cells.

However, research has begun to blur these distinctions by demonstrating the ability of both the MSC and HSC to generate osteoblasts. In addition to the MSC and HSC, recent studies have shown the osteogenic potential of other stem cell populations (*e.g.* skeletal stem cells) as well. Understanding which stem cells are responsible for osteogenesis, both during development and in cases of repair after fracture or disease, could have significant therapeutic benefit.

This chapter will give a general overview of normal bone function and repair, followed by a description of major bony defects and diseases. Current knowledge on the molecular mechanisms governing stem cell differentiation to osteoblasts and osteoblast maturation will be described. We will then discuss current treatment strategies for promoting bone repair and will describe the limitations of these strategies. Compared to current strategies, stem cells hold great potential clinically, as they have the capacity to completely regenerate bone tissue. Thus, we will discuss research findings detailing the osteogenic potential of different stem cell populations, including MSCs, HSCs and skeletal stem cells (SSCs). We will then finish this chapter by detailing the current state of stem cell treatment options for fracture repair and bony disease and discuss how research findings may be translated to improve current therapies.

NORMAL BONE ANATOMY, PHYSIOLOGY AND FUNCTION

Bone tissue supports muscles and soft tissues, protects vital organs, and harbors the bone marrow. In addition, it serves as a reservoir for calcium, phosphate, and other important ions used throughout the body. The general structure of bone can be viewed as a central marrow space surrounded by bone tissue. The bone itself is lined by the endosteum on its inner surface and the periosteum on its outer surface. The endosteum is a membranous structure that is mostly cellular, contains vasculature, and harbors osteoprogenitors that communicate with the bone marrow. The periosteum is a fibrous connective tissue covering the bone and is attached to the outer cortex *via* collagenous fibers known as Sharpey's fibers. The periosteum serves as an attachment site for ligaments and tendons, contributes to bone's blood supply and is a site for bone remodeling in response to local or

systemic stimuli. Periosteal cells can communicate with cells in the endosteum and marrow space through channels known as Volkman's canals [1].

The two main structural types of bone are cortical bone and trabecular bone. The adult human skeleton is composed of 80% cortical bone and 20% trabecular bone by weight [2]. Cortical bone forms the hard outer layer of bone, has a low porosity of 5-10%, and provides resistance to torsion and bending. Trabecular bone forms a honeycomb-like network of inter-connected trabeculae throughout the interior of the bone. This trabecular bone has a higher porosity of 50-90% and allows the bone to withstand compressive forces. Further, trabecular bone has a high rate of metabolic activity and remodeling [2]. Bone remodeling occurs in order for bone to maintain strength and mineral homeostasis. This skeletal remodeling results in regeneration of most of the adult skeleton every 10 years and is essential for the maintenance of healthy bone.

Skeletal remodeling and maintenance are regulated by four major bone cell types: osteoclasts, osteoblasts, osteocytes, and osteogenic stem cells. Osteoclasts, known to be of HSC origin, are giant, multinucleated cells that adhere to the bone by a ruffled border and resorb bone through acidification and proteolytic digestion. Osteoblasts combat osteoclast-mediated bone resorption by secreting osteoid, which mineralizes to form new bone. After secretion, osteoblasts either terminally differentiate into osteocytes and become physically embedded in the mineralized tissue, quiesce into lining cells, or undergo apoptosis. Approximately 10-20% of osteoblasts will become osteocytes, which are non-dividing cells that comprise 90-95% of the bone cell population [3]. They are smaller than osteoblasts and have a larger nucleus to cytoplasm ratio. Once they become embedded in the mineralized tissue, they develop cytoplasmic projections that intercalate throughout the bone and allow for direct communication with other osteocytes. Through this network, they are able to regulate phosphate homeostasis and transduce mechanical stress signals into biologic activity to stimulate either bone resorption or formation. Osteogenic stem cells will be discussed in further detail below, but provide the sources for osteoblasts and osteocytes. The functions and number of these various cell types can become disrupted during bony defects and diseases. Thus, understanding how to modulate and regulate these different cell types, particularly osteogenic stem cells, is essential for the development of

