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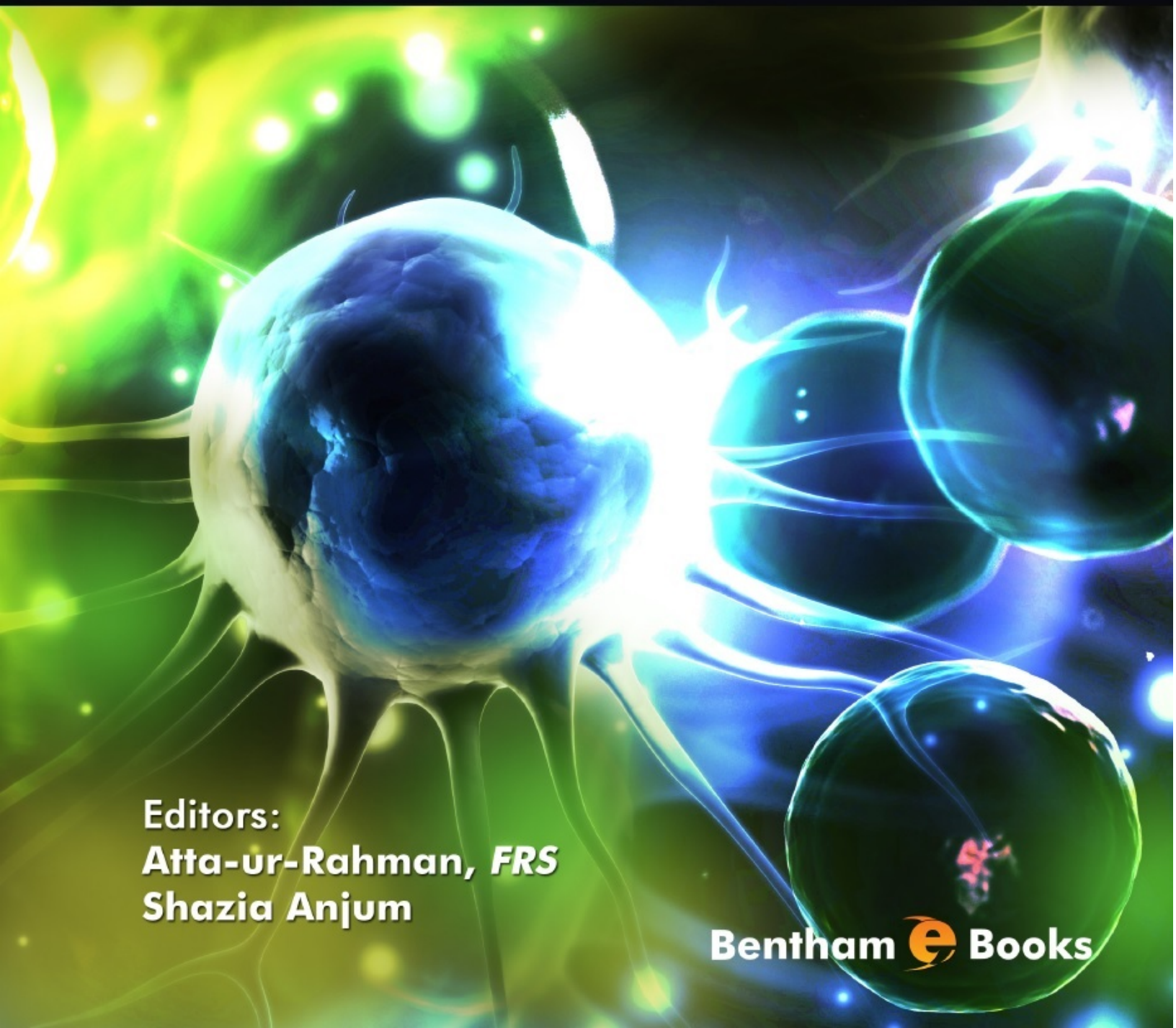
Frontiers in Stem Cell and Regenerative Medicine Research

Volume 6



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Frontiers in Stem Cell and Regenerative Medicine Research

(Volume 6)

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CONTENTS

PREFACE

Research in the rapidly emerging field of stem cells is playing a pivotal role in regenerative medicine. The first 3 chapters of this volume deal with mesenchymal stem cell research. In chapter 1, Wei Seong Toh presents the emerging role of mesenchymal stem cell (MSC) secretome as a new paradigm in treating cartilage regeneration. The study of MSC secretome allows a better mechanistic understanding of the role of MSCs in tissue repair and disease treatment.

Mauricio *et al.* describe a promising strategy for the optimization of hybrid systems through the association of biomaterials to dental pulp stem cells, in chapter 2. Dental pulp stem cells can be easily isolated from deciduous and definitive teeth. In chapter 3, Kan *et al.* describe the recent progress and the opportunities as well as challenges in MSC research.

Arteta *et al.*, in chapter 4, discuss current studies that underline the importance of liver progenitor cells (LPCs) for constructing bioartificial livers and as the source of cells for transplantation.

In the next chapter, Ward *et al.* present new exciting developments in cardiogenesis from bench-to-bedside. They review the heart development in different organisms, supplemented with insights from stem cell biology and clinical studies, which will throw light on the development of effective stem cell treatments for myocardial infarction and other cardiac diseases. Yue *et al.*, in their chapter 6, present an overview of different Ca^{2+} signalling events in the differentiation of embryonic stem cells into cardiomyocytes.

The last two chapters deal with neurodegenerative diseases. Zareen Amtul, in Chapter 7, highlights the regenerative cell-based therapies that can be used to combat neurodegenerative disorders. In the last chapter, García-Montes and Drucker-Colín discuss in detail about the central role of stem cell transplantation to cure Parkinson´s Disease. They discuss the current challenges in optimizing stem cell therapy for the treatment of Parkinson's disease.

We hope that the readers will enjoy the comprehensive reviews on new developments in stem cell and regenerative medicine research. We wish to thank the editorial staff of Bentham Science Publishers, particularly Dr. Faryal Sami, Mr. Shehzad Naqvi and Mr. Mahmood Alam for their constant help and support.

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CHAPTER 1

The Emerging Role of Mesenchymal Stem Cell Secretome in Cartilage Regeneration

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Abstract: Articular cartilage has a limited capacity to repair following injury. As a result, cartilage injuries often progress to serious joint disorders such as osteoarthritis. Mesenchymal stem cells (MSCs) are currently being evaluated in clinical trials as the therapeutic cell source for treatment of cartilage lesions and osteoarthritis. In addition to their differentiation potential, it is widely accepted that the beneficial actions of MSCs can also be mediated by their secretome. Of note, it has been demonstrated that MSCs are able to secrete a broad range of trophic factors and matrix molecules in their secretome to modulate the injured tissue environment and direct regenerative processes including cell migration, proliferation and differentiation to mediate overall tissue regeneration. The study of MSC secretome not only allows a better mechanistic understanding of the role of MSCs in tissue repair and disease treatment, but also enables the potential development of the *next-generation*, ready-to-use, highly-amenable and 'cell-free' therapeutics for clinical application. In this chapter, we present the latest understanding of MSC secretome and its components as a new paradigm for the treatment of cartilage lesions and osteoarthritis.

Keywords: Cartilage, Exosomes, Extracellular vesicles, Immunomodulation, Mesenchymal stem cells, Osteoarthritis, Secretome, Tissue regeneration.

