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Frontiers in Stem Cell and Regenerative Medicine Research

Volume 5

Editors:
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Frontiers in Stem Cell and Regenerative Medicine Research

(Volume 5)

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PREFACE

Regenerative medicine continues to be a hot and exciting field of research. In chapter 1, Shinoka *et al.* review a short history of organ and tissue regeneration, including recent breakthrough advances in the regeneration field, and the current state of vascular tissue engineering. The field of regenerative medicine is progressing with an accelerating pace; the prospect of using stem and progenitor cells clinically for vascular regeneration in treating vascular and ischemic diseases is on the horizon. Zeng *et al.* comprehensively describe the mechanisms of signaling and translation of stem/progenitor cells in vascular regenerative medicine in chapter 2.

Fortin *et al.*, in chapter 3, cover the field of functional integration of neural tissue grafted in animal models of CNS disease. The review comprises conventional and novel procedures that are being used to treat CNS disorders with neural tissue.

In chapter 4, Schulze *et al.* summarize the functionalized 3D scaffolds for template mediated biomineralization in bone regeneration. They describe both conventional and recent development in guided bone tissue engineering.

Regenerative medicine has undergone major exciting advances over the last few years in the treatment of ocular diseases. Valdez-Garcia *et al.*, in chapter 5, discuss recent development and as well as future prospects by using available bioinformatic tools for corneal endothelium differentiation. In the last chapter of this volume, Agorastou and Tsoulfas present the therapeutic strategies and the advances made in the management of hepatocellular carcinoma (HCC) using stem cells.

We hope that the readers will enjoy reading this volume which covers many important stem cell and regenerative medicine research. We would like to express our sincere thanks to 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

Tissue Engineering in Vascular Medicine

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Abstract: Tissue engineering is a major breakthrough in cardiovascular medicine that holds amazing promise for the future of reconstructive surgical procedures. The three main components used in creating a tissue engineered construct are: 1) a scaffold: used to mimic the extracellular matrix, 2) a cell type: seeded to the scaffold to help with biocompatibility and regeneration, and 3) cell signaling: communication between the cells *via* biochemical, physio-chemical signaling. Our goal in this chapter is to review the short history of organ and tissue regeneration, the advances in the regeneration field, and the current state of vascular tissue engineering.

Keywords: Biodegradable scaffolds, Bone marrow mononuclear cell, Cardiovascular disease, Cell seeding, Clinical trial, Congenital heart disease, Coronary artery disease, Electrospinning, iPS cell, Pediatric cardiac surgery, Peripheral artery disease, Single ventricle physiology, Stem cell, Tissue engineering, Tissue-engineered vascular grafts, Total cavopulmonary connection, Vasculature.

INTRODUCTION

Tissue engineering has boundless potential for ameliorating patient outcomes who suffer from cardiovascular diseases, especially patients with congenital cardiovascular anomalies. Congenital cardiovascular anomalies are the most common birth defect in America. Roughly 1% of all newborns is born, each year, with a congenital cardiovascular defect and, despite the significant advances in the surgical treatment of congenital cardiovascular conditions in the newborn period, congenital cardiovascular anomalies remains a leading cause of mortality in the pediatric population. Congenital cardiovascular reconstructive operations are

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created to maintain vascular continuity. They accomplish this through employing synthetic implants such as vascular grafts, valves, or patches. However, there are several associated complications in using these synthetic materials such as: thrombo-embolic events, infection, lack of growth potential, ectopic calcification and neointimal hyperplasia (which is related to poor durability of the synthetic implant). These aforementioned events are the six most common causes of illness and death following reconstructive surgery. In addition, current synthetic materials used in congenital operations, such as Goretex® or Dacron®, lack growth potential and require successive surgeries to continuously increase the size of the graft (by removing and implanting a larger graft) as the patient grows. Tissue engineering has the potential to drastically improve or even eliminate complications from using synthetic grafts through implanting a tissue engineered biologically active graft, instead of a synthetic graft, during the surgical operation. Tissue-engineered vascular grafts (TEVGs) are particularly attractive for repairing pediatric congenital cardiovascular anomalies because they have the ability to integrate with the host tissue, remodel from stress changes due to the hemodynamic environment, and grow with the patient; TEVGs also lack immunogenicity and have a lower incidence of infection.

Our team was the first in the world to implant TEVGs clinically in the pediatric population for the correction of congenital heart disease *via* an initial clinical trial involving 25 children; we successfully confirmed the TEVGs significant potential in a late-term follow up assessment [1 - 3]. The results of this study were promising and, after returning from the bedside to the bench and back, we further elucidated several of the findings behind the success of that first trial. Herein, we discuss our important findings, such as: materials for TEVG composition, methods to create TEVGs, the mechanism behind TEVG technology, and our unique experience at the forefront of TEVG clinical investigations.

