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# Frontiers in Stem Cell and Regenerative Medicine Research

Volume 5

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

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# **Frontiers in Stem Cell and Regenerative Medicine Research**

*(Volume 5)*

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## **Frontiers in Stem Cell and Regenerative Medicine Research**

*Volume # 5*

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# CONTENTS

<b>PREFACE</b> .....	ii
<b>LIST OF CONTRIBUTORS</b> .....	ii
<b>CHAPTER 1 TISSUE ENGINEERING IN VASCULAR MEDICINE</b> .....	3
<i>Tadahisa Sugiura, Avione Y. Lee and Toshiharu Shinoka</i>	
<b>INTRODUCTION</b> .....	3
A Brief History of Tissue Engineering .....	4
Scaffolds Used in Tissue Engineered Grafts .....	5
<i>Biodegradable Polymers for Tissue Engineered Grafts</i> .....	5
<i>Fabrication Method of Scaffold Materials for TEVGs</i> .....	6
<i>ECM Matrix-Based Natural Materials Used in TEVGs</i> .....	7
<i>Sheet-Based Techniques for Building TEVGs</i> .....	8
Cell Sources for Seeding Tissue Engineered Constructs .....	9
<i>Matured Somatic Cells for Tissue Engineering</i> .....	9
<i>Stem Cells and Progenitor Cells for Tissue Engineering</i> .....	10
Mechanism of Neotissue Formation in TEVG Remodeling .....	13
<i>Inflammatory Mediated Process</i> .....	13
<i>Endothelial-to-Mesenchymal Transition</i> .....	13
Developing Arterial TEVGs .....	15
<i>Scaffold Materials for Arterial TEVGs</i> .....	16
<i>Electrospinning Technique</i> .....	16
<i>Cell Component for Arterial TEVGs</i> .....	17
<i>Cell Source for TEVG Remodeling</i> .....	19
<i>Tissue Remodeling Process in Arterial TEVGs</i> .....	20
<i>Calcific Deposition</i> .....	21
Clinical Application of TEVGs .....	21
<i>Scaffold Fabrication</i> .....	21
<i>Cell Collection and Scaffold Preparation</i> .....	22
<i>Surgical Implantation</i> .....	22
<i>Results</i> .....	23
Perspectives for the Future .....	24
<b>CONFLICT OF INTEREST</b> .....	24
<b>ACKNOWLEDGEMENTS</b> .....	24
<b>REFERENCES</b> .....	24
<b>CHAPTER 2 STEM/PROGENITOR CELLS IN VASCULAR REGENERATIVE MEDICINE:</b>	
<b>MECHANISMS, SIGNALLING AND TRANSLATION</b> .....	32
<i>Ka Hou Lao, Xuechong Hong, Qingbo Xu and Lingfang Zeng</i>	
<b>INTRODUCTION</b> .....	33
<b>VASCULAR SYSTEM</b> .....	34
Blood Vessels and Vascular Cells .....	34
<i>Vascular Endothelial Cell</i> .....	35
<i>Vascular Smooth Muscle Cell</i> .....	37
Pathological Aberrations of ECs and SMCs in Vascular Diseases .....	38
<b>ADULT STEM/PROGENITOR CELLS IN VASCULAR REGENERATION</b> .....	39
Circulating Vascular Progenitor Cells .....	40
<i>Circulating EPCs</i> .....	40
<i>Identification and Growth of EPCs in vitro</i> .....	41
Vascular Wall Resident Progenitor Cells .....	45
Mesenchymal Stem Cells .....	46
Pericytes .....	47
<b>PLURIPOTENT STEM CELLS IN VASCULAR REGENERATION</b> .....	47
Embryonic Stem Cells (ESCs) .....	47
Induced Pluripotent Stem Cells (iPSCs) .....	48
Methods of PSCs Differentiation to Vascular Cell Lineages .....	49
<i>EB Differentiation Methods</i> .....	49
<i>Differentiation by Co-culturing with Feeder Layers Methods</i> .....	51

<i>Monolayer Differentiation Methods</i> .....	51
<i>Animal-free Differentiation Methods</i> .....	52
Molecular Mechanisms of PSCs Differentiation to Vascular Cell Lineages .....	54
<i>VEGF Signalling</i> .....	55
<i>PDGF Signalling</i> .....	55
<i>SRF-myocardin Complex</i> .....	55
<i>TGF-<math>\beta</math>/BMP Signalling</i> .....	56
<i>Wnt/Notch Signalling</i> .....	57
<i>Integrin Signalling</i> .....	58
<i>HDACs and Epigenetics</i> .....	58
<i>Non-coding RNAs (miRNAs and Long Non-coding RNAs)</i> .....	59
Therapeutic Potentials of PSCs-derived Vascular Cells .....	60
<i>Hindlimb Ischemia Model of Peripheral Artery Diseases</i> .....	61
<i>Wire-injury Model of Atherosclerotic Vascular Remodelling</i> .....	62
<i>Myocardial Infarction</i> .....	66
<i>Cerebral Ischemia Model of Strokes and Retinal Ischemia-reperfusion Injury Model of Diabetic Retinopathy</i> .....	68
<i>Current Limitations to PSCs-derived Cell Therapy</i> .....	68
<b>LINEAGE CONVERSION OF ADULT CELLS AND THEIR POTENTIALS IN VASCULAR REGENERATION</b> .....	70
Direct Lineage Conversion to Endothelial Cells .....	71
Indirect Lineage Conversion to Vascular Cells through Partially Undifferentiated State .....	73
Direct versus Indirect Lineage Conversion in Vascular Regeneration .....	73
<b>TISSUE ENGINEERING TO SUPPORT STEM/PROGENITOR CELLS IN VASCULAR REGENERATION</b> .....	74
<b>MODELLINGS FOR VASCULAR DISEASES USING STEM AND PROGENITOR CELLS</b> .....	77
Methods for Specification of Vascular Cells from PSCs .....	77
Vascular Disease Modelling Using iPSCs .....	78
<b>CONCLUDING REMARKS AND FUTURE PERSPECTIVE</b> .....	81
<b>CONFLICT OF INTEREST</b> .....	83
<b>ACKNOWLEDGEMENTS</b> .....	83
Source of Funding .....	83
<b>REFERENCES</b> .....	83