INTRODUCTION

Articular cartilage is a unique hypocellular, avascularized and aneural load-bearing tissue, supported by the underlying subchondral bone [1]. Due to the lack of vascularization, articular cartilage has a limited capacity for regeneration upon injury. Articular cartilage injuries have a high incidence and therefore a high socio-economic and healthcare impact that cannot be underestimated. In knee joint alone, ~60% of patients who underwent arthroscopy displayed cartilage lesions [2]. When left untreated, these lesions can lead to osteoarthritis (OA), an

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inflammatory and degenerative joint disease characterized by the degradation of the articular cartilage, subchondral bone, meniscus, ligaments, and the formation of painful osteophytes. OA is the most common form of arthritis affecting numerous joints including the knee joint, hip joint, and the temporomandibular joint (TMJ), and is the leading cause of disability worldwide [3, 4].

Current treatment options for articular cartilage injuries include arthroscopic lavage and debridement, microfracture, osteochondral grafting, and autologous chondrocyte implantation (ACI) [2]. While there are tissue repair with symptomatic relief, most cartilage repair techniques lead to fibrocartilaginous tissue repair that lacks the structural organization and matrix composition of the native articular cartilage.

Stem cells represent a promising cell source for cartilage repair [5, 6]. Currently, stem cells are classified into embryonic or 'pluripotent' stem cells, and non-embryonic 'somatic', 'adult' or 'tissue' stem / progenitor cells [6]. Embryonic stem cells (ESCs) are pluripotent stem cells derived from the inner cell mass of a blastocyst, an early-stage preimplantation embryo, and are defined by two distinct properties: pluripotency and unlimited self-renewal. They are able to differentiate into cell derivatives of the three primary germ layers including ectoderm, endoderm and mesoderm [7]. With advances in stem cell biology, personalized pluripotent stem cells, also known as induced pluripotent stem cells (iPSCs) can be derived from somatic cells through reprogramming using defined gene and protein factors [8]. Of note, several groups have reported differentiation of human ESCs and iPSCs to chondrocytes [9 - 12], and demonstrated the functional efficacy of these cells for cartilage repair in animal studies [13 - 16].

Adult stem / progenitor cells are undifferentiated multipotent cells present in various adult tissues as they contribute to the physiological cell turnover as well as to tissue repair. Among these adult stem cells, mesenchymal stromal/stem cells (MSCs) are the most extensively studied and used cell type in clinical trials and have been heralded as the next major development for treatment of tissue injuries and diseases (http://www.clinicaltrials.gov). Of note, MSCs are currently being evaluated in clinical trials for treatment of cartilage injuries and osteoarthritis (OA) [17, 18]. While it is clear that MSCs are able to differentiate *in vitro* into a variety of cell types including chondrocytes, osteoblasts and adipocytes, MSCs are increasingly being investigated and harnessed for their trophic functional abilities [6, 19]. This book chapter aims to discuss the role of MSCs in cartilage regeneration and to present the latest development of MSC secretome and its components as a new paradigm for treatment of cartilage injuries and osteoarthritis.

MESENCHYMAL STEM CELLS

Mesenchymal stromal/stem cells (MSCs) are multipotent adult stem cells capable of self-renewal and multi-lineage differentiation into osteoblasts, chondrocytes and adipocytes [20]. They are easily isolated from a wide variety of tissues including bone marrow, muscle, adipose tissue, blood, and synovium [21 - 23]. MSCs are isolated as a heterogeneous cell population and characterized by their ability to adhere to plastic, form colonies in colony-forming unit-fibroblast (CFU-F) assay, and differentiate into osteoblasts, chondrocytes and adipocytes [20, 24]. According to the minimal criteria defined by the Mesenchymal and Tissue Stem Cell Committee of the International Society for Cellular Therapy (ISCT), MSCs are positive for cell surface markers CD73, CD90 and CD105, and negative for CD34, CD45, CD11b, CD14, CD19, CD79a and human leukocyte antigen (HLA)-DR surface molecules [25].

MESENCHYMAL STEM CELL-BASED THERAPIES FOR CARTILAGE REPAIR

Several MSC-based strategies for cartilage repair have been reported in animal [26] and clinical studies [17]. MSCs can be used in direct cell transplantation, and/or in combination with growth factors and scaffolds [26]. Direct transplantation of MSCs occur commonly in the form of fresh marrow or monolayer expanded and selected cells [27]. The use of fresh marrow or freshly isolated mononuclear cells is gaining interest due to their rapid availability without the need for cell expansion [28]. Furthermore, fresh marrow comprises not only MSCs but also accessory cells and growth factors.

However, in all above described cell-based strategies for cartilage repair, the culture conditions remains an issue, and there is currently poor standardization for the culture conditions and the number of cells needed for transplantation with respect to various sizes and types of cartilage lesions [29]. As with all cell-based therapies, there exist significant logistical and operational challenges associated with proper handling and cell storage to maintain the vitality and viability of the cells for transplantation [30]. With advances in proteomics, it is becoming clear that MSCs not only exhibits ability to differentiate into multiple lineages, but also secrete a broad spectrum of trophic factors in the secretome that are mediating various aspects and processes of tissue repair and regeneration [19] (Fig. **1**). In the past decade, the investigation of MSC secretome has therefore gained much attention, with the interest to decipher the factor (s) mediating the biological activity of MSCs in tissue repair.

CHAPTER 2

The Potential Clinical Application of Mesenchymal Stem Cells from the Dental Pulp (DPSCs) for Bone Regeneration

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Abstract: The skeleton has the vital role of providing support and protection to all body organs, as well as to function as storage system for ions and other components essential to its homeostasis, also presenting essential function in the movement and posture of the individual. Bone fractures are a fairly common situation affecting individuals of all ages, but gain importance when concomitant pathologies are present or when the bone lesions' extension exceeds the tissues' intrinsic healing capabilities. As such, biomedical research has invested in unveiling adequate therapies to aid in those cases. The tissue engineering field has therefore evolved in the direction of developing biomaterials and scaffolds to structure, support and promote bone ingrowth, and in developing strategies to optimize these biomaterials *in vivo* performance, by including cell-based therapies and growth factors. Herein, we discuss one promising strategy for the optimization of these hybrid systems, through the association of biomaterials to a specific source of mesenchymal stem cells: the dental pulp stem cells. Dental pulp stem cells can be found in individuals of any age, and can be easily isolated from deciduous and definitive teeth, expanded and cryopreserved for further

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use. These cells are capable of differentiating towards multiple lineages, presenting great potential for osteo-differentiation. Dental pulp stem cells have been demonstrated to incorporate diverse biomaterial systems and promote mineral deposition both *in vitro* and *in vivo*, aiming at the reconstruction of osseous defects, in either experimental or clinical situations. The mesenchymal stem cells from the dental pulp can also be found and isolated from many species other than humans, granting them potential to be implemented not only in human medicine but also in veterinary care practices, and in regenerative strategies for other organs and tissues, such as dental reconstruction and nervous system regeneration.

Keywords: Adult teeth, Animal models, Biomaterials, Bone regeneration, Cell isolation, Cell sources, Ceramic biomaterials, Deciduous teeth, Dental cells, Dental pulp, Dental regeneration, Differentiation, Growth factors, *In vitro*, *In vivo*, Mesenchymal stem cells, Nerve regeneration, Stem cells, Tissue regeneration, Tooth.

INTRODUCTION

Nearly 30 years ago, Vacanti [1] described the transplantation of cells using bio-absorbable polymer matrices. That was the beginning of tissue engineering, then described as “an interdisciplinary field that applies principles of engineering and life sciences toward the development of biological substitutes that restore, maintain, or improve tissue function”. The fundamentals of tissue engineering are based on three major pillars: scaffolds, cells and regeneration factors, all deemed essential for the success of stem cells based therapies (Fig. **1**) [2 - 4].