A Brief History of Tissue Engineering

The initial concept of regenerative engineering, or tissue engineering, was proposed in the mid-1980s. At the time, a shortage of suitable donor organs was high and tissue engineering was proposed as a way to seek a solution to the problem. In 1993, Langer and Vacanti, pioneers of this new and exciting field, classified tissue engineering as an endeavor that required help across many disciplines, *i.e.* an interdisciplinary field, with the goal of creating new biological tissue to restore the function of diseased tissues or organs [4]. Tissue-engineered tissue is similar in function to host tissue in that it consists of the extracellular matrix (ECM), cells, and the signaling systems. The task of tissue engineering is

to use three main components: 1) a scaffold material, 2) a cell type, and 3) biochemical and physio-chemical signaling to induce new biological tissue formation. How those three components are combined is discussed in further detail below.

Scaffolds Used in Tissue Engineered Grafts

The TEVG scaffold provides a three dimensional structure for optimal cellular attachment to the scaffold, cell infiltration into the scaffold, and cell proliferation within the scaffold. So as not to induce an immunological response, the materials used in making the scaffold must be: 1) biocompatible to reduce inflammation, 2) possess biomimetic properties to stimulate cell proliferation, and 3) are created to have an architecturally porous structure that facilitates cellular infiltration. These three aspects of the TEVG scaffold will better help stimulate neotissue formation and subsequent integration with the native tissue [5, 6]. The scaffolds that best satisfy these requirements are made from biodegradable polymers and/or natural ECM based materials and are regularly used as the scaffolding material for TEVGs.

Biodegradable Polymers for Tissue Engineered Grafts

The goal of biodegradable polymer scaffolding is to serve as temporary architecture for cells to infiltrate and proliferate. While degrading, these polymers produce fragments which lead to a loss in their mechanical properties. This loss in mechanical properties is followed by a continuous decrease in their mass when compared to their volume (mass/volume). In addition, a crucial first step in designing a regenerative scaffold is in the selection of an appropriate scaffolding material. This selection is determined based on a group of factors such as the materials biodegradability, biocompatibility, and mechanical properties (Table **1**). Several biodegradable polymers, such as poly (ε-caprolactone) (PCL), poly (lactic acid) (PLA), and poly (glycerol sebacate) (PGS), have been investigated for TEVG applications. PCL and PLA have a successful clinical application history and are thus commonly used to construct vascular scaffolds [7]. Both PLA and PCL are maintained for a long period in the body due to their hydrophobic properties. Combining PCL and PLA with other degradable synthetic polymers will create copolymers where the degradation time and mechanical properties can be better adjusted for optimal performance of the graft, such as with poly (lactide-co-ε-caprolactone) (PLCL) which allows for fine adjustments of degradation rates and mechanical properties (Table **1**). For instance, we have studied and confirmed the feasibility of using *in-vivo* small-diameter PLA-PLCL arterial grafts in the high-pressure arterial environment [8]. Slow polymer degradation allows the

CHAPTER 2

Stem/Progenitor Cells in Vascular Regenerative Medicine: Mechanisms, Signalling and Translation

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Abstract: Endothelial cells (ECs) and smooth muscle cells (SMCs) play pivotal roles in maintaining vascular homeostasis. Their dysregulations are critical in the pathogenesis of various disease processes such as atherosclerosis which leads to severe cardiovascular diseases such as coronary heart disease and stroke. Vascular regeneration serves as an effective therapeutic strategy for these diseases. The therapeutic prospect of stem cell-based therapy, given the capacity of stem cells to replicate, to differentiate and to directly form new blood vessels, represents the ideal approach for vascular regeneration. The identification of adult stem/progenitor cells in both circulating blood and on the vessel wall indicates that endogenous stem cells have the capacity to repair injured endothelium and restore vascular homeostasis. On the other hand, pluripotent stem cells (PSCs), which have eminent capacity for self-renewal, represent the ideal candidates in regenerative medicine. There are enormous excitements surrounding the prospect of generating functional vascular cells through activating vascular lineage differentiation of PSCs (such as embryonic stem cells (ESCs) and induced PSCs (iPSCs)) for clinical cell therapy. Recent advances in induced lineage conversion from fibroblasts to ECs and SMCs present another exciting strategy in vascular regeneration. Progresses in vascular tissue engineering have further complemented the vascular differentiation and grafting of reprogrammed vascular lineage cells onto the sites of vessel injury, through scaffolds made with native matrices, synthetic polymers or decellularised tissues. Finally, the progresses made in generating more origin-specific vascular cells from patient-derived iPSCs have enabled researchers in uncovering new insights on the molecular mechanisms underlying various vascular diseases, as well as for drug discovery, drug screening, toxicity testing and personalised medicine delivery. In this chapter, we will describe different strategies and highlight the recent efforts in generating functional vascular cells from various populations of stem and progenitor cells, their underlying molecular mechanisms, and their roles in therapeutic vascular regeneration. With the field of regenerative medicine moving in an accelerating pace, the prospect of using stem and progenitor cells

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clinically for vascular regeneration in treating vascular and ischemic diseases is on the horizon.

