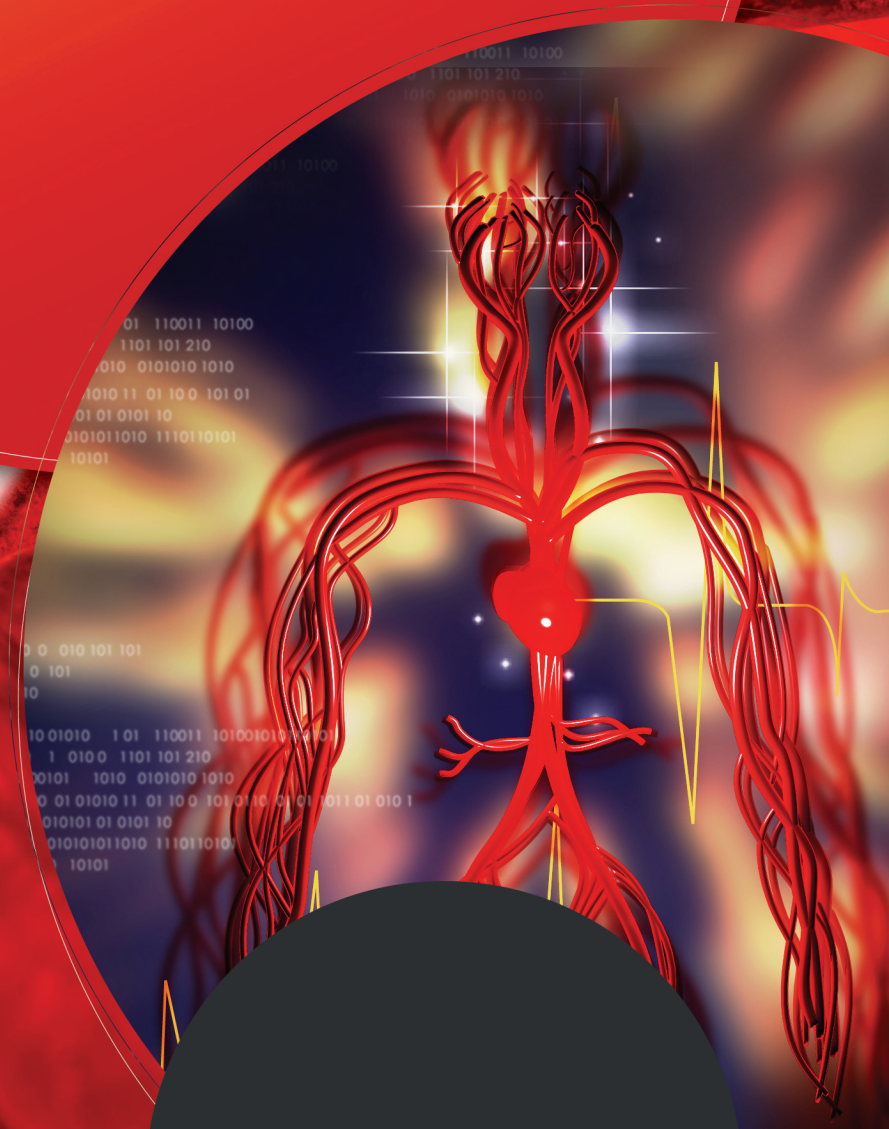
VASCULARIZATION IN TISSUE ENGINEERING



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Vascularization in Tissue Engineering

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PREFACE

Angiogenesis refers to the process of forming a new vascular system with an existing vascular network through the proliferation and migration of vascular endothelial cells on the basis of existing capillaries and/or venules. Angiogenesis is of great significance in tissue engineering, wound healing and regeneration repair. The core of tissue engineering is to establish a three-dimensional space complex of cells and biological materials, which is used to reconstruct the morphology, structure and function of the damaged tissues and achieve permanent replacement, to achieve the purpose of repairing wounds and rebuilding functions. However, there are few tissue engineering products that can be applied to clinics at present. One of the main reasons is the early vascularization of tissue engineering products. Evidence shows that when the cell mass is greater than 3 mm^3, the diffusion of interstitial fluid cannot be used to maintain the cell's survival, and the supply of oxygen and nutrients must be achieved through the regeneration of blood vessels. Because tissue engineering scaffolds do not have a vascular network, vascular regeneration has become an important factor limiting the ability of tissue engineering constructs to form tissues after implantation. Therefore, when using tissue engineering technology to construct large and complex artificial tissues or organs, how to avoid ischemic necrosis or poor healing of the central part of the defect has become the primary task of tissue engineering vascularization. At present, by constructing scaffold material-seed cell-growth factor complex, based on the research foundation of angiogenesis mechanism, the formation of neovascularization *in vitro* three-dimensional microenvironment or *in vivo* transplantation culture is the most common method for tissue engineering vascularization. Therefore, it is very important to understand the occurrence, development, physiological and pathological processes of angiogenesis for tissue engineering vascularization. In this book, the authors focus on the biological and pathological conditions of vascularization, microenvironment factors on angiogenesis, co-culture systems and scaffold materials used for angiogenesis, including: (1) biological basis of vascularization; (2) effects of microenvironment factors on angiogenesis; (3) microenvironment of pathological vascularization; (4) vascularization in co-culture systems; (5) vascularization and scaffold material.