Stem cells are responsive undifferentiated cells with varying degrees of self-proliferation and differentiation plasticity. These characteristics makes stem cells attractive as tissue regeneration motors [5]. Although the number of stem cells is higher before birth, in the adult there are still several “niches” with a significant number of stem cells [6].

The stem cells “niches” in adults include the skin, adipose tissue, peripheral blood, bone marrow, pancreas, intestine, brain, hair follicles, and others, as well as in the dental pulp [7]. In this review we focus on dental pulp derived stem cell population. Dental stem cells (DPSCs) are one good alternative to other sources’ cells due to their easy collection from healthy donors and high proliferation and differentiation ability [8 - 11], with the advantage of being collect from both infants and adults, which are frequently subjected to dental orthodontic treatments that imply the removal of healthy teeth.

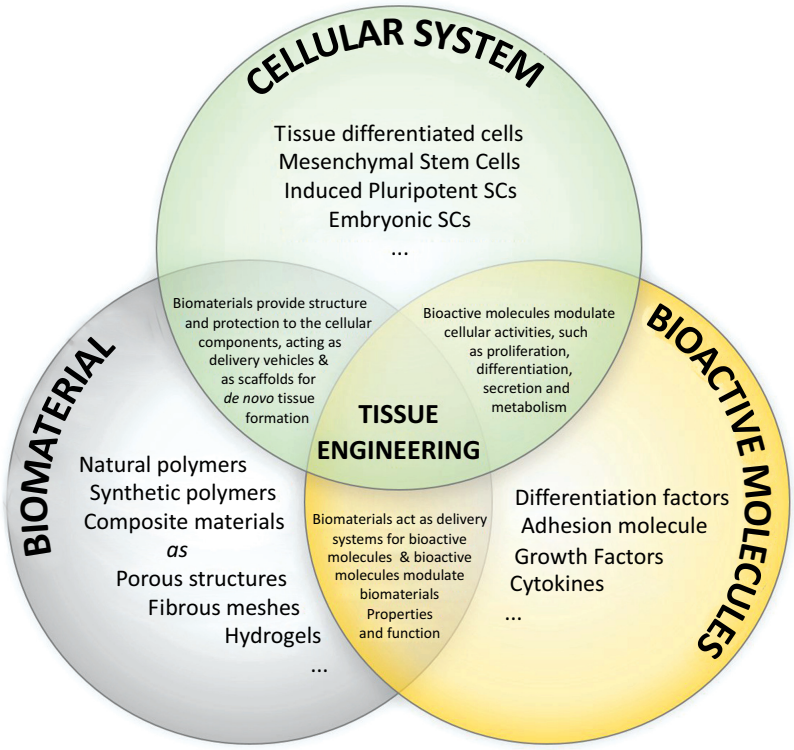

Fig. (1). The three pillars of tissue engineering – the biomaterial scaffolds that provide structure and a protective environment for the tissue regeneration; the cells that motor the regeneration itself; and the regeneration factors that promote and enhance cell growth and differentiation.

DENTAL STEM CELLS

Tooth Anatomy and the DPSCs Niche

Human teeth are an incredibly specialized organ composed of the crown (visible part that protrudes from the gingiva), the neck (the transition area), and the root (anchored to the alveolar bone). The external layer of the tooth is composed by enamel that covers and provides protection to the intermediate dentine part, and the inner pulp. Throughout life, human exhibit two series of teeth: the initial deciduous teeth (that start sequentially erupting around six months of age), followed by permanent / term or definitive teeth. The latest set of dentition starts developing from the tooth germ at approximately six years of age and sequentially 'pushes out' the deciduous dentition, in approximately six years (with exception for the 3rd molars or 'wisdom teeth' that, when present, arise around the twentieth year of age) [12].

CHAPTER 3

Mesenchymal Stem Cells in Regenerative Medicine: The Challenges and the Opportunities

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Abstract: Regenerative medicine generally aims at re-establishing the normal functions by replacing the damaged body parts through stem cells or other biomedical approaches. The tremendous opportunities of mesenchymal stem cells (MSCs) in regenerative medicine have long been recognized. However, this potential "off-the-shelf" therapeutic product is still facing daunting challenges. In this chapter, we intend to update the recent progresses and highlight the opportunities and the key challenges in the field. Our discussion has direct implications for identifying new directions for the future basic research as well as clinical applications of MSCs.

Keywords: Autoimmune diseases, Bone and cartilage diseases, Cell surface markers, Cell therapy, Cell transplantation, Clinical trial, Degenerative diseases, Immunomodulation, Mesenchymal stem cells (MSCs), "Off-the-shelf" therapeutic product, Pre-clinical investigation, Regenerative medicine, Safety profile, Tissue repair, Tissue engineering, Translational applications.

INTRODUCTION

Regenerative medicine deals with the process of engineering, replacing, or regenerating human cells, tissues or organs to restore or establish normal function. Regenerative medicine, in its broad sense, is not a new medical discipline, since the first successful bone graft (bone from a dog's skull used to repair defect in human cranium) documented by van Meenerenin in 1668, the first thyroid transplant was performed by the Swiss surgeon Kocher in 1883, and the first

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corneal transplant was performed by Zirm in 1906. However, the term of "regenerative medicine" was coined much later by Kaiser in 1992 [1].

The first multipotent adult stem cell population, the hematopoietic stem cell (HSC), which gives rise to all the blood cells, was discovered in the 1950s. Subsequent investigations, in the 1960s -1970s, from Friedenstein and colleagues found that bone marrow also contains non-hematopoietic clonogenic stromal cell population [2 - 4], referred to as colony-forming unit-fibroblasts (CFU-F). These studies revealed the plasticity of these marrow cells, *i.e.*, these cells possess the multipotent properties of adult stem cells. For this reason, these cells were later called mesenchymal stem cells (MSCs) [5, 6].

Confusingly, there are many slightly different terms or definitions, such as mesenchymal stromal cells (MSCs) [7], multipotent adult progenitor cells (MAPCs) [7], multipotent adult stem cells (MASCs) [8], multilineage progenitor cells (MLPC) [9] and skeletal stem cells (SSCs) [10], that presumably refer to similar, if not exactly the same cell population [11], and there are also other clearly distinct MSC subpopulations or MSC-like cells, such as very small embryonic-like (VSEL) stem cells [12, 13], multilineage-differentiating stress enduring (Muse) cells [14] and unrestricted somatic stem cells (USSC) [15] that are presumably with unique cell surface markers and differential potentialities. All these cause a great deal of controversies.

Nevertheless, since their original description, the presence of MSCs or MSC-like cells has been proven in almost all adult tissues such as adipose tissue [16, 17], muscle [18], peripheral blood [19], lung [20], heart [21], corneal stroma [22], dental pulp [23], placenta [24], endometrium [25], amniotic membrane [26], and Wharton's jelly [27]. More importantly, this adult stem cell population possesses many desirable features, such as easy accessibility without any significant ethic concern, excellent safety profile (non-tumorigenic and non- or low-immunogenic), ability to exert trophic effects, self-renewal and multipotent differentiation, anti-apoptotic and immunomodulatory effects, and capacity for migration to the injury site, *i.e.*, homing [28], and participating in regeneration in a variety of tissues (Table **1**). It is no wonder that the translational potential of cells was almost immediately recognized, especially for regenerative medicine.