CHAPTER 2

Stem Cell Therapy for Brain Injury in Neonates

Marisa J. Pacella[1,*], Martha Douglas-Escobar[2], Tong Zheng[3] and **Michael D. Weiss[1,3]**

[1] *Department of Pediatrics, University of Florida, Gainesville, FL, USA*

[2] *Department of Pediatrics, University of California San Francisco, CA, USA*

[3] *McKnight Brain Institute, University of Florida, Gainesville, FL, USA*

Abstract: Neonatal brain injury encompasses hypoxic-ischemic encephalopathy (HIE), perinatal stroke, and white matter injury. The estimated incidence of HIE in developed countries is 1.5 in 1,000 live births. Hypothermia therapy improves neurodevelopmental outcomes in only 53% of treated HIE patients. Perinatal stroke occurs in 1 per 1,600 births and is a major cause of cerebral palsy and developmental impairments. Periventricular white matter injury affects 50% of premature neonates and accounts for 90% of neurologic deficits in survivors.

Stem cell therapy has emerged as a potential treatment for neonatal brain injury. This review will present the state of the art for stem cell therapy in neonatal brain injury.

Keywords: Cerebral palsy, Hypoxic ischemic encephalopathy, Induced pluripotent stem cell, Mesenchymal stem cell, Neonatal brain injury, Neural stem cell, Periventricular leukomalacia, Stem cell therapy, Stroke, Umbilical cord blood, Umbilical cord mesenchymal stem cells.

INTRODUCTION

Brain injury may occur during the neonatal period, resulting in long-term disabilities. Causes of neonatal brain injury include hypoxic-ischemic encephalopathy (HIE), perinatal stroke, and periventricular leukomalacia.

[*] **Corresponding author Marisa J. Pacella:** Department of Pediatrics, University of Florida, PO Box 100296, Gainesville, FL 32610, USA; Tel: +352-273-8985; Fax: +352-273-9054; Email: mpacella@ufl.edu

Atta-ur-Rahman & Shazia Anjum (Eds.)

HIE is a major cause of neurological disabilities in term neonates, despite the recent widespread use of hypothermia therapy. The estimated incidence of HIE in developed countries is 1.5 per 1,000 live births [1]. Although therapeutic hypothermia offers neuroprotection, the benefits are modest. Only 53% of treated neonates show improvement in neurodevelopmental outcomes. Perinatal stroke, which has an incidence of 1 per 1,600 births [2], is a major cause of cerebral palsy (CP) and developmental impairments. Currently, therapies do not exist for acute or chronic stroke in this population. In addition, periventricular white matter injury affects 50% of premature neonates with birth weights less than 1,500 grams and accounts for 90% of neurologic deficits in survivors [3].

The neonatal brain may be an optimal candidate for cell-based therapies due to the inherent plasticity at this developmental stage. This review will examine the various stem cell types that are currently being explored for neonatal brain injury in basic science models of neonatal brain injury. First, we will review the types of stem cells. Then, we will discuss the potential application of stem cells, in particular their application to the field of neonatal neurology. Finally, we will summarize both planned and on-going human clinical trials.

WHAT IS A STEM CELL?

Russian histologist Alexander Maksimov first coined the term “stem cells” in 1908. Since then, scientists have recognized the existence of multiple types of stem cells. Although each type of stem cell has unique properties, they all, by definition, possess the abilities of continuous self-renewal and potency, or the capacity to differentiate into one or more mature cell lines. Stem cells can be obtained from a variety of sources including bone marrow, adult and fetal brains, umbilical cord blood (UCB), the umbilical cord (UC) and placental tissue, adipose tissue, and induced progenitor cells from fibroblast (Fig. **1**). Stem cells collected from the patient’s own body are referred to as autologous, whereas cells from a donor are allogeneic.