### **CHAPTER 3 FUNCTIONAL INTEGRATION OF NEURAL TISSUE GRAFTED IN ANIMAL MODELS**

<b>OF CNS DISEASE</b> .....	107
<i>Jeff M. Fortin, Brent A. Reynolds and Loic P. Deleyrolle</i>	
<b>INTRODUCTION</b> .....	107
<b>A RETROSPECTIVE OF NEURAL CELL REPLACEMENT THERAPY (CRT)</b> .....	108
<b>CURRENT NEURAL CELL REPLACEMENT THERAPY (CRT)</b> .....	109
Neural Cell Types .....	111
<i>Primary Cells</i> .....	111
<i>Embryonic Stem Cell (ESC)-Derived Neural Cells</i> .....	112
<i>Primary Neural Stem Cells</i> .....	113
<i>Induced Pluripotent Stem Cells (iPSCs) and Direct Reprogramming</i> .....	114
Survival of Grafted Neural Tissue .....	114
<i>Cell Delivery Routes</i> .....	116
Mechanisms and Preclinical Efficacy (Performance) of Neural Cell-Based Brain Therapy .....	117
<i>Cell Replacement/ Functional Integration</i> .....	117
<i>Trophic Support</i> .....	118
<i>Enzyme Replacement</i> .....	119
<i>Immunomodulatory Mechanisms</i> .....	119
<i>Remyelination</i> .....	119
<b>CONCLUDING REMARKS</b> .....	120
<b>CONFLICT OF INTEREST</b> .....	120
<b>ACKNOWLEDGEMENTS</b> .....	121
<b>REFERENCES</b> .....	121

<b>CHAPTER 4</b>	<b>FUNCTIONALIZED 3D SCAFFOLDS FOR TEMPLATE-MEDIATED BIOMINERALIZATION IN BONE REGENERATION</b>	130
	<i>Basma El Khaldi-Hansen, Fatma El-Sayed, Dorothee Schipper, Edda Tobiasch, Steffen Witzleben and Margit Schulze</i>	
	<b>INTRODUCTION</b>	130
	<b>FUNCTIONALIZED 3D SCAFFOLDS</b>	131
	Scaffold Materials and 3D Fabrication	131
	<i>Commercial Scaffolds</i>	131
	<i>Glasses and Ceramics</i>	132
	<i>Natural and Synthetic Polymers</i>	133
	<i>Hybrid Materials</i>	134
	Scaffold Functionalization	136
	<i>3D Bulk Functionalization</i>	137
	<i>Surface Functionalization</i>	140
	<b>TEMPLATE-MEDIATED BIOMINERALIZATION</b>	143
	Templates	143
	<i>Overview</i>	143
	<i>Templates in Biological Structures</i>	146
	Template-mediated Mineralization	148
	<i>Calcium Carbonate</i>	148
	<i>Silica/Silicates</i>	149
	<i>Hydroxyl Apatite</i>	150
	<i>Nanocomposites</i>	151
	<i>Bioactive Glasses</i>	154
	Bone Mineralization	156
	<i>Bone Composition</i>	156
	<i>Bone Mineralization</i>	157
	<b>BONE REGENERATION THERAPIES</b>	157
	Cell-Based Strategies for Bone Regeneration	157
	Three-dimensional Scaffolds Enhance Bone Regeneration	160
	Clinical Studies for Bone Regeneration	162
	<b>CONCLUSION</b>	164
	<b>CONFLICT OF INTEREST</b>	165
	<b>ACKNOWLEDGEMENTS</b>	165
	<b>REFERENCES</b>	165
<b>CHAPTER 5</b>	<b>CURRENT STATE AND FUTURE PERSPECTIVES IN CORNEAL ENDOTHELIUM DIFFERENTIATION</b>	179
	<i>Jorge-Eugenio Valdez-García, Judith Zavala and Victor Trevino</i>	
	<b>INTRODUCTION</b>	180
	<b>CURRENT KNOWLEDGE OF CORNEAL ENDOTHELIUM DEVELOPMENT AND EMBRYOLOGICAL MOLECULAR SIGNALING</b>	181
	Corneal Embryology	181
	Signaling Pathways Leading to CE Formation	182
	Molecules Involved in the Maintenance of CE Characteristics	184
	Molecular Markers of CECs	186
	<b>CELL THERAPY APPROACHES FOR CE RESTORATION</b>	187
	CEC Isolation and Harvest	187
	Potential of Stem Cells in CE Restoration	188
	<b>BIOINFORMATIC APPROACHES ASSISTING IN IN VITRO STEM CELL-DIRECTED DIFFERENTIATION</b>	194
	Genomic Technologies	195
	Bioinformatics Programming Tools	196
	Differential Expression	196
	Principal Components Analysis	196
	Multi-Step Approaches	197
	Advances in Corneal Endothelium	199
	<b>CONCLUSION</b>	199
	<b>CONFLICT OF INTEREST</b>	200