Recent years witnessed the incredible progression and transformation of regenerative medicine, which mainly reflects the tremendous advances in the stem cell field. In fact, armed with MSCs, regenerative medicine is now unquestionably an active branch of translational research that could offer solutions and hope for people who have conditions that are currently beyond repair. The typical approaches of modern regenerative medicine include:1) the injection of stem cells

or progenitor cells (cell therapies), 2) the induction of regeneration by biologically active molecules administered alone or as a secretion by infused cells (immunomodulation therapy), and 3) transplantation of *in vitro* grown organs and tissues (tissue engineering).

Table 1. Advantages and disadvantages of the different types of stem cells.

Stem cells	Advantages	Disadvantages	References
embryonic stem cells(ES)	pluripotent, relatively easy to propagate	ethical concerns, ability to form tumors including teratoma	[34, 35]
induced pluripotent stem cells (iPSC)	Similar to ES but less controversial, no ethical concerns	low efficiency, genomic insertion, incomplete reprogramming	[36, 37]
mesenchymal stem cells(MSC)	easy accessibility, no ethic concern, excellent safety profile, trophic effects, multipotent, anti-apoptotic, immunomodulatory, homing	heterogeneous, long-term expansion may cause phenotypical changes and senescence	[38 - 40]

The unique features of MSCs and the enormous unmet needs of regenerative medicine strongly justified the tremendous worldwide effects that have devoted to realize the potentials of MSCs for treatment of a variety of different clinical disorders (see Table **2** for the ongoing clinical trials). In fact, the scope of the opportunities, including basic researches [29], translational researches [30] and clinical applications [31], are overwhelmingly broad to be covered at a reasonable depth. Therefore, in this chapter, we will have to focus only on the selected subtopic, *i.e.*, 1) perspective of MSCs in regenerative medicine, 2) the phenotypical characteristics of MSCs and the related challenges, and 3) the clinical opportunities and the challenges. In this section, we focus only on regenerative medicine, especially the highly impact disorders.

On the other hand, MSCs are also facing daunting challenges despite decades of intense research and many ongoing clinical trials [32, 33] (Table **2**). In fact, the practical and theoretical challenges that have bottlenecked the field so far are almost as great as the opportunities, if not more overwhelmingly. Therefore, similarly, we will focus only on the most important challenges.

Overall, our main aims of this chapter are to outline the current understanding of the selected field, update the recent progress and highlight the opportunities as well as the key challenges. Hopefully, our discussion will help clarify the confusion, put the key issues in right perspective and identify new fruitful directions for the future research as well as clinical applications.

CHAPTER 4

Liver Regeneration: An update on the Role of Non-Parenchymal Cells

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Abstract: Many advances have been made during last years in the field of liver regeneration. Current studies underline the importance of liver progenitor cells (LPCs) as one of the sources for constructing bioartificial livers (BAL) and as source of cells for transplantation. However, the liver microenvironment is also formed by non-parenchymal cells (NPCs) that interact with LPCs and parenchymal cells during liver regeneration. Recent advances in liver tissue engineering have shown the importance of NPCs in extracorporeal systems, such as bioreactors for BAL or in several systems of 3D culture in combination with either hepatocytes, hepatoblast or LPCs. A precise knowledge of the functional role and the relationships between the diverse liver cell types is of great importance in the development of a liver organoid. Thus, this review focuses on the role of NPCs during liver regeneration in regard to their relationship with LPCs, and their potential use in 3D and extracorporeal systems in order to improve their efficacy and thus, their potential to be in the clinical setting.

Keywords: 3D co-culture, Bioartificial liver, Bioreactor, Extracellular matrix, Hepatic stellate cells, Hepatic stem cells, Hepatocytes, Kupffer cells, Liver progenitor cells, Liver regeneration, Liver sinusoidal endothelial cells, Non-parenchymal cells.

INTRODUCTION

The liver is a complex organ with an impressive regenerative potential to recover normal liver function after injury. Even though hepatocytes are the main contributors to normal cell turnover and to liver regeneration (LR) after partial hepatectomy (PH), when the regenerative capacity of the liver is impaired, liver progenitor cells (LPCs) become activated [1, 2], proliferate and differentiate.

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LPCs consist in a bipotential progenitor cells which differentiate into hepatocytes as well as into cholangiocytes [3] depending on the signals they receive from other liver cells present in the liver microenvironment, including liver sinusoidal endothelial cells (LSECs), liver macrophages, Kupffer cells (KCs), and hepatic stellate cells (HSCs), and also by signals from the extracellular matrix (ECM) they produce [2]. In fact, activating signals from such various sources can easily reach the LPCs in liver diseases of diverse etiologies [4, 5].

The liver microenvironment is a complex milieu composed of different cell types which actively interact with each other in a well-orchestrated cellular and molecular cross-talk in the different liver cell compartments [6]. Interleukin-6 (IL-6), Tumor Necrosis Factor-alpha (TNF-α) [7], Osteopontin, Transforming Growth Factor beta (TGFβ) [8], TNF-like weak inducer of apoptosis (TWEAK) [9], Hepatocyte Growth Factor (HGF) [10] are just a few examples of cytokines and growth factors produced by the liver cells, capable of promoting LPC-mediated regeneration [11]. The joint action of these soluble factors together with cell-cell interaction and with the surrounding ECM will decide the fate of LPCs either into mature hepatocytes or cholangiocytes.

Nowadays, very active research is being carried out concerning the potential use of NPCs and LPCs in the construction of bioartificial livers and 3D co-cultures systems for clinical application. As a result many *in vitro* and *in vivo* models are being developed to look deep into the mechanisms of liver injury and generate new and improved models in order to apply them on a daily basis-clinical setting. Even though this active investigation has led to a significant increase in our knowledge in the last decade, more research is needed to comprehend the intricate network of cells and molecules that form the liver microenvironment.

Non Parenchymal Cells within the Liver Microenvironment

The liver is mainly formed by parenchymal cells, namely hepatocytes, and NPCs consisting in LSECs [12], KCs [13], HSCs [14], lymphocytes and biliary epithelial cells [15, 16] (Fig. **1**). Additionally, even though the liver contains less proportion of ECM compared to other organs, liver ECM plays an important role in maintaining the differentiated phenotype of hepatocytes and NPCs [17, 18]. In this review we will focus our attention on the main NPCs leaving out the lymphocytes for further studies due to their complexity in function and cell components, and also the biliary component, which has been already extensively reviewed [19, 20].

The endothelial cells lining the hepatic sinusoids differ in many aspects from other endothelial cells. They are fenestrated and do not possess a regular basement membrane. Additionally, LSECs express multiple endocytic receptors and surface

molecules which endow them with the ability to regulate the homeostasis and the immunological function of the liver [21 - 23].