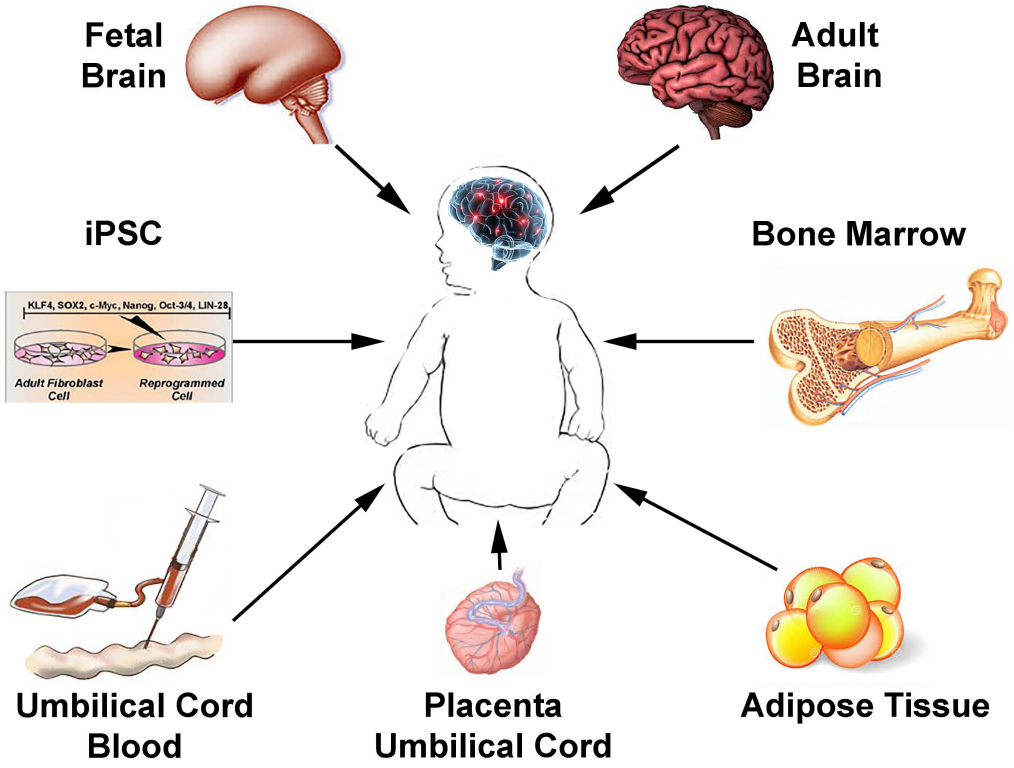

Fig. (1). The potential sources of stem cells for neonatal brain injury are shown graphically. Sources of stem cells include fetal and adult brain tissue, bone marrow, adipose tissue, UC and placental tissue, UCB, and induced progenitor stem cells derived from fibroblast.

SOURCES OF STEM CELLS

Mesenchymal Stem Cells

Mesenchymal stem cells (MSCs) are a class of stem cells found in the stroma, or connective tissue, of multiple organs within the body. These tissues include bone marrow and UCB plus more recently menstrual blood, adipose tissue, UC tissue, and endometrium. MSCs are currently studied not only for their proliferative and multilineage differentiation capacity in transplantation medicine but also for their immunomodulatory properties and homing ability in regenerative medicine [4].

Bone marrow MSCs are located around sinusoidal blood vessels within the marrow [5]. To isolate and harvest the cells, bone marrow is aspirated from a

CHAPTER 3

Models of Hereditary Neurodegenerative, Neurological, and Psychiatric Diseases Based on Induced Pluripotent Stem Cell-Derived Neurons

Sergey P. Medvedev[1,2,3,4] and **Suren M. Zakian**[1,2,3,4,*]

[1] *Federal Research Center Institute of Cytology and Genetics, Siberian Branch, Russian Academy of Sciences, Novosibirsk, Russia*

[2] *Institute of Chemical Biology and Fundamental Medicine, Siberian Branch, Russian Academy of Sciences, Novosibirsk, Russia*

[3] *State Research Institute of Circulation Pathology, Novosibirsk, Russia*

[4] *Novosibirsk State University, Novosibirsk, Russia*

Abstract: Neurodegenerative, neurological, and psychiatric diseases (NNPDs) significantly contribute to disability and mortality worldwide. Despite numerous studies, only few effective therapeutic methods and drugs have been found so far even for the most common diseases, such as Alzheimer's and Parkinson's diseases. The lack of effective therapeutic methods is mainly explainable by the absence of relevant *in vitro* model systems to study pathological phenotypes at the cellular and molecular levels. Induced pluripotent stem cells are a unique source of different types of patient-specific neurons that can reproduce *in vitro* the specific features of individual diseases. In this chapter, the cell models based on patient-specific induced pluripotent stem cell-derived neurons and their use in the search for new drugs and toxicological studies are described in detail. In addition, the utility of genome engineering for the creation and study of cell models for NNPDs is discussed.