Though this book, readers will have a better understanding of the occurrence, development, physiological and pathological processes of angiogenesis, and know more about ways and means of angiogenesis. The authors sincerely hope that this book will add further insight into basic and applied researchers as well as clinicians involved in tissue engineering vascularization, thus contributing to further advances in regenerative medicine.

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FOREWORD

When Dr. Cai asked me to write this foreword, I was greatly honored to have the opportunity to introduce this eBook.

As one of the research hotspots of life science in the 21st century, tissue engineering is a new subject combining cell biology and material science to construct tissue or organs *in vitro* or *in vivo*. The method is to plant seed cells on scaffold materials to form an active complex, which finally establishes tissues and organs with normal physiological functions and is applied to the treatment and repair of human diseases. At present, the construction of skin, bone and cartilage in vitro has made great progress, and has been widely used in clinical. However, how to build a blood supply system that can transport nutrients, oxygen and remove metabolize waste for the body, to ensure the survival of implanted artificial tissue in the body and play its normal physiological function, which is a major difficulty of tissue engineering, as well as a key issue of regenerative medicine.

Vascularization, existing in both physiological and pathological procedures, is a considerable complicated process which is regulated and controlled by a variety of biological factors via diverse pathways and mechanism. It's a complex and multi-step physiological process, which must be carried out under a strict micro-environment. At present, in addition to conventional treatment methods, plenty of vascular diseases such as arterial ischemic disease, peripheral arterial disease, pulmonary hypertension, limb ischemia, myocardial infarction and cerebral infarction were treated with endothelial progenitor cells possessing the characteristics of stem cells in clinical work, which have a very broad prospect. Therefore, the exploration of the vascularization of tissue engineering will greatly promote the research results of regenerative medicine to clinical application.

It is generally known that seeding cells, growth factors, scaffold materials and microenvironment are the four basic elements of tissue engineering. This eBook is a comprehensive and systematic introduction to the vascularization of tissue engineering from these four aspects. Firstly, it details the whole process of physical vascularization (vascularization and angiogenesis) and the role of various growth factors. Then, the effect of the physiological microenvironment and pathological microenvironment on the vascularization in tissue engineering is elaborated, such as related mechanism, pathophysiological features and so on. Besides that, various common seeding cells, typical co-culture system model and multifarious scaffold materials of vascularization in tissue engineering are also introduced in detail.

In conclusion, I am excited about this eBook. Because it not only systematically and comprehensively introduces the mature theoretical knowledge, methods and technology of vascularization in tissue engineering, but also shows the latest progress achievements and future development in the world. I believe it will be beneficial to all those who have an interest in vascularization in tissue engineering and will lay a crucial and solid theoretical foundation for making future progress of tissue engineering vascularization.

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CHAPTER 1

Biological Basis of Vascularization

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Abstract: Vascularization, existing in both physiological and pathological procedures, is a considerable complicated process which is regulated and controlled by a variety of biological factors *via* diverse pathways and mechanism. It is crucial to understand these processes and factors well if we intend to unveil the mystery of vascularization. More importantly, understanding basic processes offer tools and thinking directions with which researchers are able to design various methods to achieve vascularization, which is called vascular tissue engineering. In this part, major procedures of physical vascularization (vasculogenesis and angiogenesis) and growth factors are introduced, as well as their roles and new research outcomes in vascularization. These factors include vascular endothelial growth factor(VEGF), basic fibroblast growth factor(bFGF), platelet-derived growth factor(PDGF), angiopoietin1, angiopoietin2 and others(junctional molecules, integrals *etc*.). Lastly, some frequently used markers and testing methods about vascularization research are briefly introduced.

Keywords: Angiopoietin, Angiogenesis, FGF, PDGF, Vascularization, VEGF.