ACKNOWLEDGEMENTS .....	200
REFERENCES .....	200
<b>CHAPTER 6 THE ROLE OF STEM CELLS IN THE MANAGEMENT OF HEPATOCELLULAR</b>	
<b>CARCINOMA</b> .....	209
<i>Polyxeni Agorastou and Georgios Tsoulfas</i>	
<b>INTRODUCTION</b> .....	209
Hepatocellular Carcinoma (HCC) .....	209
Stem Cells .....	211
Stem Cells and Cancer .....	213
<b>CANCER STEM CELLS AND HEPATOCELLULAR CARCINOMA</b> .....	214
Human Liver Progenitor Cells .....	214
Cancer Stem Cell (CSC) Signaling in Liver Cancer .....	216
Isolation of Cancer Stem Cells – Challenges Involved .....	218
<b>TREATMENT STRATEGIES FOR HCC USING STEM CELLS</b> .....	218
Affecting Epigenetic Changes .....	219
Stem Cell Therapy and Liver Regeneration .....	219
Hepatic Stem Cell Transplantation .....	220
Stem Cells and Tissue Engineering .....	222
Stem Cells and Liver Fibrosis/Cirrhosis .....	223
Cancer Stem Cells (CSCs) and HCC .....	225
<b>CONCLUSION</b> .....	225
<b>CONFLICT OF INTEREST</b> .....	226
<b>ACKNOWLEDGEMENTS</b> .....	226
<b>REFERENCES</b> .....	226
<b>SUBJECT INDEX</b> .....	236

## 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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## 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<sup>®</sup> or Dacron<sup>®</sup>, 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 ( $\epsilon$ -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- $\epsilon$ -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

## 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 19<sup>th</sup> 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 19<sup>th</sup> century [11]. In the early 20<sup>th</sup> 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

## 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 1990<sup>th</sup>, 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 21<sup>st</sup> 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,  $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$ ,  $\beta$ -tricalcium phosphate ( $\beta$ -TCP),  $\text{Ca}_3(\text{PO}_4)_2$ , and biphasic calcium phosphate (BCP), a mixture of HA and  $\beta$ -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 <sup>®</sup>	Cermisys	Granulate 500-1000 $\mu$	60% HA 40% $\beta$ -TCP
4Bone <sup>®</sup>	Augma Biomaterials	Granulate 1000-2000 $\mu$	60% HA 40% $\beta$ -TCP
MBPC+ <sup>®</sup>	Biomatlante	Granulate 500-1000 $\mu$	20% HA 80% $\beta$ -TCP
OsboneS <sup>®</sup>	Curasam AG	Granulate 300-600 $\mu$	> 95% HA < 5% TCP

### ***Glasses and Ceramics***

The scientific interest of chemists and material scientists in the development of



**CHAPTER 5****Current State and Future Perspectives in Corneal Endothelium Differentiation****Jorge-Eugenio Valdez-García<sup>\*</sup>, Judith Zavala and Victor Trevino***Tecnologico de Monterrey, Ophthalmology Research Chair, Monterrey, Mexico*

**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<sub>0</sub> phase of the cell cycle [9]. In healthy conditions, the average density of CECs is ~3,000 cells/mm<sup>2</sup>. 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<sup>2</sup> [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].

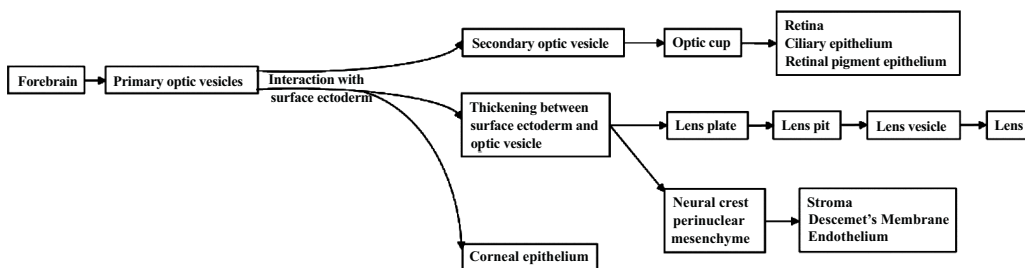


Fig. (1). Schematic representation of the embryological development of corneal layers.

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

# 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

## SUBJECT INDEX

### A

Acid 5, 6, 17, 51, 75, 134, 137, 138, 139, 140, 141, 142, 150, 151, 157, 183, 189, 192, 219  
 glycolic 6, 75, 137, 138  
 hyaluronic 139, 141, 142, 157  
 lactic 5, 6, 17, 134, 137, 138, 140  
 malic 150  
 phosphoric 150, 151  
 (RA), retinoic 51, 183, 189, 192, 219  
 Ac-LDL uptake 59, 62, 63, 64, 65, 66  
 Activated ECs 38  
 Activin 50, 51, 52, 57, 64  
 Adipose 46, 47, 135, 138, 158, 179, 189, 191, 192, 212  
 stem cells (ASCs) 135, 158, 189, 192  
 tissue 46, 47, 138, 158, 179, 189, 191, 192, 212  
 Adventitia 35, 39, 45, 46  
 tunica 35  
 Alginate solutions 134  
 Allodynia 110  
 Amniotic cells 71  
 human 71  
 Analysis, over-representation 193, 194, 196  
 Analytical methods 131, 133  
 Angiogenesis 33, 35, 40, 41, 44, 45, 56, 135, 140, 158, 160, 164  
 Anterior descending coronary artery, left 66  
 Antithrombogenic property 11  
 Apatite, nanohydroxyl 153, 154  
 Arteriogenesis 35, 70  
 Artificial stem cells type 160  
 Aspirin 161  
 Astrocytes and ependymal cells 212  
 Atherosclerosis 32, 33, 38, 39, 40, 78, 81  
 Atherosclerotic CVD 15  
 Atomic force microscopy (AFM) 137, 156  
 ATPase pump 184, 185  
 ATP pump 186, 189, 190