Keywords: Embryonic stem cells, Endothelial cells, Induced pluripotent stem cells, Ischemic diseases, Lineage conversion, Stem cells, Tissue engineered blood vessels, Transdifferentiation, Vascular differentiation, Vascular progenitor cells, Vascular regeneration, Vascular smooth muscle cells.

INTRODUCTION

Human blood vessels consist of a monolayer of endothelial cells (ECs) that is often associated with mural cells including smooth muscle cells (SMCs) and pericytes, which together compose the indispensable part of the vascular system. The vascular system acts as the conduit transporting oxygen, nutrients, hormones and growth factors while removing metabolic waste products throughout the whole body. Vascular integrity hence plays an essential role in organ development as well as in the maintenance of tissue homeostasis. Vasculature has high plasticity, most notably in its ability in *de novo* vessel formation (vasculogenesis) and sprouting (angiogenesis), which are both important in healthy tissue repair. Disruption in vascular homeostasis can lead to numerous cardiovascular pathologies [1].

Cardiovascular disease is the leading cause of death in the world. Endothelial dysfunction and vascular remodelling are critical in the development of atherosclerosis, which can proceed to a whole range of associated cardiovascular diseases including coronary heart disease, stroke, peripheral vascular diseases (PVDs), unstable angina and sudden cardiac death [2, 3]. Many of these conditions are the results of disrupted vascular supply caused by vascular remodelling associated with atherosclerosis, leading to irreversible cell losses and organ failure. Vascular regeneration, which includes the restoration of normal vascular function and structure, the rejuvenation of vascular senescence and the growth of *de novo* blood vessels, represents an effective therapeutic strategy in relieving symptoms of tissue ischemia and preventing the eventual target-organ damages. However, difficult challenges confer upon vascular regeneration, mainly due to inadequate means to efficiently and rapidly regenerate ECs for replacement therapy.

Traditionally, cells within the adult vasculature were considered to be terminally differentiated. The principal sources of vascular regeneration were considered to be the recruitment of bone marrow-derived circulating progenitors and the re-entry of mature cells on the vascular wall back into the cell cycle. Recent efforts

in investigating the dynamic nature of blood vessels have also established vessel wall as a reservoir of multipotent resident stem/progenitor cells that can be differentiated towards ECs and SMCs. Their endogenous activities could be important in repairing injured endothelium, thereby restoring the integrity of the vessel and its function. Moreover, enormous interests are also bestowed upon activating vascular lineage differentiation of pluripotent embryonic stem cells (ESCs) and the more lineage-committed "adult" stem/progenitor cells (*e.g.* circulating and vascular wall resident stem/progenitor cells, mesenchymal stem cells (MSCs) and endothelial progenitor cells (EPCs)) for vascular repairs and for generation of tissue-engineered blood vessels (TEBVs) [4, 5]. Recent advances in somatic cell reprogramming into functional vascular cells, either through the intermediate pluripotent state (induced pluripotent stem cell (iPSCs)) or by direct lineage conversion, offer additional strategies for vascular regeneration [6, 7]. Therefore a lot of efforts have also been placed on designing the most robust and efficient strategies in generating key vascular cells from these various potential cell sources. Major strides in the development of advanced vessel scaffolds made from biodegradable polymers, native matrices or other biological materials have further broadened the toolkits for vascular regenerative cell therapy. Finally, researchers continue to uncover novel insights in the molecular mechanisms underlying many vascular pathogenesis through employing patient-specific iPSC-derived vascular cells as *in vitro* disease models. This approach is remarkably useful in identifying new drug targets and enabling the delivery of 'personalised medicine' by offering new patient-specific pharmacological, genetic and cellular therapies. This chapter will discuss the recent advances in vascular differentiation of adult stem/progenitor cells and pluripotent stem cells (PSCs), direct lineage conversion of somatic cells, vascular tissue engineering and the uses of iPSC-based vascular disease modelling to uncover novel therapeutic strategies.

VASCULAR SYSTEM

Blood Vessels and Vascular Cells

The vascular system comprises three anatomically and functionally distinct components: arteries, veins and capillaries. The arterial system is primarily involved in the delivery of oxygenated blood and nutrients from the heart to target tissues and organs. It has higher blood pressure and has a higher composition of SMCs underlying the endothelium. The capillaries allow the exchange of oxygen and nutrients with metabolic wastes between the blood and the tissues. The venous system functions by carrying deoxygenated blood, waste products, and other factors released by the tissues back to the heart. Veins tend to have larger