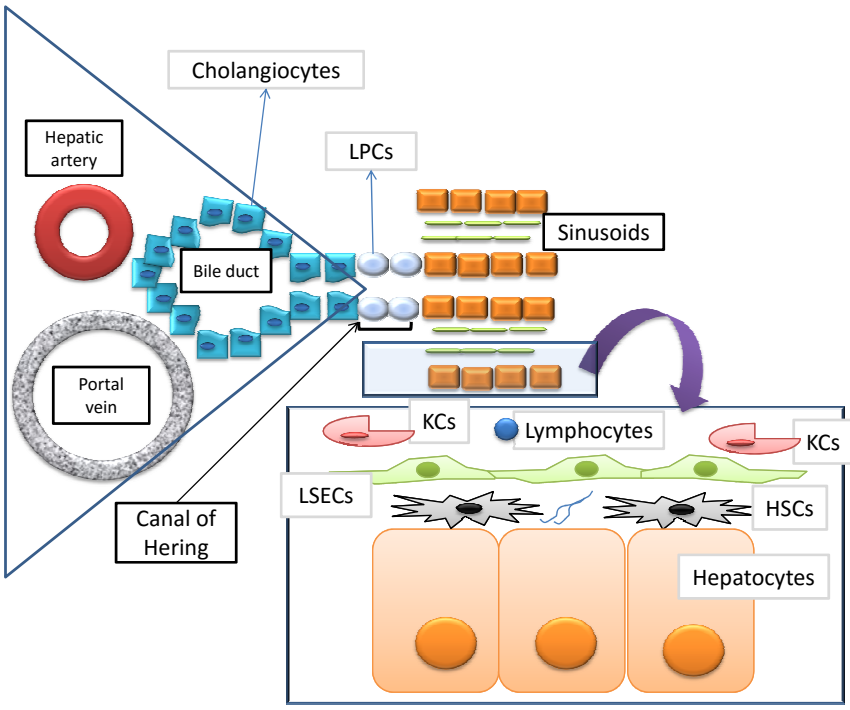

Fig. (1). Schematicview of the liver sinusoids and portal triad. Liver progenitor cells (LPCs) are located in the canal of Hering in the area of the bile ducts. LPCs are in close vicinity with nonparenchymal cells (NPCs) which include liver sinusoidal endothelial cells (LSECs), Kupffer cells (KCs), hepatic stellate cells (HSCs) and lymphocytes (NK cells, NKT cells, neutrophils, among others).

Liver macrophages/Kupffer cells lie in the hepatic sinusoids in close contact with LSECs and represent the bigger population of resident macrophages of the body [24]. They stand as a first line of defense against circulating pathogens and are able to initiate an inflammatory response in the liver by releasing multiple mediators involved in pathological processes [25].

The HSCs are resident perisinusoidal cells which possess a key role in normal and injured liver. HSCs take part in the diseases causing chronic liver injury by its contribution to portal hypertension, increased fibrogenesis, amplification of inflammation and altered matrix degradation [26].These myofibroblast-like liver non-parenchymal cells mediate the ECM deposition and remodeling in the

CHAPTER 5

Cardiogenesis and Repair: Insights from Development and Clinical Trials

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Abstract: Cardiovascular diseases are major contributors to global mortality. Myocardial infarction represents a significant complication of one such disease that affects a very large population worldwide, with the ischemic region and the resultant scar tissue generated reducing cardiac function and becoming a focus for recurrent infarctions. Several stem cell therapy approaches aimed at regenerating the non-functional myocardium have emerged using multipotent and pluripotent stem cells. However, many of the pre-clinical and clinical trials have not yielded the anticipated outcomes, and so different strategies are now being explored to achieve regeneration. The failure of these stem cell therapies may be partially attributable to the dearth of information on human cardiac developmental and regenerative pathways. However, numerous studies have investigated cardiogenesis and heart regeneration in model organisms, which have provided considerable insights into the processes of cardiac development, and other studies on the differentiation of pluripotent stem cells have largely corroborated these findings. Here we review heart development in different organisms, supplemented with insights from stem cell biology and clinical studies, which will underpin the development of effective stem cell treatments for myocardial infarction and other cardiac insults.

Keywords: Cardiogenesis, Chicken, CVD, EPC, Fruit fly, HSC, Human, Mesoderm, Mouse, MSC, Myocardial infarct, Regeneration, Stem cells, Toad, Zebrafish.

INTRODUCTION

Cardiovascular diseases (CVDs) – including coronary heart disease, ischemic heart disease, cardiomyopathies, cardiac dysrhythmias, cerebrovascular disease,

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peripheral arterial disease, rheumatic heart disease, congenital heart disease, valvular heart disease, deep vein thrombosis and pulmonary embolisms – are major causes of mortality worldwide. In 2008, CVDs were the leading cause of mortality due to non-communicable diseases globally (17 million deaths/57 million total) [1], while in the USA the deaths attributable to non-congenital CVDs were 236.1 per 1000 in 2009. CVDs also affect the quality of life and productivity of affected individuals [2]. Due to these glaring statistics, extensive research has been directed towards understanding CVDs and developing effective treatments.

Ischemic heart disease (IHD) is one of the major CVDs in terms of morbidity and mortality. IHD is caused by a reduction of blood flow due to atheroma, thrombosis or spasm, or decreased blood oxygenation due to anemia, carboxy-hemoglobulinemia and other causes. One of the main complications of ischemic heart disease is myocardial infarction (MI), a consequence of ischemia-induced cardiomyocyte death, which typically occurs due to the rupture or erosion of a coronary artery plaque. MIs initially affect the sub-endocardial myocardium, but prolonged ischemia can extend this to the sub-epicardial myocardium. Complications arising due to MI include heart failure, myocardial rupture, aneurysmal dilatation, ventricular septal defect, mitral regurgitation, cardiac arrhythmias, atrial fibrillation, conduction disturbances and post MI pericarditis. It was estimated that in the USA alone the number of individuals who have suffered MI was 7.6 million and heart failure was 5.1 million [2]. Following a MI episode a cascade of events occurs in the myocardium leading to activation of repair mechanisms, including within non-infarcted myocardium. This is principally mediated by myofibroblasts that proliferate in response to tumor growth factor β (TGFβ), leading to formation of scar tissue due to the expression of type I and fibrillar collagen [3 - 5]. The process also involves the activation of matrix metalloproteinases (MMPs) that facilitate the migration of circulatory inflammatory cells such as neutrophils and monocytes to the infarct site, where they participate in the phagocytosis and proteolytic digestion of infarcted tissue [4, 6]. The current treatment of MI involves revascularization, therapeutic vasodilation and surgical interventions [7]. The management of this condition also includes changes in lifestyle to reduce risk factors associated with further episodes. These existing approaches provide relief from symptoms and survival benefits, but fail to restore the damaged myocardial tissue to a healthy and fully functional state.

Regenerative medicine represents the means to effectively treat CVDs, providing the ability to generate new myocardial tissue *in situ*. However, to do so effectively requires a detailed understanding of cardiogenesis and cardiac stem cells. A variety of experimental systems, including both model organisms and cultured

stem cells, continue to provide important information in this regard. This Chapter reviews these experimental systems, their application to studying cardiogenesis and cardiac repair, as well as the essential lessons learned for clinical applications.

MODEL ORGANISMS IN THE STUDY OF CARDIOGENESIS

Introduction to Cardiac Development

A wide variety of model organisms have been studied to understand the mechanisms involved in embryonic development, which has provided comprehensive insights with relevance to human development. For cardiogenesis, these include invertebrate species like insects, such as fruit fly (*Drosophila melanogaster*) [8 - 10], along with several vertebrate species, most notably zebrafish (*Danio rerio*) [11 - 18], toad (*Xenopus* sp.) [19], chicken (*Gallus gallus*) [20 - 32], and mouse (*Mus musculus*) [33, 34]. Collectively, studies in those model organisms have revealed a high degree of conservation of the mechanisms involved in cardiogenesis during evolution, and allowed the dissection of the chronology, morphogenesis, cellular ontogeny and molecular pathways underlying this process.

Fruit Fly Cardiac Development

Heart tube development in fruit fly has provided a unique model system for the study of cardiogenesis. Its simplicity has facilitated the identification of the key factors involved, while its accessibility has meant that the morphological events during heart formation have been able to be dissected at the single cell level.