### B

Bioactive glass (BG) 133, 135, 139, 154, 155, 156  
 Bioartificial liver support system 209  
 Biodegradable 3, 5, 6, 10, 18, 34, 75  
 polymers 5, 6, 34  
 scaffolds 3, 10, 18, 75  
 scaffolds approach 75  
 Bioinformatic(s) 179, 181, 195, 196, 199  
 tools, available 179  
 Biomineralization 130, 131, 143, 144, 145, 146  
 Biphasic calcium phosphate (BCP) 132  
 Blood outgrowth endothelial cells (BOECs) 42, 43  
 Blood perfusion 61  
 Blood vessels 9, 10, 18, 19, 34, 37, 60, 66, 74, 75, 77, 140, 152, 160, 162  
 native 19, 75, 77  
 tissue-engineered 34, 66, 74  
 Blood vessels and vascular cells 34  
 BM-derived cells 11  
 Bone cells 153, 156, 161, 164  
 native 164  
 Bone defects 130, 160, 161, 162, 164, 165  
 critical size 160, 161  
 Bone generation 156  
 Bone marrow (BM) 3, 10, 11, 14, 22, 33, 40, 41, 43, 45, 46, 75, 76, 135, 138, 152, 158, 159, 160, 161, 179, 188, 189, 191, 192, 211, 212, 214, 217, 219, 220, 221, 222, 223, 224  
 and lympho-hematopoietic cells in blood 212  
 cells 11, 75, 224  
 mononuclear Cells 11  
 stem cell 179  
 Bone morphogenic proteins (BMPs) 44, 51, 137, 161  
 Bone sialoprotein 157  
 Bone tissue 130, 134, 136, 153, 155, 156, 160, 162, 164

- regeneration 134, 153, 155, 162, 164
  - repairing 156
  - Bowman's layer 180
- C**
- Calcific aortic valve disease (CAVD) 80
  - Calcium carbonate 146, 148
  - Calcium chloride 148, 150, 187
  - Calcium phosphate 150, 152, 157
    - cements 152
  - Calcium silicate hydrates (CSH) 150
  - Cancer stem cell (CSCs) 209, 213, 214, 215, 216, 217, 218, 219, 225
  - $\epsilon$ -caprolactone 5, 6, 155
  - Carcinogenesis 217, 218
  - Cardiac muscle cells 37
  - Cardiac surgery 3
  - Cardiomyocytes 53, 66, 71, 73, 74, 78
  - Cardiovascular cells transplantation approach 67
  - Cardiovascular disease 3, 15, 24, 32, 33, 36, 41, 48
  - CARG elements 56
  - Carotid arteries 7, 8, 73, 77
  - Cavopulmonary connections, total 3, 12, 21, 22
  - CEC density 180
  - CEC Isolation 186, 187
  - CE-like cells 186, 190, 192, 193, 199
    - generated 193
    - induced 190
  - Cell 3, 5, 6, 7, 9, 13, 15, 17, 33, 40, 53, 56, 58, 74, 75, 82, 107, 108, 109, 111, 112, 113, 114, 115, 116, 117, 118, 120, 137, 139, 141, 142, 143, 158, 180, 182, 184, 185, 187, 191, 194, 213
    - adhesion 115, 137, 141, 142, 194
    - attachment 75, 139, 142, 143
    - cycle 17, 33, 180, 182, 184
    - damage 187
    - differentiation, vascular 53, 56, 58
    - differentiation progenitor cells transform 13
    - infiltration 5, 6, 7
    - proliferation 5, 184, 185, 213
    - replacement 116, 117, 118
    - replacement therapy (CRT) 107, 108, 109, 112, 113, 114
    - seeding 3, 7, 9, 15, 75
    - therapy 40, 74, 82, 108, 111, 117, 120, 158, 191
  - Cell lineages 10, 48, 49, 54, 160, 211, 224
    - vascular 48, 49, 54
  - Cells-derived iPSCs 50, 65
  - Cells display 67
  - Cells for tissue engineering 12
  - Cellulose 137, 138
  - Central nervous system (CNS) 107, 108, 110, 115, 119, 120
  - Chemical vapor deposition (CVD) 15, 141
  - Chitosan 6, 76, 133, 134, 139, 140, 146, 147, 149, 150, 154
  - Cholangiocytes 214, 215, 219
  - Circulating 40, 41, 42, 43, 44
    - angiogenic cells 42, 43
    - EPCs 40, 41, 42, 44
    - vascular progenitor cells 40
  - Cirrhosis 209, 210, 215, 219, 223, 224, 225
  - Cobblestone morphology 36, 42, 62, 63, 64, 65
  - Collagen 7, 13, 18, 20, 36, 37, 49, 51, 62, 63, 64, 65, 66, 76, 115, 135, 137, 140, 156, 157
    - gel 7, 36, 115
  - Collagenase 187
  - Colony-forming unit-endothelial cells 42
  - Computer added design (CAD) 131, 139
  - Congenital cardiovascular anomalies 3, 4
  - Connective tissues 35, 180
  - Contact-inhibited phenotype 184, 185
  - Contractile 18, 37, 79
    - proteins 37
    - SMCs 18, 37, 79
  - Corneal 179, 180, 181, 183, 184, 185, 186, 187, 188, 190, 191, 192, 193, 194, 198, 199
    - blindness 179, 180, 187
    - endothelial cells (CECs) 179, 180, 181, 184, 185, 186, 187, 188, 190, 191, 192, 193, 194, 198, 199
    - endothelium 179, 180, 181, 183, 199
    - layers 181, 184
  - Coronary artery disease 3, 43
  - Crystalline genes 183
  - Cytokines 11, 13, 14, 36, 37, 38, 49, 51, 53, 54, 64, 65, 75, 130
    - pro-inflammatory 36, 37