CHAPTER 3

Functional Integration of Neural Tissue Grafted in Animal Models of CNS Disease

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Abstract: Studies on neural cell replacement therapy appear in the literature as far back as the 19th century. While FDA-approved clinical trials have been ongoing since the 1980s, pre-clinical and clinical outcomes have been variable, and the field is still widely considered to be in its infancy today. Stem cells have properties that are suited for repair of the injured central nervous system (CNS), but a primary question is how these cells can best be grafted to produce long term functional benefit to the host environment. Among the challenges in neural cell transplantation is controlling the ultimate characteristics of grafted cells, pertaining to their survival, phenotypes and performance. This chapter will discuss phenotypic fates and functional integration of neural tissue grafted in animal models of CNS disease, with focus on researchers' current ability to anticipate graft behavior. Topics will encompass conventional and novel procedures used to treat CNS disorders with neural tissue. We will give attention to neural stem and precursor cells derived from adult, fetal and embryonic sources, as well as induced pluripotent sources, and finally the differentiated progeny of these cells.

Keywords: Astrocytes, Cell replacement therapy, Embryonic stem cells, Enzyme replacement, Graft, Immunomodulation, Induced pluripotent stem cells, Neural stem cells, Neurogenesis, Neuroinflammation, Neurons, Precursor cells, Regenerative medicine, Remyelination, Spinal cord injury, Stroke, Subgranular zone, Subventricular zone, Tissue culture, Transplant, Trophic support.

INTRODUCTION

Hundreds of millions of people around the world currently suffer from neurological disease [1]. Endogenous stem cells in the adult mammalian [2, 3]

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and adult human [4] central nervous system (CNS) may, in part, enable neurological injuries to repair themselves. Neural stem cells (NSCs) are found in at least two adult human neurogenic regions - the periphery of the anterior lateral ventricles (the subventricular zone (SVZ) of the forebrain [5]; the site of origin for olfactory bulb neurons) and the hippocampal dentate gyrus (the subgranular zone [4, 6]; a brain area involved in learning and memory). Additionally, undifferentiated cells in other areas of the CNS may also account for new neuron formation [7, 8]. Unfortunately, adult neurogenesis is likely not robust enough to address the severity of many injuries [9, 10]. As another option, NSCs and precursor cells (NPCs) can be harvested from a donor, and then expanded in tissue culture for the purpose of later transplantation.

Neural cell replacement therapy (CRT) is a promising method to help regenerate the afflicted CNS, and the promise of this approach has inspired enormous amounts of global research. In light of the numerous types of neurodegenerative diseases and neurological insults diagnosed prodigiously on an annual basis, it would seem that these research efforts are well placed. In addition to the pain and suffering of those physically afflicted, there is a significant burden placed on the lives of family and friends, as well as a financial burden placed on the community.

A RETROSPECTIVE OF NEURAL CELL REPLACEMENT THERAPY (CRT)

Documented experiments in CRT in the CNS date back as far as the late 19th century [11]. In the early 20th century, researchers grafted fetal and adult neural tissue into the brains of rodents, learning a good deal about axonal growth and other processes [12]. A principle impediment revealed in these early transplants was low or highly variable donor cell survival, which remains a problem today [13]. Neurological disorders such as spinal cord injury (SCI), stroke, Alzheimer's disease, Parkinson's disease (PD), amyotrophic lateral sclerosis (ALS) and more have been studied as applications for cell therapy. Many of the cell types used in pre-clinical studies have been of non-CNS origin, including embryonic stem cells (ESCs), induced pluripotent stem cells (iPSCs), human umbilical cord blood stem cells and mesenchymal stem cells. But various other experiments have grafted cells of CNS origin, as well, including fetal and adult differentiated and undifferentiated cells. Human fetal neuroectoderm-derived neural stem cells (NSCs) have provided particularly favorable results to support the use of cell transplantation in treating neurological disorders. However, these cells have been difficult to expand to sufficient numbers *in vitro*, and have failed to efficiently

differentiate into desired cell types when transplanted [14]. Indeed, a common practice has been to implant heterogeneous populations of cells that are uncommitted or undefined, in animal CNS injury models [1, 9, 13, 15, 16], resulting in a lack of control over donor cell phenotypic fate as a recurring hindrance in the field [17]. The data has often been difficult to interpret, given the dynamic and unpredictable phenotypic nature of the grafted material [18 - 22].

An early NSC transplant into a human patient was in 2006, for a six-year-old boy with Batten disease (juvenile Neuronal Ceroid Lipofuscinosis (NCL)) who had lost the ability to walk and talk [23], resulting neither in negative side effects nor any long-term benefit. Since 1987, the team of Robert Breeze and Neil Rosenberg has implanted human fetal dopaminergic neurons in more than 60 patients with PD [24]. Using these differentiated neurons for PD meant that each patient required several aborted fetuses as sources of donor material, which raised ethical and scientific concerns. The need for a renewable and more standardized source of cells, with fewer ethical and legislative complications, has since been significantly resolved by the use of fetal and adult neural stem and precursor cells. Three entities currently culturing and implanting their own renewable lines of human neural stem and precursor cells into human patients include Neuralstem Inc., StemCells Inc. and ReNeuron. Neuralstem Inc. is sponsoring an ALS trial. StemCells Inc., who completed a clinical trial in 2009 for the treatment of infantile NCL [15], is now sponsoring trials for SCI and Pelizaeus–Merzbacher disease [25]. And ReNeuron is treating stroke patients with its own proprietary cell line.