The heart or ‘dorsal vessel’ in the fruit fly consists of two types of cells, namely the cardioblasts and pericardial cells, each of which develop from cardiac progenitors in the lateral mesoderm as longitudinal rows (Fig. **1A**). The cardioblasts develop segmentally and migrate to the midline to form the lumen of the dorsal vessel (Fig. **1B**). The fully-developed adult heart consists of this simple linear tube, divided into anterior and posterior chambers, in which the contractile cardiomyocytes are derived from the cardioblasts and are flanked by pericardial cells (Fig. **1C**) [35, 36].

The molecular events co-ordinating the regulated movements of cardiomyocytes in fruit fly are highly conserved, with their vertebrate homologs shown to be involved in cardiogenic specification and heart development (Table **1**). The *tinman* gene, encoding a homolog of NKX2.5, was found to be expressed in the dorsal tip of the mesoderm, which contains the cardiac precursor cells and continues to be expressed in the heart progenitors until late embryonic stages [37]. The induction of *tinman* requires expression of *twist* in the presumptive

CHAPTER 6

The Role of Ca^{2+} Signalling in the Differentiation of Embryonic Stem Cells (ESCs) into Cardiomyocytes

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Abstract: Embryonic stem cells (ESCs) possess the capability of self-renewal and pluripotency. They can propagate indefinitely and differentiate into any type of cell derived from the endoderm, mesoderm and ectoderm. This makes them ideal for basic research as well as for the development of treatments and cures for a variety of different diseases. This is especially so for developing methods to treat heart disease, where they might be used for screening cardiovascular drugs or for improving transplantation procedures in a clinical application. Thus, understanding the pathways involved in the regulation of the differentiation of ESCs into cardiomyocytes and developing efficient and reliable methods to induce differentiation, are key to the progress of cardiac research. In this chapter, the main methods currently used to induce the differentiation of ESCs into cardiomyocytes are reviewed. These include the use of embryoid body (EB)-dependent cultures, two-dimensional (2D) monolayer cultures, and the co-culture of human ESCs (and iPSCs) with mouse visceral endoderm-like stromal (END-2) cells. We also present a broad overview of the different Ca^{2+} signalling events that are known to occur during the differentiation of ESCs into cardiomyocytes. These include: 1) Ca^{2+} mobilization from the endoplasmic reticulum (ER)/sarcoplasmic reticulum (SR) mediated by inositol 1,4,5-trisphosphate receptors (IP_3Rs) and ryanodine receptors (RyRs); 2) the possible involvement of cluster of differentiation 38 (CD38)/cyclic adenosine diphosphate ribose (cADPR) signalling; and 3) the influx of Ca^{2+} from the extracellular medium *via* L-type Ca^{2+} channels (LTCCs), store-operated Ca^{2+} entry (SOCE), transient receptor potential vanilloid 1 (TRPV1), and transient receptor potential canonical 3 (TRPC3) channels. Moreover, the role of the sarco/endoplasmic reticulum Ca^{2+}-ATPase (SERCA), Na^+/Ca^{2+} exchanger (NCX) and calreticulin in regulating cardiomyocyte differentiation by maintaining Ca^{2+} homeostasis is also described. Understanding how Ca^{2+} signalling regulates the differentiation of ESCs into cardiomyocytes might provide valuable clues for the development of efficacious treatments for cardiovascular disease.

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Keywords: Ca^{2+} signalling, Calreticulin, Cardiomyocyte differentiation, CD38, Co-culture, Embryoid body, Embryonic stem cells, Heart disease, IP_3Rs, LTCCs, Monolayer culture, NCX, Orai1, Pluripotency, RyRs, Self-renewal, SERCA, SOCE, Spontaneous beating, STIM1, Synchronous beating, TRPC3, TRPV1.

INTRODUCTION

Heart disease, also called cardiovascular disease, is one of the leading causes of death in the world today. Because the human heart has a very limited capacity for self-repair, there are few effective treatments for congestive heart failure, congenital heart disease, and heart attacks due to atherosclerosis [1, 2]. In the most severe cases, the only option is a heart transplant. However, there is a paucity of healthy organs from recently deceased donors that are suitable for transplantation and various post-operative complications can occur, such as organ rejection [3]. Therefore, it is imperative to develop alternative methods for treating heart disease. When embryonic stem cells (ESCs) were first isolated and characterized, and their potential for cell therapy was recognized, a lot of effort was subsequently spent in developing methods for treating cardiovascular disease *via* cell replacement. ESCs are cells in the inner cell mass (ICM) of blastocysts that possess the capacity of self-renewal and pluripotency [4, 5]. Thus, ESCs can divide in an unlimited manner and can differentiate into all the derivatives of the three germ layers (*i.e.*, the ectoderm, mesoderm and endoderm) [6, 7]. This means that ESCs hold great potential in cell therapy for the treatment of various kinds of diseases, and in heart disease in particular. Following the discovery of ESCs, the conversion of already-differentiated cells into so-called induced pluripotent stem cells (iPSCs) was made possible. For example, mouse and human fibroblasts were converted to iPSCs *via* the introduction of just four transcription factors, Oct3/4, Sox2, C-myc and Klf4 [8, 9], and human somatic cells were dedifferentiated into iPSCs with a different combination of factors, *i.e.,* Oct4, Sox2, NANOG and LIN28 [10]. iPSCs exhibit the same morphology and growth properties as ESCs, which makes them very useful in drug discovery, disease modelling and transplantation medicine. However, there are a number of major obstacles in the application of both ESCs and iPSCs in cell therapy. These include how the proliferation of transplanted cells might be controlled and how functional specifically-differentiated cardiomyocytes might be stably obtained. Therefore, a better understanding of the cellular and molecular mechanisms involved in the regulation of ESC and iPSC differentiation into cardiomyocytes, and the development of new methods of differentiation, are required before cells can be used for cardiovascular drug discovery and ultimately for the treatment of heart disease.

Similar to the *in vivo* heart development of vertebrate embryos, the differentiation of ESCs into cardiomyocytes is a multistep process, which is regulated by various signalling pathways, including the BMP/SMAD, Wnt, FGF/FGFR, and Notch/ RBP-J pathways [11 - 15]. Following the identification of these regulatory mechanisms, a variety of cardiomyocyte differentiation protocols were developed to specifically generate nodal, atrial, or ventricular-like cells. In addition, Ca^{2+} is known to act as an important second messenger in many cellular activities in cardiomyocytes, including excitation-contraction (EC) coupling and cell viability [16]. Accumulating evidence indicates that Ca^{2+} signalling also plays a key role in regulating the differentiation of ESCs into cardiomyocytes [17 - 20]. In this chapter, we review the methods currently used to induce the differentiation of cardiomyocytes from ESCs as well as the role of Ca^{2+} signalling in this process.

METHODS TO INDUCE *IN VITRO* CARDIOMYOCYTE DIFFERENTIATION

ESCs provide a viable system for generating cardiomyocytes *in vitro* and thus facilitate the study of cardiogenesis with genetic manipulation or pharmacological treatment [21]. In order to be applied effectively in drug screens and cardiovascular disease modelling and therapy, functionally mature cardiomyocytes need to be generated in large numbers. Currently, there are three main methods used for inducing the differentiation of ESCs into cardiomyocytes: (1) embryoid body (EB)-dependent culture; (2) two-dimensional (2D) monolayer culture; and (3) co-culture of ESCs with mouse visceral endoderm-like stromal (END-2) cells [22, 23]. The differentiation state of cardiomyocytes obtained *via* these methods can be verified by analyzing the expression of cardiomyocyte-specific markers *via* RT-PCR and immunohistochemistry; by determining the sarcomeric organization of the myofibrils in these cells; by measuring their Ca^{2+} handling signature; and by measuring their electrophysiological properties to determine if they show the distinct action potential phenotypes of nodal, atrial, and ventricular-like cells (Fig. **1**). In addition, the percentage of spontaneously beating cells and the beating frequency at early stages, as well as the presence of synchronous beating at later stages, are used as a measure of successful cardiomyocyte differentiation [22, 24]. The development of synchronous beating is associated with the formation of gap junctions; thus, the expression of the gap junction protein connexin 43, is also used as a means to determine the differentiation state of cardiomyocytes [25].