**D**

Deacetylation 147  
 Decellularised scaffolds 75, 76  
   engineering human 76  
 Decellularized tissues 8, 140  
 Dental pulp 48, 179, 193, 194  
   cells 48  
   stem cells (DPSC) 179, 193, 194  
 Derived RPE cells 83  
 Descemet's membrane 180, 182, 190  
 Dexamethasone 139  
 Differential scanning calorimetry (DSC) 137  
 Direct lineage conversion 34, 70, 71, 73  
 Diseases 3, 4, 15, 32, 33, 38, 61, 79, 110, 112, 162, 163, 164, 179, 181, 210, 212  
   congenital heart 3, 4  
   coronary heart 15, 32, 33  
   ischemic 33, 38  
 DNA synthesis 184, 185, 194  
 Donor tissue 8, 15, 24, 199  
 Dopamine-producing fetal cells 112  
 Dopaminergic 111, 113, 114  
   donor cells 111  
   neurons 113, 114  
 Dysfunctional 38, 80  
   iPSCs-derived cells 80  
   vascular cells 38

**E**

EC 52, 55, 58, 59, 78  
   differentiation 55, 58, 59, 78  
   progenitors 52  
 ECs and mural cells 68  
 Electron configuration 141  
 Electrospinning 3, 6, 15, 16, 17, 133, 138, 142, 155  
   technique 15, 16, 17  
 Electrospun composites 135, 136, 142  
 Embryogenesis 179, 182, 183, 194, 197, 199  
 Embryoid bodies (EB) 49, 58, 62, 63, 64, 65, 66  
 Embryological origin 181  
 Embryonic stem cells (ESCs) 12, 32, 33, 34, 47, 48, 49, 51, 52, 59, 64, 78, 107, 108, 112,

158, 160, 163, 164, 179, 189, 192, 193, 194, 197, 198, 199, 211, 222, 223, 224  
 Endotemplating 144  
 Endothelial cells 9, 10, 13, 17, 32, 33, 41, 43, 66, 71, 140, 158, 161, 162, 163, 180, 182, 188, 219  
   native 13  
 Endothelial cells (ECs) 8, 9, 10, 13, 17, 18, 19, 32, 33, 36, 38, 39, 40, 41, 42, 43, 45, 47, 48, 50, 52, 53, 54, 56, 58, 60, 61, 63, 64, 65, 66, 67, 68, 71, 72, 73, 77, 81, 140, 158, 160, 161, 163, 179, 180, 182, 219  
 Endothelial 8, 10, 11, 19, 34, 40, 41, 42, 43, 44, 45, 46, 57, 58, 59, 64, 66, 160, 191  
   colony-forming cells (ECFCs) 41, 42, 43, 44, 45, 64, 66  
   differentiation 57, 58, 59  
   outgrowth cells (EOCs) 42, 43  
   progenitor cells (EPCs) 8, 10, 11, 19, 34, 40, 41, 42, 43, 44, 45, 46, 66, 160, 191  
 Endothelialization 11, 17, 19, 20  
 Engineered blood vessels 33, 34, 74, 82  
 Engineering scaffold 135  
 Enzyme replacement 107, 119  
 Ependymal cells 212  
 Epidermal growth factor (EGF) 184, 185, 187, 189  
 Epigenetic 12, 58, 59, 73, 80, 114, 192, 197, 209, 216, 218, 219, 222  
 Epithelium 180, 182, 186, 189  
 ESCs, cells resemble 48  
 Ethylene glycol 153, 155  
 Expressed genes, differential 196  
 Expression 12, 18, 48, 56, 58, 59, 193  
    $\alpha$ -SMA 18  
   gene 12, 48, 56, 58, 59, 193  
 Extracellular matrix (ECM) 3, 4, 8, 9, 10, 13, 18, 19, 37, 49, 52, 54, 76, 115, 139, 180, 182, 194

**F**

Femoral artery 62, 64  
   wire-injured 64  
 Fetal ventral midbrain cells 111  
 Fibroblast growth factor (FGFs) 50, 51, 66, 115, 162, 185  
 Fibronectin 10, 42, 49, 50, 51, 66, 76, 115

Fibrosis 210, 219, 221, 223, 224  
Fluorescence-activated cell sorting (FACS) 49, 50, 69  
Focal adhesion kinases (FAK) 58  
Foot necrosis 62, 63, 64

## G

Gastric epithelial cells 48  
Gene 193, 194, 195  
    expression Omnibus (GEO) 193, 195  
    ontology 193, 194  
Generating vascular cells 51, 81, 83  
Genomic technologies 195  
Glaucoma 181, 185  
Glial fibrillary acidic protein (GFAP) 190  
Glycogen synthase kinase (GSK) 51, 189, 190  
Graft 23, 24, 116  
    -related Complication 23, 24  
    survival 116  
Green fluorescent protein (GFP) 39  
Guided differentiation of MSCs 138, 139

## H

HA–collagen scaffolds 135  
Haemophilia 80  
Hepatic 209, 212, 215, 219, 220, 221, 223  
    resection 209, 219, 221, 223  
    stem cell 212, 215  
    stem cell transplantation 220  
Hepatitis 210  
    B virus (HBV) 210  
    C virus (HCV) 210  
Hepatoblasts 214, 215  
Hepatocellular carcinoma 209, 214, 216  
Hepatocytes 48, 71, 209, 212, 214, 215, 219, 220, 221, 222, 223, 224, 225  
Heterogeneity 214  
Hexagonal morphology 184, 188  
Human fetal 108, 111  
    mesencephalic cells 111  
Human 48, 71, 72, 73, 111, 114, 197, 214  
    fetal striatal cells 111  
    fibroblasts 48, 71, 72, 73, 114  
    liver progenitor cells 214  
    primordial germ cells 197

Huntington's disease (HD) 111, 119  
Hutchinson-Gilford progeria syndrome (HGPS) 39, 79  
Hybrid scaffolds 134, 138, 164  
Hydrogels 76, 137, 139, 140, 154  
Hydroxyl apatite 150, 151, 152, 156, 157  
    structure of 151