CURRENT NEURAL CELL REPLACEMENT THERAPY (CRT)

There are ample challenges to be addressed in current neural CRT, and high on the list is the need to ensure the reliability of renewable cell lines that are cultured extensively (*i.e.*, eventual transplant material). Renewable cell sources that are now available include those of the aforementioned companies (who have also demonstrated long term *in vitro* stability for their proprietary lines) and induced pluripotent cells, which will likely gain increased clinical use before long. Standardization is another challenge. Cell culture techniques used among the scores of facilities performing trials and experiments may best be standardized where possible. The field may also benefit by standardizing procedures for actually implanting the cells. Standardized practices would allow for consistency between different sites conducting transplant studies, and thereby easier interpretation of data. But room should be left for outside-of-the-box thinking, as well. Another obstacle is cell portability. This refers to the ease with which cells

CHAPTER 4

Functionalized 3D Scaffolds for Template-mediated Biomineralization in Bone Regeneration

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Abstract: Three-dimensional scaffolds are known to directly influence proliferation and differentiation of mesenchymal stem cells into bone tissue due to their properties such as stiffness and topography. While conventional methods for chemical induction of differentiation processes are based on incorporation of growth factors and/or cytokines *via* blending or adhesion onto the scaffold surfaces, novel approaches use template-mediated biomineralization to mimic the stimuli stem cells receive in their natural niche. This chapter summarizes recent progresses in guided bone tissue engineering with particular focus on design and functionality of three dimensional scaffolds, chemical templates and promising approaches for the corresponding cell-based approaches for future therapies.

Keywords: Biomineralization, Bone, Scaffolds, Stem cells, Template-mediation, Tissue engineering.

INTRODUCTION

Bone defects due to ablative surgery, injuries and to pathological or physiological bone resorption still represent a major challenge for dental and orthopaedic surgeons. The need for bone regeneration in cranial, oral and orthopaedic surgery is one of the central issues in clinical practice [1]. Bone defects due to tumor resections, large size fractures which do not heal without external support, major trauma are patterns for bone reconstruction. Current clinical treatments to repair bone defects are problematic and often yield poor healing due to the complicated anatomy and physiology of bone tissue, as well as the limitations of medical technology. Replacement of bone defects by autogenous bone require

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considerable amount of the autograft and therefore associated with significant donor site morbidity, including infection, pain, and hematoma formation whereas allografts introduce the possibility of immune rejection and infections. Despite significant advancements in the material science technology including computer added design (CAD), a material fulfilling all requirements of a bone substitute has not yet been developed [2].

The history of scaffold development includes three main steps: in the 1990th, scaffolds of the first generation just had to be inert materials, to avoid serious interference with natural tissue. The development of the second generation in the beginning 21st century was mainly focused on biocompatibility issues [3]. The third generation is aimed to mimic the natural bone structure in both directions: to imitate the three dimensional bulk and the surface of natural bone [4]. To realize this, biomineralization processes with special focus on initial steps were extensively studied to specify the natural multi-step process of bone formation [5]. Significant effort could be achieved when the function of so-called templates have been discovered that are able to initiate and significantly influence biomineralization processes [6]. In the following, several aspects in current scaffold production will be addressed: first, a brief overview will be given on scaffold materials, their synthesis and fabrication methods. Secondly, the scaffold functionalization strategies will be discussed, mainly divided into bulk and surface functionalization. Third, biomineralization of natural bone and artificial inorganic salts is discussed including analytical methods that are used to study apatite formation. Finally, the most important cell culture aspects are presented, with special focus on signal transduction during differentiation of mesenchymal stem cells for cell-based therapies in bone regeneration.

FUNCTIONALIZED 3D SCAFFOLDS

Scaffold Materials and 3D Fabrication

Commercial Scaffolds

The main principle behind tissue engineering involves growing the required cells *in vitro* into an appropriate three-dimensional scaffold for organ or tissue regeneration. Cells randomly migrate to form two-dimensional layers. The required growth to form 3D structures has to be stimulated and can be achieved by using porous materials, the scaffolds, to which the seeded cells attach and colonize [7].