Keywords: Induced Pluripotent Stem Cells, Neurodegenerative, Neurological and Psychiatric diseases, Modeling.

[*] **Corresponding author Suren M. Zakian:** Federal Research Center Institute of Cytology and Genetics, Siberian Branch, Russian Academy of Sciences, pr. Akad. Lavrentieva 10, Novosibirsk, 630090 Russia; Tel: +7-383-3634937, Fax: +7-383-3304560; Email: zakian@bionet.nsc.ru

Atta-ur-Rahman & Shazia Anjum (Eds.)

INTRODUCTION

Neurodegenerative, neurological, and psychiatric diseases (NNPDs) are a very broad and heterogeneous group of diseases with yet absent efficient therapies for most of them or at least the methods for alleviating their severe symptoms. Heterogeneity of NNPDs appears at several levels as well as the problems in studying these diseases. NNPDs comprise both sporadic diseases and genetically determined hereditary illnesses. The hereditary variants of NNPDs account for approximately 1-10%. In turn, the genetic causes underlying NNPDs development are also rather variable. For example, it is known that over 300 mutations in four genes (*APP*, *MAPT*, *PSEN1,* and *PSEN2*) are associated with the hereditary form of Alzheimer's disease [1, 2]. These facts contribute to the complexity in searching for the drugs and other therapeutic tools for NNPDs, and the overall problem comprises the following aspects: (1) poor availability (both qualitative and quantitative) of biopsy specimens for clinical examination, especially, for rare hereditary disease forms; (2) difficulties in correct diagnosing and clarification of the correlations between phenotypic manifestations and specific genetic features of patients; and (3) a typical situation when the NNPDs pathogenesis is studied at terminal disease stages, which leaves the mechanisms vague that underlie disease development.

The *in vivo* and *in vitro* model systems able to reproduce the necessary pathological states under laboratory conditions may give solutions to the aforementioned problems [3]. The use of laboratory animals (from the fruit fly to non-human primates) as *in vivo* models has provided significant information about NNPDs. However, the problem in using the animal models arises due to certain differences at the level of physiology of organs and their systems as well as physiology and biochemistry at the cellular level, which stem from the tremendous evolutionary distance between the animals and human.

In vitro human cell cultures can replace animal models for probing of pathological phenotypes. The human immortalized cell lines (for example HEK293 or HeLa) expressing mutant proteins are applicable for this purpose. However, patient-specific neurons would be a more suitable solution, since they precisely match the type of cell that manifests the pathological phenotype. In addition, they replicate

the natural characteristics of neurons regulatory pathways as well as physiological level of neuron genes expression, including mutant genes. One of the potential sources for such neurons is pluripotent cells, *i.e.*, the cells that are able to differentiate into derivatives of all three primitive germ layers (ectoderm, mesoderm, and endoderm). Quite recently, the only type of pluripotent cells accessible for researchers was embryonic stem cells (ESCs), obtainable from preimplantation embryos. The use of these cells is limited because of ethical issues associated with the death of embryos at the moment cell lines are produced as well as with the impossibility to produce the cell lines carrying certain mutations that cause a particular disease without using genome editing technologies.