EB-dependent differentiation involves the 3D suspension culture of EBs, and recapitulates to a certain extent the normal growth environment of these cells by providing similar temporal and spatial cues that might occur during early embryogenesis. Using this method, cells derived from all three germ layers, including cardiomyocytes, can be generated [26]. During the formation of

CHAPTER 7

Regenerative Cell-Based Therapies to Combat Neurodegenerative Disorders

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Abstract: This chapter is a brief overview of the burden of neurodegenerative diseases, notably Alzheimer's disease, on society, the short falls of current drug therapies and emphasis on the reasons to increase our efforts in developing regenerative medicine-based stem cell therapeutics. The chapter further provides caution on unsettled translational concerns that are necessary to settle before proceeding with this innovative treatment to the clinic, in addition to highlighting the promising basic strides made in stem cell therapy of neurological disorders.

Keywords: Alzheimer's disease, Amyotrophic lateral sclerosis, Cell-based therapy, Embryonic stem cells, Epilepsy, Huntington's disease, Induced neural progenitor cells, Induced pluripotent stem cells, Neural stem cells, Neuro-degenerative disease, Parkinson's disease, Regenerative therapy, Spinal muscular atrophy, Transplantation.

INTRODUCTION

Approximately fifty million people around the world are affected by devastating neurodegenerative disorders that cost society in physician visits, hospitalization, medication, home care, and loss of productivity over 600 billion USD/year [1 - 4]. With the rise in life expectancy and aging population, the occurrence of age-related neurodegenerative disorders (such as Parkinson's disease, Alzheimer's disease, Huntington's diseases, amyotrophic lateral sclerosis, multiple sclerosis, Friedreich's ataxia and lysosomal storage disorders (for example Battens disease) is predicted to increase inexorably with enormous economic and human costs. Ironically, these neurodegenerative diseases lack effective treatment options for patients.

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Alzheimer's disease, for instance a progressive neurodegenerative ailment, is the frequent reason of age-related dementia and in the U. S. alone affects over 5 million people [5]. Over 95% of the cases of multifactorial Alzheimer's disease are sporadic in nature. Clinically Alzheimer's disease gradually impairs cognition, ability to learn, and the performance of daily activities. Pathology of Alzheimer's disease includes depletion of large cortical cholinergic projection neurons, deposition of the extracellular amyloid β-peptide containing plaques (in brain parenchyma and blood vessels) [4, 6] intracellular neurofibrillary tangles, neuroinflammation, vascular impairment and synaptic and neuronal degeneration.

All amyloid disorders share compromised proteostasis as one of the key features (amyloidosis refers to the abnormal protein folding and aggregation of amyloidogenic proteins) that result due to the formation of insoluble aggregates. Just like amyloid plaques and neurofibrillary tangles are the misfolded protein aggregates in Alzheimer's disease, mutated superoxide dismutase is in amyotrophic lateral sclerosis [7], huntingtin and α-synuclein are in Huntington's disease and Parkinson's disease [6, 8], respectively. The aggregation process can be caused by the overproduction or poor clearance of these amyloidogenic proteins. Several reports suggest that in Alzheimer's disease, Parkinson's disease, and Prion disease, amyloid oligomers are the most toxic species rather than the insoluble fibrillar species [7, 9 - 11]. The particular amyloidogenic proteins that assemble and differ among amyloid disorders but they all share identical structural motifs. These structural features are used to target amyloidogenic proteins for therapeutic purposes.

Although a variety of drugs for proteostasis can relieve some of the symptoms associated with these ailments but largely these drugs provide only moderate and temporary relief to the sufferers. As a matter of fact, due to the strong faith in the Amyloid Cascade Hypothesis, the bulk of Alzheimer's disease research during the last decades has been Aβ-based, which is unfortunately not supported by the failure of late-stage clinical trials [12 - 14]. Clinical trials on prodromal familial Alzheimer's disease cases began with the rationale that amyloid β-peptide triggers the disease and hence initiation of the Aβ immunotherapies, several years before any clinical symptoms of the disease appear, would be effective. So far, all efforts to develop medications that aim Aβ or other pathogenic pathways in sporadic cases of Alzheimer's disease have not been successful in late-stage clinical settings, with the very latest example is solanezumab by Eli Lilly phar-maceutical company [12, 15]. Several promising therapeutic candidates were supposed to be revolutionary medicines but have been unsuccessful in current trials on human (*reviewed in* [12]). Presently there is neither a cure nor an effective prevention for many of these diseases. Plausibly, a viable treatment for Alzheimer's disease has been to boost the production of cerebral acetylcholine neurotransmitter to

augment the loss of cholinergic neurons [16], however, this is not a curative measure either.

It has been challenging to learn about the cell biology of human neurodegenerative diseases until recently. Stem cell technology; such as neural stem cells (NSCs), mesenchymal stem cells (MSCs) [17], human embryonic stem cells (hESCs), pluripotent stem cells or induced pluripotent stem cell-derived neural cells (iPSCs) [18] that are differentiated into diverse types of specific neural and glial phenotypes have become an exceptionally appealing strategy, over the last couple of decades, to study and intervene neurodegenerative disorders [19, 20]. Neural cells have been successfully produced in the brain using both mesenchymal and neural stem cell transplantation and even *via* systemic injection of small molecular compounds [21]. Next to stimulating enthusiasm in developing therapeutics for neurodegenerative diseases, cell-replacement therapies also help to interpret the typical roles of neural genes in both neurodegenerative conditions as well as in neural development. Stem cells research seems to be an ideal translational therapy to progress into an effective treatment for patients suffering from Alzheimer's disease, Parkinson's disease, Huntington's disease [22, 23], spinal muscular atrophy [24] and amyotrophic lateral sclerosis [25, 26] (Table **1**). In fact, treatment trials for amyotrophic lateral sclerosis, based on stem cell therapy have already been permitted by US Food and Drug Administration [27, 28].

Table 1. Cell based therapies applied in Parkinson's disease, amyotrophic lateral sclerosis, epilepsy, Alzheimer's disease and spinal muscular atrophy.

Donor cells	Model	Graft/Injection site	Therapeutic outcome	Possible mechanism	Ref.
Fetal DA neurons (mouse)	6-OHDA rat	-	Reduced apomorphine-induced rotation	-	[62]
Embryonic NSC (human)	6-OHDA macaque	-	Lowered Gomez-Mancilla dyskinesia score	-	[63]
Embryonic DA neurons (rate)	6-OHDA rat	-	Improved survival, reinnervation, behavioral recovery	Direct communication between DA neurons and FGF-2	[64]
Mesenchymal stromal cells	ALS human	-	Minor improvement	-	[65]

CHAPTER 8

Stem Cell Transplantation: Is this the Future Solution for Parkinson's Disease?

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Abstract: Significant effort in the past three decades has been dedicated to the concept of restoring normal neurological functions through stem cell transplants, in devastating neurodegenerative diseases including Parkinson's disease. The aim of this chapter is to present an objective and up-to-date progress in this field. Specifically, the successes as well as the failures and the lessons learned from these outcomes towards further achievements in regenerative therapy are discussed. We build an easily understandable progression from basics to more complex issues and the current challenges in optimizing stem cell therapy in treatment of Parkinson's disease.