Current bone tissue engineering strategies are very successful in fabrication of scaffold materials that exhibit attractive properties for bone repair, such as their ability to stimulate osteogenesis, support hydroxyapatite (HA) formation and incorporation into the natural bone tissue. This is well documented for different material classes, *i.e.* bioactive polymers and glasses which are studied as scaffold materials [8]. The scaffold material has to exhibit certain properties: the architecture has to provide cavities for vascularization (in particular in large scale defects) and tissue reformation. The material should be developed with a porous structure of pore sizes adjusted to for the transport of nutrients and metabolic products without a loss in mechanical stability. Furthermore, scaffolds are required to show cyto- and tissue compatibility for applied cells to attach, grow and differentiate. Bioactivity is also required to allow interactions between the scaffold and the cellular components which can be modified with cell-adhesive ligands to enhance attachment and controlled release. For hard tissue regeneration, the scaffold should possess adequate mechanical stability and the feature of the biomaterial should be in accordance with the host tissue [9]. The current commercially used 3D-structured scaffold materials are based on calcium phosphate-based bioceramics, such as HA, $Ca_{10}(PO_4)_6(OH)_2$, β-tricalcium phosphate (β-TCP), $Ca_3(PO_4)_2$, and biphasic calcium phosphate (BCP), a mixture of HA and β-TCP (Table **1**) [10].

In the following an overview is given about the most important three material classes studied for scaffold development: glasses and ceramics, natural and synthetic polymers and so-called hybrid materials that consist of inorganic and organic building blocks.

Table 1. Commercial scaffolds for bone regeneration.

Product	Producer	Shape	Material Composition
Maxresorb®	Cermisys	Granulate 500-1000 µ	60% HA 40% β-TCP
4Bone®	Augma Biomaterials	Granulate 1000-2000 µ	60% HA 40% β-TCP
MBPC+®	Biomatlante	Granulate 500-1000 µ	20% HA 80% β-TCP
OsboneS®	Curasam AG	Granulate 300-600 µ	> 95% HA < 5% TCP

Glasses and Ceramics

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CHAPTER 5

Current State and Future Perspectives in Corneal Endothelium Differentiation

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Abstract: Regenerative medicine in ocular diseases has shown major advances over the last few years. The most critical progress has been achieved for diseases of the cornea and retina, for which the transplantation of local and differentiated stem cells (SC) is being studied. The treatment of the cornea is aimed at restoring corneal clarity after severe injuries and diseases. Corneal blindness is the fourth leading cause of visual impairment worldwide, and access to corneal transplantation surgery, the main treatment for corneal blindness, is difficult given the shortage of tissue donors. For this reason, the development of alternative therapies using SC is of special interest. The corneal endothelium (CE) is the innermost layer of the cornea, and it is in contact with the aqueous humor. It consists of a monolayer of polygonal cells which maintains an optimal hydration state and clarity in the cornea through an active ATP-Na/K pump. This tissue possesses limited mitotic ability. Therefore, when major injury occurs, it can only be treated with a corneal graft. Recent advances have shown potential in harvesting corneal endothelial cells (CECs) in order to obtain enough quantities to perform a transplant. However, these strategies are still limited by the need for tissue donors, as well as by the long time lapses required to proliferate the CECs. SC isolated from different sources, including adipose tissue and dental pulp, are being investigated in regenerative medicine given their potential to differentiate into other cell lines. For use in CE restoration, a broad analysis must be performed, taking into account CE embryological pathways, current reports in SC differentiation into ocular tissues, and recently available bioinformatic tools, which can be used for differentiation assays. This review encompasses the present knowledge of CE development and embryological molecular signaling, recent reports in SC differentiation into CE, and the available bioinformatic tools used to direct *in vitro* SC differentiation.

Keywords: Bioinformatics, Bone marrow stem cell, Cornea, Corneal endothelium, Dental pulp stem cell, Differentiation, Embryogenesis, Embryonic stem cell, Growth factors, Induced pluripotent cells, Mesenchymal stem cell.

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INTRODUCTION

The cornea is transparent tissue that, in conjunction with the sclera, forms the outer part of the eye. It is an avascular connective tissue that functions as a primary barrier against mechanical and infectious damage to the internal ocular structures. It is organized into five layers: the epithelium, Bowman's layer, stroma, Descemet's membrane, and the endothelium [1]. The epithelium acts as a biodefense system and helps to maintain the corneal surface's optical smoothness. Bowman's layer is the interface between the epithelium and the stroma, and it is formed by collagen fibers that are randomly arranged. The stroma is composed of keratocytes and the extracellular matrix, and it accounts 90% of the corneal thickness. It provides structural strength, shape, and transparency to the cornea. The endothelium is the internal monolayer of cells covering the posterior surface of Descemet's membrane, and it is in contact with the aqueous humor; its main function is to regulate corneal hydration through an adenosine triphosphate (ATP) and bicarbonate-dependent pump, which allows the eye to perform its visual function properly [2]. It also serves as a system through which nutrients are passed and waste is removed through simple and facilitated diffusion and active transport [3]. The molecular characteristics of corneal endothelial cells (CECs) include: apical tight junctions (zonula occludens or ZO-1), which allow the tissue to function as a barrier; aquaporin integral proteins (AQP-1) which, together with the ATP bicarbonate-dependent, pump participate in the fluid movement across the endothelium; the secretion of collagen type VIII, the main component of Descemet's membrane; and the expression of the neuron-specific enolase (NSE), a neural crest protein [4 - 8].