In 2006, Takahashi and Yamanaka published their paper reporting a method for generating the cells very similar in their properties to the pluripotent cells from mouse somatic cells by ectopic overexpression of the four genes *Oct4*, *Sox2*, *Klf4,* and *c-Myc* [4]. This cell type is referred to as induced pluripotent stem cells (iPSCs). Using the factors *Oct4*, *Sox2*, *Klf4, and c-Myc,* iPSCs was generated from various differentiated cell types of the mouse [5 - 8] and human [9 - 11]. In addition, the team of American researchers headed by J.A. Thomson [12] succeeded in generating human iPSCs using somewhat altered set of genes (*OCT4, SOX2, NANOG,* and *LIN28*). Later, numerous research teams discovered that mouse and human iPSCs can be produced with the help of ectopic overexpression of a rather wide set of genes, which includes; the genes encoding transcription factors (for example, *UTF1*), nuclear orphan receptors (*Esrrb* and *Nr5a2*), human telomerase catalytic domain, and *SV40 large T antigen* [13 - 16]. In addition, iPSCs can be generated using certain sets of microRNAs and low-molecular-weight chemical compounds [17, 18]. Numerous studies conducted after the first work by Yamanaka allowed the technology for iPSC generation to be considerably modified. Along with the new and more efficient gene sets for reprogramming, the vector systems allowing for removal of exogenous DNA from cell genomes or avoiding its integration at all have also been used. For example, *PiggyBac* DNA transposons, which can be completely eliminated from cell genomes, were actively used in iPSC generation as well as nonintegrated vectors, such as plasmids, episomes, minicircle vectors, Sendai virus vectors, synthetic

CHAPTER 4

Prospects of Regenerative Medicine in the Treatment of Pulmonary Diseases

Alexander V. Averyanov*

Federal Research Clinical Center under Federal Medical Biological Agency of Russia, Moscow, Russia

Abstract: Respiratory diseases represent a wide array of most common diseases with great social and financial burden. Despite the obvious progress in the treatment of patients with respiratory pathology, achieved in the last decade, there are still many incurable disorders such as emphysema, pulmonary fibrosis, cystic fibrosis, acute respiratory distress syndrome, pulmonary hypertension, etc. The results of treatment of these disorders are far from perfect and often the only today alternative is lung transplantation. Regenerative medicine could become a new solution, offering real tools for severe and progressive lung diseases. To date, numerous preclinical studies have proven the effectiveness of regenerative technologies in different animal models and in addition quite a few clinical trials have been started with the first results. This review critically evaluates the data obtained in the experimental and clinical studies, with the emphasis on advances and limitations of regenerative medicine.

Keywords: Airway engineering, ARDS, COPD, Emphysema, Pulmonary fibrosis, Pulmonary hypertension, Regenerative medicine, Respiratory diseases, Sepsis, Stem cell therapy.

Within the last decades, a remarkable progress was observed in the development of new drugs and new technologies in the field of respiratory medicine that resulted in significantly improved outcomes of treatment of numerous pulmonary diseases. However, there is a range of lung pathologies characterized by high rate

* **Corresponding author Alexander V Averyanov:** Federal Research Clinical Center under Federal Medical Biological Agency of Russia, Moscow, Russia; Tel: +7(495) 395 05 11; Fax: +7(495) 395 64 30; Email: averyanovav@mail.ru

Atta-ur-Rahman & Shazia Anjum (Eds.)

of mortality and morbidity for whose treatment no effective strategies developed yet. Those include severe pneumonia, sepsis, acute respiratory distress syndrome, lung cancer, COPD and emphysema, pulmonary hypertension, cystic fibrosis, and interstitial lung disease, *e.g.* disorders whose actuality is still high and treatment results are rather unsatisfactory. In many ways, this problem persists due to the lack of efficient medical solutions that could effectively influence lung inflammation, fibrosis, and mechanisms of airway tissue repair mechanisms. Drugs currently used to treat inflammatory respiratory diseases have serious limitations. Antibiotics could suppress infection but do not affect systemic inflammatory response. In contrast, corticosteroids and cytostatic agents are able to decrease chronic inflammation and delay lung fibrosis. However, long-term treatment with these medications could have severe adverse effects. Indeed, lung or heart-lung transplantation could represent a single alternative for treatment of end-stage pulmonary disease. Since early 1990's, over 28,000 lung transplantations were performed worldwide [1]. Regenerative medicine including introduction of heterogeneous types of stem cells, development of new biomaterials, bioscaffolds and their combinations could represent alternative to lung transplantation and promising tool in the treatment of pulmonary diseases. However, results of first clinical trials reduced initial optimism achieved in preclinical studies. This review summarizes recent experimental and human research findings in the area of stem cell therapy and airway engineering for the above-mentioned respiratory diseases. Systematic literature review was performed in PubMed (http://www.ncbi.nlm.nih.gov/pubmed) and e-library (http://elibrary.ru) among articles published between January 1990 and February 2015 and all records on Clinical Trials (https://clinicaltrials.gov) found by keywords: stem cells, lung disease, COPD, pulmonary fibrosis, pulmonary emphysema, pulmonary hypertension, acute lung injury, pulmonary infection, lung tissue engineering. In the analysis of experimental research the focus was on the publication of the last three years. Because of the small number, all available clinical studies with published results were accepted.