## I

Immature 211, 217  
    cells, unspecialized 211  
    epithelial cells 217  
Immune 12, 61, 69, 114, 119, 131, 164, 209, 212, 213, 217, 220, 221, 223, 224  
    rejection 12, 131, 209, 221  
    response 61, 69, 114, 213  
    system 119, 164, 212, 217, 220, 223, 224  
Immunodeficient mice 43, 60, 61  
Immunogenic antigens 69  
Intramyocardial injection 64, 65, 67  
Ionic liquids 144, 146, 148, 149  
Isolation of cancer stem cells 218

## K

Keratocytes 180, 181, 186

## L

Lens 182, 183  
    placode 182, 183  
    plate 182  
    vesicle 182  
Leukemia inhibitory factor (LIF) 49, 119  
Limbal stem cell (LSC) 193  
Lineage 49, 50, 51, 70, 72, 73  
    conversion approaches 72, 73  
    conversion of adult cells 70  
    vascular 49, 50, 51  
Liver 209, 214, 215, 216, 217, 219, 220, 221, 222, 223, 224, 225  
    cancer 216, 217, 224, 225  
    cancer cells 216, 217  
    fibrosis 209, 223  
    progenitor cells 209, 215, 225  
    transplantation 214, 221, 222, 223, 224, 225

orthotopic 209, 219, 220  
 Low-density lipoprotein (LDL) 36  
 Lympho-hematopoietic cells 212

## M

Magnetic activated cell sorting (MACS) 49, 69  
 Markers 42, 214  
   hematopoietic stem cell 42  
   phenotypic 214  
 Mesenchymal cells 13, 20, 161, 189, 193  
   adult 189  
 Mesenchymal stem cells (MSCs) 10, 11, 34, 39,  
   46, 47, 48, 56, 108, 119, 130, 131, 140,  
   157, 158, 159, 160, 161, 162, 163, 179,  
   188, 189, 191, 193, 196, 212, 224  
 Mesenchyme, periocular 182, 183, 184  
 Micro-grafted rat dopaminergic cells 115  
 Mineralization processes 145, 147, 148, 154  
 Mitogen-activated protein kinase (MAPK) 225  
 ModRNA 49, 54, 78  
 Monolayer 50, 51, 52, 53  
   cultures 52, 53  
   differentiation approach 50  
   differentiation methods 51, 52  
 Mononuclear cells 3, 10, 11, 42  
 Motor neurons 113, 114, 118  
 Multi-lineage differentiation ability 46  
 Mural cells 33, 68  
   hESC-derived 68  
 Myocardial 46, 64, 66, 67, 78  
   infarction (MI) 46, 64, 66, 67, 78  
 Myocardin 37, 55, 56, 57, 59

## N

Neointima 44, 46, 64  
   formation 44, 46  
   hyperplasia 64  
 Neotissue 9, 13, 18, 19, 20  
   cells 19  
   formation 9, 13, 20  
   hyperplasia 13, 18  
 Neovascularisation 40, 63  
 Nerve growth factor (NGF) 187  
 Neural 56, 59, 73, 74, 107, 108, 110, 113, 116,  
   117, 119, 120, 181, 182, 184, 190, 212

cell replacement therapy 107, 108  
 crest cells 181, 182, 184, 190  
 crest stem cells 56, 59  
 CRT 110, 116, 117  
 stem cells (NSCs) 73, 74, 107, 108, 110,  
   113, 119, 212  
 tissue 107, 116, 120  
 Neurogenesis 107, 108, 113  
 Neurological disorders 108, 116, 117  
 Neuronal ceroid lipofuscinosis (NCL) 109, 119  
 Neuron-specific enolase (NSE) 180, 186, 189,  
   191  
 New bone tissue formation 161  
 Next-generation sequencing (NGS) 195, 196,  
   199  
 NSCs and precursor cells 108  
 Nuclear magnetic resonance (NMR) 133, 137

## O

Ocular development master gene 183  
 Oligodendrocytes 110, 114, 120, 212  
 Osteoblasts 138, 139, 141, 142, 153, 156, 157,  
   158, 159, 161, 162  
 Osteocalcin 157  
 Osteoclastogenesis 21  
 Osteoclasts 153, 156, 157  
 Osteogenesis 21, 133, 135, 140, 141, 161  
 Osteogenic differentiation 21, 134  
 Osteonecrosis 162, 164  
 Osteoporosis 152, 153, 163, 164  
 Osteoprogenitor cells 158

## P

Pancreatic stem cells 212  
 Parkinson's disease (PD) 108, 109, 111, 112,  
   119  
 Pediatric cardiac surgery 3  
 Periocular mesenchyme cells 183, 198  
 Peripheral 3, 11, 19, 33, 40, 42, 45, 60, 61, 62,  
   141, 158, 159, 212, 213, 222, 223  
   artery disease 3, 60, 61  
   blood 11, 19, 40, 42, 45, 158, 159, 212, 213,  
   222, 223  
   blood MNCs 42