The corneal endothelium (CE) does not possess mitotic ability in adults. This is because CECs are arrested in the G_0 phase of the cell cycle [9]. In healthy conditions, the average density of CECs is ~3,000 cells/mm^2. When this tissue is injured as the result of a mechanical trauma, a chemical burn, post-surgical complications, or corneal pathologies like Fuch's dystrophy, the CEC density can decrease, making it difficult to maintain corneal clarity. The result is opacity, which can lead to blindness. In order to preserve ocular transparency, the CEC density must remain above ~500 cells/mm^2 [10].

Corneal endothelial diseases that require corneal transplant include Fuchs' dystrophy, bullous pseudophakic keratopathy, posterior polymorphous dystrophy, congenital hereditary endothelial dystrophy, iridocorneal endothelial syndrome, and some intermediate forms. Corneal blindness represents the fourth leading cause of blindness worldwide (5.1%), and it is a major cause of visual impairment

following cataracts, glaucoma, and age-related macular degeneration [11]. Although penetrating keratoplasty has been the standard procedure for most diseases of the cornea, it is restricted due to a lack of donors. Moreover, the outcomes are not usually expected due to several factors, including the risk of immune-mediated graft rejection, and a significant increase in the prevalence of glaucoma following transplantation [12, 13]. The emergent strategies in the field of cell biology, as well as the tissue cultivation of CECs, aim to produce transplantable endothelial cell sheets. For this purpose, the development of novel strategies has focused on the use of cultured CECs, CESC, and SC of extra-ocular origin [14]. This chapter is focused on reviewing the current strategies used to reprogram SC from diverse tissue sources. For this purpose, knowledge of the embryological events that dictate CE formation is crucial. Likewise, the use of bioinformatics tools has proved to ease the processing of large amounts of data resulting from these assays, and they are useful when designing induced culture media for differentiation approaches.

CURRENT KNOWLEDGE OF CORNEAL ENDOTHELIUM DEVELOPMENT AND EMBRYOLOGICAL MOLECULAR SIGNALING

Corneal Embryology

The embryological origins of the major ocular structures are diverse. The central part of the cornea, including the endothelium and keratocytes, is derived from neural crest cells. Fig. (**1**) shows the embryological origin of corneal layers. The retina and the epithelial layers of the iris and ciliary body are derived from the anterior neural plate, the lens from the surface ectoderm, and the corneal epithelium from the epidermal ectoderm [15].

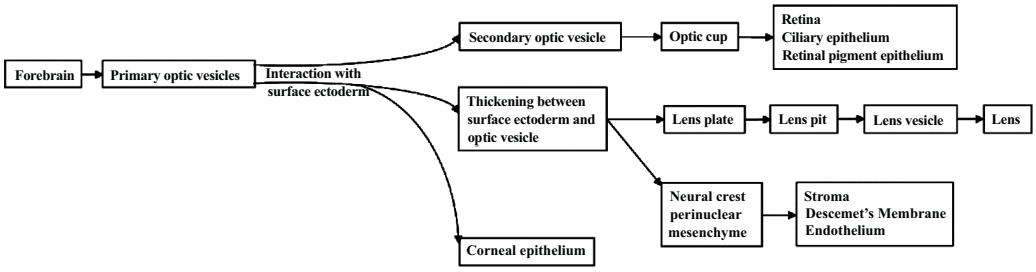

Fig. (1). Schematic representation of the embryological development of corneal layers.

The eye starts developing between the second and third week of gestation, when the embryo is around 2.6 mm in length [16]. The primary optic vesicles developed in each side of the forebrain grow and reach the surface ectoderm, giving rise to

CHAPTER 6

The Role of Stem Cells in the Management of Hepatocellular Carcinoma

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Abstract: Despite the significant advances in the management of hepatocellular carcinoma (HCC), the results are still far from satisfactory. One of the reasons for this is the need to identify the mechanisms involved in the molecular pathogenesis of HCC, so as to be able to provide patient-targeted therapies. The difficulty in this endeavor lies in the multitude of pathways involved and the challenge of finding out how each one of them fits into the larger picture.

As is the case in several different organs, stem cells appear to have a critical role in the evolution of the liver and its neoplasms, as well as in key aspects of hepatic regeneration and response to various injury mechanisms. The goal of this chapter is to present basic principles of stem cells and identify pathways involved in the development of stem cells, which can at the same time affect the evolution and diagnosis of HCC. Finally, therapeutic strategies involving stem cells will be presented, in an effort to identify future challenges.