CHRONIC OBSTRUCTIVE PULMONARY DISEASE AND LUNG EMPHYSEMA

Chronic obstructive pulmonary disease (COPD) is one of the most common

diseases, affecting more than 10% of adults older than 40 years [2]. The burden of COPD, especially in underdeveloped and developing countries, is steadily growing and WHO predicts the disease will be the third leading cause of death by 2030 in the world's population [3].

COPD results from a lung injury that exceeds the ability of the lung repair mechanisms to restore the tissue structure and function [4]. The airway inflammation due to exposure to tobacco smoke and other pollutants is thought to be the major cause of lung damage in COPD.

Pulmonary emphysema (PE), a phenotype of chronic obstructive pulmonary disease (COPD), is a pathologic condition characterized by abnormal and permanent enlargement of the airspaces distal to the terminal bronchioles that leads to destruction of airspace walls and usually to a progressive airflow restriction [5]. Accelerated apoptosis of the airway epithelial cells and vascular endothelial cells, disruptions in the tissue maintenance and repair are the dominating mechanisms of the tissue damage and loss of elastic recoil in PE [6]. COPD patients have also been shown to have significantly reduced counts of circulating endothelial progenitors as compared to normal subjects. This phenomenon is considered as one of the mechanisms for development of emphysema [7, 8].

The modern approaches to the management of patients with COPD include smoking cessation, drug therapy with bronchodilators and inhaled steroids, pulmonary rehabilitation, noninvasive ventilation, and long-term oxygen for severe respiratory failure. In patients with severe emphysema, the technologies such as a lung volume reduction surgery and endobronchial valves and coils may be considered. Unfortunately, these methods do not affect the mechanisms of regeneration in the damaged lung, but only reduce the rate of the disease progression and frequency of exacerbations. Indeed, the cell therapy approaches aimed at recovering the lung tissue regenerative potential have been suggested to be helpful in the COPD treatment.

Preclinical Studies

In a mouse model of elastase-induced pulmonary emphysema, Ishizawa *et al.*

CHAPTER 5

Esophageal Tissue Engineering

Todd Jensen, Wael Sayej and **Christine Finck***

Connecticut Children's Medical Center Department of Surgery, 282 Washington Street Hartford, CT 06106, USA

Abstract: The focus of the chapter is to review current novel tissue engineering approaches for the esophagus. Patients suffering from esophageal defects and disease are commonly treated with surgical intervention, which can lead to a host of short and long term complications including the need for repeated surgery. The diseased segment or defect is replaced with either stomach, colon or small intestine from that patient, which lacks the motility to function as an esophageal replacement. Therefore, a new surgical therapeutic option is needed that can be tailored to each patient and be comprised of autologous cells. Many researchers have evaluated the use of native or synthetic matrix as a scaffold for bridging the gap. The cells used on these scaffolds have ranged from cells isolated from the native esophagus, pluripotent stem cells, mesenchymal stem cells and even fibroblasts. When these scaffolds and cells have been introduced in animal models, those scaffolds seeded with cells show a positive outcome with integration into host tissue and lack of a large immune response. The opposite was seen when scaffolds were implanted without any cells seeded on them. These implantation studies have also been done in larger animals and allowed to incubate for months and demonstrate anatomy similar to that of native esophagus but further studies are needed to better understand the mechanism for this result as well as what combinations of cells and scaffolding yield a positive result for the patient.

Keywords: Atresia, Autologous cells, Biopsy, Caustic injury, Decellularized, Digestive system, Eosinophilic esophagitis, Epithelial cells, Epithelial reprogramming, Esophageal cancer, Esophagus, Extracellular matrix, Fistula, Implantation, Mesenchymal stem cells, Pluripotent stem cells, Recellularized, Regenerative, Smooth muscle, Synthetic scaffold, Tissue engineering.