vascular diseases (PVDs) 33, 62, 141  
PGA scaffold 12, 18, 75  
Plaque cells 39  
Platelet-derived growth factor (PDGF) 47, 49, 55, 66, 185  
Pluripotency factors 73, 74  
Pluripotent cells 12, 109, 179, 186, 192, 194, 211  
    induced 109, 179, 192  
    non-immunogenic 192  
Pluripotent stem cells (PSCs) 12, 32, 33, 34, 40, 47, 48, 49, 51, 52, 53, 54, 56, 57, 60, 66, 69, 75, 77, 81, 82, 107, 108, 114, 158, 160, 163, 222, 223  
Polyelectrolyte multilayers 143  
Polyglycerols 154  
    hyperbranched 154  
Polygonal cells 43, 179, 190  
    yielded 190  
Polygonal morphology 63, 65, 187, 190, 191  
Polymers 5, 6, 7, 76, 135, 137  
Primary Neural Stem Cells 113  
Principal component analysis (PCA) 197, 199  
Principal components (PC) 196, 197  
Progenitor cells 8, 10, 11, 19, 33, 34, 32, 39, 40, 41, 43, 45, 46, 47, 50, 66, 77, 160, 212, 214, 216, 217, 218, 219, 224  
    adult multipotent 40  
    adventitial 46  
    cardiovascular 50  
    c-Kit<sup>+</sup> resident 45  
    ductular 224  
    endothelial 8, 10, 11, 19, 34, 40, 41, 66, 160  
    hematopoietic 43, 160  
    hepatic 214, 219  
    resident 45  
    smooth muscle 40  
    vascular 19, 33, 39, 40, 66  
    vascular wall resident 45, 46  
    vein-derived adventitial 46  
Progenitors 48, 51, 62, 157, 212, 213  
    -derived EC-like cells 62  
Progerin 79  
Promoter regions 55, 56, 59  
Protein kinase A (PKA) 55, 77  
Proteoglycans 18, 37, 156  
PSC-derived 52, 60, 61, 62, 63, 64, 65, 66, 68, 81

cells 52, 62, 63, 64, 65, 66, 68, 81  
vascular cells 61  
vascular cells in repairing endothelium 62  
vascular cells/progenitors 62  
cells 60  
PSCs 49, 54, 60, 69, 77  
    -derived Vascular Cells 60, 69  
    differentiation 49, 54, 77  
Pulmonary hypertension 38, 39

## R

Reactive oxygen species (ROS) 39  
Regeneration 3, 10, 17, 36, 68, 117, 160, 189, 191, 209, 210, 211, 214, 215, 220, 221, 222, 224  
    hepatic 209, 210, 221, 222  
Regeneration process 219, 220, 225  
Regenerative medicine 32, 48, 49, 68, 70, 81, 83, 107, 112, 141, 158, 179, 186, 192  
Remodeling, delayed tissue 6, 16  
Remyelinated host cells 117  
Remyelination 107, 119, 120  
Retinal pigment epithelial (RPE) 69

## S

Scaffold (s) 5, 6, 7, 8, 9, 13, 15, 20, 21, 75, 76, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 153, 154, 223  
    biodegradable polymer 153, 223  
    bulk 136, 137, 139, 140  
    composite 134, 138  
    degradation 13, 20  
    development 131, 132  
    fabrication 21, 134, 139, 142  
    functionalization 136, 140  
    functionalization strategies 131  
    materials 5, 6, 15, 131, 132, 133, 138, 140  
    matrix 137, 141  
    natural ECM-based 7, 75  
    porous 140, 154  
    surfaces 130, 133, 136, 139, 141, 142, 143  
    tubular 9, 21, 76  
SC differentiation approaches 194  
Selective 139, 142

- laser ablation (SLA) 139, 142
- laser sintering (SLS) 139, 142
- Serum response factor (SRF) 37, 55, 56, 59
- Silk fibroin (SF) 6, 135, 151
- Single-photon emission computed tomography (SPECT) 70
- Single ventricle physiology 3, 21
- Small intestinal submucosa (SIS) 8
- SMC differentiation 59
  - of neural crest stem cells 59
  - regulator genes 59
- SMC markers 37, 38, 63, 65, 66
- SMC proliferation 20
- Smooth muscle 9, 10, 11, 13, 18, 19, 20, 21, 32, 33, 34, 35, 37, 38, 39, 40, 45, 46, 47, 48, 50, 51, 52, 53, 54, 55, 56, 61, 63, 65, 66, 67, 72, 73, 75, 76, 77, 79, 81, 158, 160
  - cells (SMCs) 9, 10, 11, 13, 18, 19, 20, 21, 32, 33, 34, 35, 37, 38, 39, 40, 45, 46, 47, 48, 50, 51, 52, 53, 54, 55, 56, 61, 63, 65, 66, 67, 72, 73, 75, 76, 77, 79, 81, 158, 160
  - lineage cells 45
  - progenitor cells (SMPCs) 40, 46
- Sodium dodecyl sulphate 150
- Solubility 145, 147
  - increased 147
- Somatic cells 10, 12, 34, 48, 73, 82, 114, 160, 192, 194, 222
  - initial 73
  - mature 10
  - patient-derived 82
  - reprogrammed 160
  - reprogrammed patient-derived 82
- Specification of vascular cells 77
- Spinal cord injury (SCI) 107, 108, 113, 118, 120
- SRF-myocardin 56
- Stem cell(s) 10, 12, 32, 34, 40, 41, 44, 46, 47, 56, 66, 73, 74, 107, 108, 113, 130, 135, 138, 139, 141, 143, 158, 160, 162, 189, 192, 193, 198, 211, 212, 213, 214, 216, 217, 219, 220, 222, 223, 224, 225
  - adipose 135, 189, 192
  - adipose-derived mesenchymal 141
  - adipose tissue-derived 162
  - adult neural 113
  - adult tissue 214
  - allogeneic 212
  - attachment 143
  - differentiation 135, 139, 198, 214, 217
  - disorder 213
  - ectomesenchymal 158
  - endogenous 32, 107
  - endogenous hematopoietic 223
  - endothelial 189
  - human embryonic 66
  - hematopoietic 41, 211, 212, 214, 222
  - human mesenchymal 138, 143
  - human umbilical cord blood 108
  - limbal 193
  - marrow-derived 220, 222, 223, 224
  - mesodermal 40
  - multi-potent 44, 212
  - neural 73, 74, 107, 108, 212
  - pluripotent 12, 32, 34, 47, 66, 222, 223
  - progenitor 214
  - putative periductular 224
  - population 220
  - proliferation 216
  - signaling 225
  - stimuli 130
  - therapy and liver regeneration 219
  - transplanting 219
  - umbilical cord 212
  - undifferentiated 160
  - vascular wall resident 46
- Stem/Progenitor Cells
  - adult 32, 34, 39, 81, 112, 214
  - hepatic 214
  - multipotent resident 34
  - neural 112
  - vascular wall resident 34
- Stromal cells 48, 51, 65, 187
  - adipose 48, 65
- Stromal fibroblasts 186, 187
- Strontium 152, 153
  - effects of 153
  - Strontium usage 153
- Support stem/progenitor cells 74
- Synthetic 4, 18, 32, 37, 132, 133, 144, 145
  - implants 4
  - polymers 32, 132, 133
  - routes 144, 145
  - SMCs 18, 37