Keywords: Basal ganglia, Chromaffins, Cell replacement, Grafts, Parkinson's Disease, Stem cells.

INTRODUCTION

Parkinson's Disease (PD) is the second most frequent neurodegenerative disease after Alzheimer's disease [1]. Epidemiological studies have calculated that 1-3% of 60-year-old people is affected with PD and it is predicted that this population will be doubled in 2030 [2]. In coincidence with tau pathologies, PD increases with age, and may affect up to a 9.3% in ages between 65 to 75, males being the most affected group [3]. Clinical motor symptoms of PD are directly associated with the degeneration of dopaminergic (DAergic) neurons in the *Substantia Nigra pars compacta* (SNpc) and are correlated with the appearance of intra-cytoplasmic inclusions called Lewy bodies (LBs) [4, 5]. Usually a patient diagnosed with this pathology becomes clinically detectable as a consequence of a 30% of dopamine (DA) deficit in the striatum. The decreased number of DAergic neurons in the

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SNpc, discovered by Ehringer and Hornykiewickz in PD patients could summarize the last 50 years of research, since these studies provided the cue to treat PD. After Cotzias demonstrated that Levodopa (L-DOPA) restores the locomotion in PD patients in the 60´s [6], there was no other successful procedure to restore dopamine until the adrenal medulla (AM) cell transplantation in PD patients in the early 80´s [7, 8]. The revolutionary idea opened the possibility to treat neurodegenerative diseases such as Alzheimer disease and Parkinson's disease. After the initial grafts performed in humans, many cells have since been tested to restore the SNpc [8, 9]. It has therefore become increasingly important to understand the mechanisms through which these cells cause repair in PD as well as the specificities of the host zone. At present there are many questions on hold and new technologies such as neuro navigators, cell sorting techniques, PET Scans, better resonators, etc. have been developed to help improve implants and selectively restore DAergic neurons. Despite all these efforts, there still remain many unraveled questions about the necessary modifications required to improve the results of grafts when PD appears [10, 11]. Understanding changes induced by loss of DA in the striatum is vital to determine how to increase bioavailability of grafted cells. Currently there are new strategies to unmask the complex function of the striatum [12, 13]. Common questions such as does grafting restore normal activity of the neural circuits? Does graft life correspond to the clinical improvement? How can we avoid the damage caused by alpha synuclein in the environment?, What properties do cells need in order to integrate into the existing circuits? These and other questions have been repeatedly asked in this area of research. In summary, nowadays there are few possibilities to restore nigrostriatal innervation, but novel cell grafts may make it possible. Many cells such as pluripotent stem cells (PSCs), multipotent stem cells (MSCs), olfactory bulb (OB) cells, NTCells are still being studied, but even if we designed the perfect neuron to restore a neural circuit, there still would be a great need to deeply study proliferation and differentiation in order to be able to maintain and control the grafted cells.

Parkinson's Disease

James Parkinson describes *paralisis agitans* in the essay “The shaking palsy” in 1817, and the symptoms were later integrated by Martin Charcot who named it Parkinson´s Disease (PD) [14]. PD diagnosis is based on clinical evaluation of motor disabilities such are bradykinesia (slowness of movement), akinesia (poverty spontaneous movements and difficulty in initiating a movement), muscle rigidity, postural instability and resting tremor. These symptoms are results of the subsequent loss of DA neurotransmission and are related to the infiltration of LBs (LBs) and Lewy neurites (LNs) [5, 15]. A clinical consensus has been adopted to report the progress and quantify the symptoms of this illness in 1987, this is the

Unified Parkinson Disease Rating Scale (UPDRS) which still remains the most accepted scale in the world. The UPDRS consist of four parts: The parts I, II and III contain 44 questions measured on a 5-point-scale (0-4). These parts measure behavior and mood, daily life activities and motor skills. The last part of the scale is focused on complying with the requirements for adjunct therapy as well as the complications. According to the infiltration of LBs, Braak has described 6 stages, where stages 1-2 are preclinical and 3-6 correspond to LBs and LNs infiltration into the striatum [4]. The Striatum is a large subcortical structure extensively innervated by DAergic, serotoninergic, glutamatergic, and GABAergic inputs (see details in Basal Ganglia section). As a neurodegenerative disorder in PD mainly the nigrostriatal fibers degenerate but the atrophy of nucleus basalis Meynert is also correlated with late Braak stages 5 and 6, and patients with this atrophy also suffer dementia associated with PD [4]. In PD conditions, the indirect pathway is facilitated and the direct pathway seems to be weakened, resulting in hypokinesia [16, 17] Fig. (**1**).

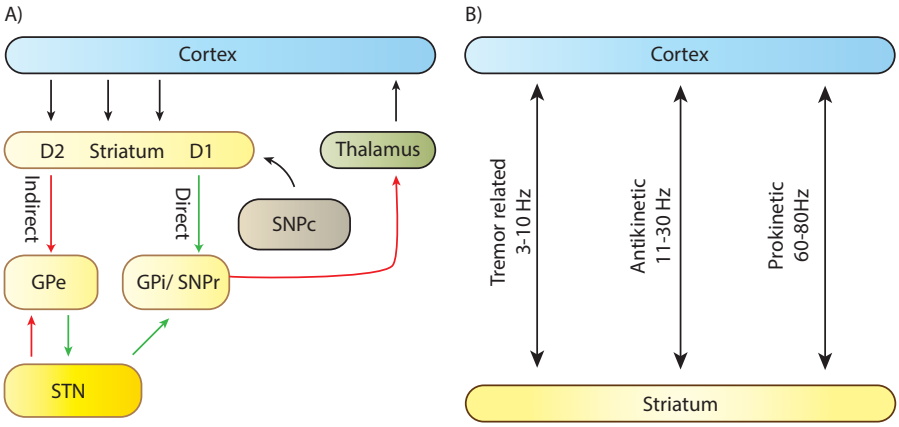

Fig. (1). Different schematic representation of basal ganglia. **A**) The Classical basal ganglia representation. SNpc; *Substantia Nigra pars compacta.* GPe; *Globus Pallidus externus.* GPi; *Globus Pallidus internus.* SNpc; *Substantia Nigra pars compacta.***B**) The Oscillatory model which correlate the tremor with an oscillation of 3-10 Hz. In the Parkinsonian state beta oscillations (11-30 Hz) are elevated. The pro-kinetic oscilation is increased in dyskinesia 60–80Hz.

Parkinson's Disease Treatment

When PD is clinically detected, L-DOPA is administered as treatment. L-DOPA is still the gold standard of treatment and is combined with aromatic amino acid decarboxylase inhibitors to avoid the peripheral metabolism of L-DOPA [18]. There is a large sum of evidence showing that with long term exposure to L-DOPA there is a loss of effectiveness during the first 2-5 years and within the following few years increases in dosage are necessary reaching at times 1 gram a day although the maximal recommended dose is 400mg/day [18, 19]. The

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Atta-ur-Rahman, Ph.D. in organic chemistry from Cambridge University (1968), has 1080 international publications in several fields of organic chemistry including 751 research publications, 37 international patents, 69 chapters in books and 221 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" 54 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.

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As recognition of her contributions to science, she has been awarded with 03 International awards like Fellowship from the *Islamic World Academy of Sciences*, Postdoctoral fellowship from the *Ministry of Culture and Education, Spain* and a Young Chemist Award from the *Third World Academy of Sciences, Italy*. She also has several national awards to her credit.