* **Corresponding author Christine M. Finck:** Connecticut Children's Medical Center Department of Surgery, 282 Washington Street Hartford, CT 06106; USA; Tel: 860-545-8477; Email: cfinck@connecticutchildrens.org

Atta-ur-Rahman & Shazia Anjum (Eds.)

INTRODUCTION

The esophagus is a tubular structure and while it appears simple in design, it is complicated in structure and function. The primary purpose of the esophagus is to transport food from the oropharynx to the stomach for digestion. However, disorders of the esophagus, whether congenital or acquired, can greatly impact quality of life. We will discuss the basic anatomy and diseases of the esophagus and then reflect on optimal tissue engineering approaches to solve some of the challenges that arise when esophageal function and structure are compromised.

REVIEW OF ESOPHAGEAL ANATOMY AND HISTOLOGY

The esophagus ranges in length from 10-12cm at birth and increases to 20-25cm at adulthood. The primary function is to provide a conduit for food to travel from the mouth to the stomach for processing and digestion. This process consists of rhythmic contractions and is controlled by the sympathetic and parasympathetic branches of the nervous system [1]. The esophagus is comprised of three basic layers including the mucosa, submucosa and muscle layers (Fig. **1**) [2]. The mucosa is lined with stratified epithelium which is maintained by a population of highly proliferative progenitor basal cells (p63+) present at the deepest point of the epithelial layer. These basal cells have been demonstrated to be a progenitor-like cell and therefore can be regenerative for the mucosal layer [3]. The submucosa contains blood vessels, glands, and fibroblasts and is the layer of tissue responsible for the secretion of mucus to facilitate movement of food down the esophagus. Lastly, the muscular layers are comprised of longitudinal and circular muscle layers, which are needed to propagate peristalsis.

DISORDERS OF THE ESOPHAGUS

Disorders of the esophagus can range from congenital defects, iatrogenic injury, inflammation and even cancer. Many of the current approaches to treating these conditions result in prolonged morbidity and mortality and are a significant healthcare cost. In some of these conditions, replacement of a small or large section of the esophagus with autologous engineered tissue would be beneficial.