Keywords: Bioartificial liver support system, Cancer stem cell, Cirrhosis, Complication, Epigenetic changes, Hepatic resection, Hepatocellular carcinoma, Hepatocytes, Immature cells, Immune rejection, Liver failure, Liver fibrosis, Liver progenitor cells, Multipotent cells, Orthotopic liver transplantation, Regeneration, Signaling pathways, Stem cells, Targeted therapy, Tissue engineering, Tolerance, Treatment, Tumor angiogenesis.

INTRODUCTION

Hepatocellular Carcinoma (HCC)

Hepatocellular carcinoma (HCC) is the sixth most common cancer world-wide

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with approximately 700,000 new cases a year, with increasing numbers in Europe and the United States [1]. There are various etiologies leading to HCC, with significant geographical variation. In parts of the world, such as eastern Asia and sub-Saharan Africa, chronic infection with hepatitis B virus (HBV) and aflatoxin exposure predominate, whereas in other parts of the world, such as Europe, Japan and North America, risk factors such as alcohol consumption and hepatitis C virus (HCV) infection are the main causes of HCC [2]. One of the more recent changes in the last decade is the significant increase of non-alcoholic steatohepatitis (NASH), which together with obesity, diabetes, dyslipidemia and hypertension constitute major components of the metabolic syndrome, essentially a disease of our times [3 - 6]. These are more prominent in Western societies and NASH is becoming one of the main etiologies for liver disease in general, and HCC in specific.

Most of the etiologies mentioned above have one thing in common, which is the fact that they can lead to cirrhosis. Cirrhosis represents essentially a terminal stage, where the liver has lost its normal architecture and instead of the normal and functional hepatic lobules, the predominant feature is that of fibrosis with multiple unsuccessful attempts at hepatic regeneration which lead to the formation of cirrhotic nodules, a typical element of cirrhotic architecture. Cirrhosis is the end-result of a chronic process of hepatic injury from a variety of agents (whether it is a virus or a toxin or a change in the metabolism among others), all of which lead to the same final, non-reversible stage. Despite the fact that the normal architecture has been lost, the liver continues to attempt to regenerate unsuccessfully, which is essentially a preneoplastic condition, as 80% of HCC cases develop in cirrhotic livers [7]. Identifying the full range of molecular changes leading to neoplastic transformation, would provide critical information in preventing the development of HCC or at least being able to manage it at an earlier stage. Moreover, knowledge of those molecular elements that are unique to every patient and every tumor, would allow a more patient-oriented and patient-targeted approach. The importance of this can be seen by the fact that, even in the cirrhotic patients who receive regular follow-up and screening, only about 40-60% of HCC cases are diagnosed at an early enough stage to be amenable to curative treatments [8].

The effort to approach the evolution, diagnosis and management of HCC at a molecular level has the role of stem cells at its core, as their immense capabilities and potential make them key players in this effort.

Stem Cells

Stem cells are unspecialized immature cells that can renew themselves through cell division for long periods of time, thus having a high proliferative potential and a self-renewal capacity. They are also able to undergo terminal differentiation to generate mature functional cells of different cell lineages. There are different types of stem cells that develop at different time points of the human development continuum and with varying abilities. Totipotent stem cells can become any cell in the body or placenta, while pluripotent cells can become any cell in the body and multipotent cells can become any cell within a specific germ layer or cell lineage [9]. Stem cells are found at all stages of development, from embryonic stem cells (ES) that can differentiate into all specialized cells in the human body, to adult stem cells which are capable of regenerating their tissue of origin (Table **1**) [10 - 13]. Embryonic stem cells are found at the blastocyst stage, four to five days after the union of the sperm and the egg and before the embryo implants in the uterus, and have the advantages of strong proliferation and differentiation potential, as well as the ability to be cultured to a clinically-relevant level; the main disadvantage are the ethical issues involved in their use [14]. The latter are not a problem with adult stem cells, which are easily accessible, but do not have the same potential for proliferation and differentiation as the embryonic ones [15, 16]. Adult stem cells are multipotent cells for the main part that are required for normal cellular turnover and regeneration, such as with the hematopoietic stem cells in the bone marrow (Table **2**). They are undifferentiated cells for the most part, that are able to maintain their numbers through division, whereas their progeny can evolve into various lineages. The fact that their divisions are at a slow rate, leads to reduced DNA mutation acquisition. On the other hand, embryonic stem cells are considered to be pluripotent for the most part, as they have the potential to generate all cell types in any type of organ or tissue in the body [17].

Table 1. Adult *vs.* Embryonic stem cells.

Adult Stem Cells	Embryonic Stem Cells
Less potential for differentiation and self-renewal	Extensive ability to proliferate and differentiate
Easily available	Ethical limitations
Can be used for autologous transplantation	Risk of graft rejection
Possibility of dedifferentiation	Inability to always control the differentiation process, may end up with a tumor
Quality not guaranteed for clinical need	Can be cultured in scale for clinical need

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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.

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