**T**

Target cells 71, 73, 162, 196, 198, 199  
  type 196, 198  
Targeted therapy 209  
TEBVs, generating autologous 76, 77  
Telomerase 190  
Teratoma formations 68  
Terminal cells, converted 73  
TEVG 5, 9, 10, 11, 13, 14, 17, 19, 20, 21, 22, 23  
  implantation 11, 21, 22, 23  
  remodeling 13, 14, 19, 20  
  research 9, 10  
  scaffold 5  
  tissue remodeling 13, 17, 21  
Therapeutic potentials of PSCs-derived vascular cells 60  
Therapeutic strategies, effective 32, 33, 81  
Thickening fraction 65, 66  
Three-dimensional scaffolds 130, 131, 160  
  enhance bone regeneration 160  
Thromboresistance 9, 10, 17  
Tissue 110, 158, 179, 185  
  endogenous 110  
  ocular 179, 185  
  oral 158  
Tissue culture 107, 108  
Tissue donors 179, 186, 188, 199  
Tissue-engineered blood vessels (TEBVs) 34, 63, 66, 74, 75, 82  
Tissue engineered 5  
  grafts 3, 4, 5, 6, 7, 9, 10, 11, 12, 15, 19, 20, 21, 22, 23, 24  
  vascular grafts (TEVGs) 3, 4, 5, 6, 7, 9, 10, 11, 12, 15, 19, 20, 21, 22, 23, 24  
Tissue regeneration 3, 131  
Topography 6, 130, 133, 141, 142  
  unique 6  
Totipotent stem cells 211  
Trabecular meshwork 182, 189, 190  
Transanastomotic outgrowth 19, 20  
Transcriptional states 196  
Transcription factors 21, 48, 58, 70, 71, 73, 114, 138, 160, 182, 183, 194, 196, 197, 199, 222, 225  
Transmural ingrowth 19, 20

Transplant 110, 219, 220, 224  
  allogeneic stem cell 219  
  autologous stem cell 219  
  cells 110  
  multipotent cells 110  
  stem cells 224  
  xenogeneic stem cell 220  
Transplantation 61, 69, 70, 211  
  autologous 69, 70, 211  
  therapeutic potentials of 61  
Transplanted hPSC-derived cells 70  
Transplanting 39, 60, 61, 67, 110  
  multipotent cells 110  
  PSCs-derived vascular cells in scaffolds 60  
Tumor 209, 225  
  angiogenesis 209  
  -Initiating Stem-like Cells (TISCs) 225  
Tumorigenicity 69, 81

**U**

UCB-endothelial progenitor cells 191  
Umbilical cord 45, 158, 159

**V**

Vascular 3, 7, 24, 32, 33, 34, 35, 36, 38, 39, 44, 46, 47, 48, 49, 50, 51, 53, 55, 57, 58, 60, 61, 62, 63, 65, 66, 68, 70, 74, 76, 77, 78, 79, 81, 82, 118, 161  
  development 47, 48, 58  
  differentiation 32, 33, 34, 49, 51, 53, 60, 70, 82  
  diseases 32, 38, 39, 46, 60, 62, 74, 77, 80  
  endothelial growth factor (VEGF) 36, 44, 46, 50, 51, 55, 57, 62, 63, 65, 66, 76, 77, 78, 118, 161  
  grafts 4, 10, 74, 75, 77, 82  
  homeostasis 32, 33, 36  
  lineage differentiation, activating 32, 34  
  pathologies 61, 68, 78, 79, 81, 82  
  progenitor cells (VPCs) 19, 33, 39, 40, 49, 50, 60, 61, 63, 66  
  progenitors 45, 46, 48, 51, 57, 62, 68  
  regeneration 16, 32, 33, 34, 39, 40, 43, 47, 50, 60, 61, 67, 70, 71, 73, 74, 76, 78, 81  
  remodelling 33, 36, 38, 39, 62, 63, 64

SMCs (VSMCs) 21, 37, 38, 39, 55, 78  
smooth muscle cells 33  
system 33, 34, 35, 74, 77  
Vascular cells 11, 12, 32, 34, 35, 38, 39, 47, 50,  
51, 53, 54, 57, 60, 68, 72, 73, 74, 75, 77,  
78, 80, 81, 82  
better differentiated 51  
cells-derived 35, 74  
functional 34, 80, 82  
functional mature 74  
generating functional 32, 81  
mature 11, 12  
types 53, 68, 73, 78  
tissue 3, 7, 24, 32, 34  
Vascular lineage cells 32, 49, 50  
reprogrammed 32  
Vasculogenesis 11, 33, 35, 41, 44, 46, 74, 161

**W**

Williams-Beuren syndrome (WBS) 79  
Wnt signalling 57

**X**

X-ray diffraction (XRD) 137, 152

**Z**

ZO-1 180, 186, 189, 190, 193  
expression of 186, 189



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