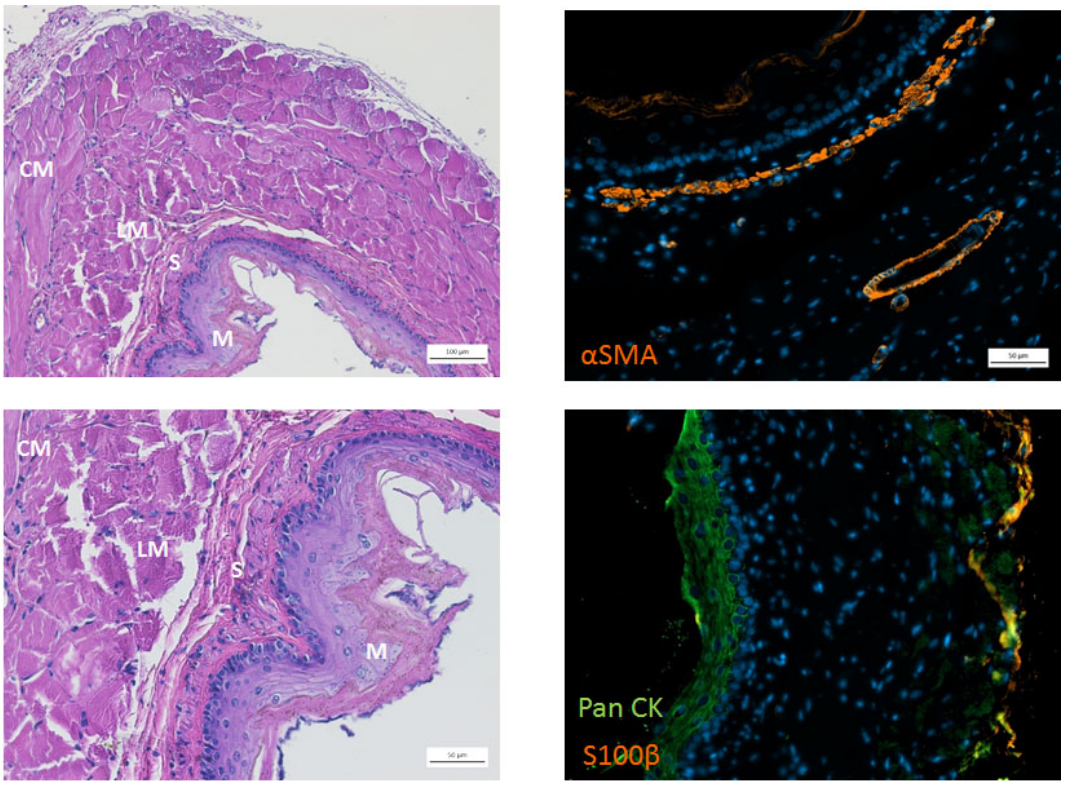

Fig. (1). Normal esophagus histology. A) H&E staining of normal esophagus reveals the mucosal (M), submucosal (S), longitudinal muscle (LM) and circular muscle (CM) layers. Immunofluorescence staining reveals epithelium staining positive for Pan Cytokeratin (Pan CK) and muscle layers as well as blood vessels staining positive for αSMA. In addition, nervous system cells are present in the peripheral layer as evidence by S100β staining. 100X and 200X Magnification.

Congenital Defects

The most common congenital malformation of the esophagus is esophageal atresia (EA) which occurs in 1 in 4,000 live births (5). There are 5 types of EA and the type most relevant to tissue engineering approaches is long gap EA (Fig. **2**). Left untreated, long gap EA is non-survivable as nutrition cannot get to the digestive tract and mucous secretions from the mouth cannot be cleared. Currently, the only course of treatment that allows the patient to eat normally without the use of a feeding tube is surgery. This entails placement of a gastrostomy tube along with attempting to replace the missing segment with other tissue from the GI tract or mechanically stretching the esophageal ends to bridge the gap. For autologous

SUBJECT INDEX

S

T

ATTA-UR-RAHMAN, *FRS*

Atta-ur-Rahman, Ph.D. in organic chemistry from Cambridge University (1968), has 1020 international publications in several fields of organic chemistry including 727 research publications, 37 international patents, 68 chapters in books and 188 books published largely by major U.S. and European presses. He is the Editor-in-Chief of eight European Chemistry journals. He is Editor of the world's leading encyclopedic series of volumes on natural products "Studies in Natural Product Chemistry" 50 volumes of which have been published under his Editorship by Elsevier during the last two decades.

Prof. Rahman won the UNESCO Science Prize (1999) and was elected as Fellow of the prestigious Royal Society (London) in July 2006. He has been conferred honorary doctorate degrees by many universities including (Sc.D.) by the Cambridge University (UK) (1987). He was elected Honorary Life Fellow of Kings College, Cambridge University, UK, conferred the TWAS (Italy) Prize and the Austrian government has honoured him with its high civil award ("Grosse Goldene Ehrenzeischen am Bande") (2007). He is Foreign Fellow of Chinese and Korean Academy of Sciences, Foreign Fellow of the Chinese Chemical Society and former President of Pakistan Academy of Sciences.

SHAZIA ANJUM

Prof. Shazia Anjum obtained her Ph.D. in organic chemistry from H. E. J. Research Institute of Chemistry, International Centre for Chemical and Biological Sciences, Karachi University, Pakistan, where she received her PhD in 2000 under the great tutelage of Prof. Dr. Atta-ur Rahman. She has authored and co-authored more than 100 research papers, a US patent, has edited 03 books and has published 02 chapters in international books. She has accomplished the synthesis of several naturally occurring aminoglycosides- that can be used as antibiotics. Dozen of students have completed their MS degrees under her supervision and couple of others are pursing for their PhD degrees.

Presently, she is the Professor of the Chemistry Department and the Director of Cholistan Institute of Desert Studies, the Islamia University of Bahawalpur, Pakistan. She is experienced medicinal and natural product chemist. As recognition of her contributions to science she has been awarded with 2 International awards from Ministry of Culture and Education, Spain and Third World Academy of Sciences Young Chemist Award, Italy. She is also recipient of several national awards.