1. INTRODUCTION OF BIOLOGICAL VASCULARIZATION

It is generally acknowledged that the mechanism of neovascularization comprises two aspects---vasculogenesis and angiogenesis [1, 2]. Supposed as the main mechanism of the construction of vascular networks in the embryonic stage, vasculogenesis occurs in the bone marrow as endothelial progenitor cells (EPCs) gradually migrating, differentiating and finally reforming new vessels, besides it also exists in adult tissue especially in the ischemic area [3, 4]. In the embryo, the first vascular network is built when somites beginning to form by the process of vasculogenesis. Locating between two germ layers, the first blood islands which form the inner layer of the yolk sac occur by *in situ* differentiation from the extra-embryonic mesoderm. In the embryonic stage, vascular network remodeling is characterized as the change of number and/or location of vascular segments to

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improve functional adaptability without evident network expansion. Vascular fusion can reduce the number of vascular segments and therefore give rise to larger vessels. Larger vessels in other regions are remodeled into a network of smaller vessels which subsequently increase the number of vessels segments. These biological processes result in a big transformation of primary plexus to enter into a more complexly structured secondary stage. Further expansion of primary and secondary vascular plexus during postnatal life occurs with the process of angiogenesis. Angiogenesis generally refers to the process of new blood vessels formation based on pre-existing ones (Shown as Fig. **1a-c**). The process of angiogenesis comprises two distinct mechanisms, sprouting of endothelial cells (ECs) and splitting of vessel lumens by intussusceptive microvascular growth (IMG).

1.1. Vasculogenesis

1.1.1. Embryonic Vasculogenesis

Vasculogenesis is a process in which blood vessels differentiate from *in situ* into ECs. Initially, the term was used for embryonic development and the presence of angioblasts, which were entirely associated with prenatal growth. "Vasculogenesis" was first brought out by Werner Risau [5] to term the development of mesodermal vascular plexus by differentiation of vascular fibroblasts. Angioblasts are thought to accumulate in the angiogenesis area. Vasculogenesis that occuring embryogenesis and extraembryonic are often accompanied by hematopoiesis, thus the term "angioblasts" has also been proposed. Elementary observations in Sabin [6] strongly suggest that both hematopoietic cells and ECs origins from the angioblasts. The existence of hemangioblasts *in vivo* has developed. At present, instead of defined as an actual ordinary cell precursor, it's rather more like a competitive cell that can generate hematopoietic cells and ECs to local environmental signals [7].

In early mouse embryos, angioblasts originated from decentralized progenitors in the lateral plate mesoderm and expressed Flk-1 (VEGFR-2) and Brachyury (Bry)genes in turn [8]. Bry gene was inhibited and Flk-1 was activated as development progressed. The dynamic changes of these progenitor cell differentiation markers were as follows: Bry +/Flk-1 -, Bry-/ Flk-1 -, Bry +/Flk-1-, Bry +/Flk-1 -, and Bry +/Flk-1 -. These groups represent different phases of differentiation of vascular mother cells. In addition to Flk-1 and Bry genes, they also display diverse sequences of other genic groups [9]. The existence of vascular mother cells has been verified *in vitro* though collecting endothelial and hematopoietic descendants of colony-forming cells (CFCs) derived from embryoid [10, 11]. Embryoid bodies obtained from suspension cultured embryonic stem cells established a model that simulates many cell differentiation

in multi-aspect during early somatic embryogenesis [12]. *In vitro*, fast breeding CFCs that produce hematopoietic colonies functioned equally to vascular mother cells. The vascular mother cells have multi-directional differentiation potentials to form ECs, smooth muscle cells and hematopoietic cells under specific conditions [8, 9, 11]. The commitment of angiogenic cells is controlled by transcription factor of etv2/er71gene, the upstream of core genes in the development of EC lineage [13], and activation of endothelial cell line specific genes (endothelin, endothelin and VE cadherin) and hematopoietic and/or hematopoietic (hematopoietic, hematopoietic and SCL) lineages [14]. ETv2 guide differentiation of endothelial and hematopoietic lineage *via* regulating ETS associate genes necessary for downstream stimulation of hematopoietic and endothelial differentiation [14].

Members of the TGF-beta superfamily participated in mesoembryonic expression of Bry, for instance BMP (bone morphogenetic protein) and lymph node and activin signals, as a guide between self-renewal of pluripotent stem cells [15, 16]. Fibroblast Growth Factor-2 (FGF-2) and BMP4 are key signaling ingredients that stimulate the development of embryonic mesoderm, thus accelerate ECs and blood cells production [17, 18]. TFIIS, subtype of TCEA3 which express in mesoderm was proved to drive the production mesoderm EC. As shRNA transfection reduced the expression of TCEA3 in mouse embryonic stem cells, the expression of Bry marker in mesoderm increased, the expression of multipotent genes in mesoderm decreased, the differentiation of EC boosted and the production of vascular endothelial growth factor (VEGF) A increased [19]. Therefore, ECs can bypass a hemangioblast intermediate directly from mesodermal angioblasts. EC differentiation in the period of embryonic process has been demonstrated to be produced by vascular mother cells directly from the mesoderm [20, 21]. These ECs can further form tubules during mesoderm culture *in vitro*. Although current evidence supports the existence of angioblasts, it has been a challenge to isolate these cells and determine their exact location in developing embryos.

Embryonic EC are considered be descendants of angioblasts [22]. Angioblasts were found to transform phenotype in mice: initially expressed tal-1/flk-1, then CD31 was obtained, while the expression of tal-1 was reduced [21]. This phenotypic change of angioblasts was observed during the formation of cardiac ducts, dorsal aortas, interlaminar vessels and main veins in different embryos. During these events, cells processes migration, isolation and assemble into tube/vessel as they differentiate into mature ECs. Instead of classical growth factors such as platelet-derived growth factor (PDGF), VEGF and FGF, notch and ephb2/ephb4 signaling pathways was considered the key components to regulate dorsal aortic angiogenesis [23, 24]. It has been proved that basement-membrane

CHAPTER 2

Effects of Microenvironment Factors on Angiogenesis

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Abstract: Angiogenesis is a vital step for complete organ engineering, and the microenvironment is one of the four basic elements of tissue engineering. Microenvironment factors such as oxygen content and stress are key dynamic factors that can trigger the variations of angiogenesis. We may induce the formation of beneficial blood vessels and prevent the formation of pathological blood vessels by precisely regulating the microenvironment. In this chapter, we will elaborate the interaction between vascular endothelial cells and the extracellular microenvironment and summarize the influence of various microenvironment factors on angiogenesis. The finding that microenvironment factors play such a concerted role in angiogenesis suggests that incorporating microenvironment factors into tissue engineering might accelerate the development of novel therapeutics.

Keywords: Angiogenesis, Endothelial cell, Extracellular matrix, Microenvironment, Stiffness.

1. INTRODUCTION

Regenerative medicine technology is one of the most effective and promising methods to repair tissue trauma and function reconstruction. However, the researching results that can be transferred into clinical practice are extremely limited. The lack of nutrition and oxygen supply will lead to the failure of transplantation therapy. In this regard, researchers proposed several schemes that can promote the vascularization of tissue engineering products, including directly inoculating endothelial cells (ECs) onto scaffold materials to generate vascular structure before implantation. However, vascular structure in function was still very limited, for the reason that the scaffold material could not provide the appropriate microenvironment for the growth of related cells and new vessels.

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Xiaoxiao Cai (Ed.)

Endothelial cells (ECs) are the vascular network's basic units, which constitute the lining of blood vessels throughout the body to the fine vascular networks which perfuse all tissues [1]. ECs line vessel walls as a monolayer by adhering to the basal lamina [2]. The vessels in different tissues and organs range in diameter from approximately 8 μm for capillaries to 2.5 cm for the aorta [3] The microvasculature system comprises only the intima and specialized SMCs, which offer both stability [4] and quiescence to capillaries [5]. As a result of their distinctive location between the volatile, intricate vascular wall and the flowing blood, ECs are subjected to forces from extracellular matrix (ECM) layers as well as surrounding tissues, such as muscle-mediated vessel contraction, physical forces from the residual hoop stress of the vessel wall, and physical inputs that originate from local ECM, which can impinge on the vessels [6]. Taken together, these microenvironment factors have a central role in managing physiological and pathological-related EC behavior, from the molecular to the tissue level.

EC is the basic unit of cell-based support therapy for cardiovascular and ischemic diseases. Guiding ECs biological behavior and optimizing angiogenesis by regulating the microenvironment factors has a great potential value in the treatment of vascular diseases, tumor diseases and so on. In this chapter, the effects of microenvironment factors on the angiogenesis in tissue engineering will be summarized.

2. EFFECT OF EXTRACELLULAR MATRIX ON ANGIOGENESIS IN TISSUE ENGINEERING

2.1. Properties of the Extracellular Matrix of Vessels

The ECM, composed of different functional proteins, is a highly dynamic structure which exists in all tissues and sustains continuous regulated remodeling. The remodeling is carried out by specific enzymes, such as metalloproteinases, accompanied by ECM degradation [7]. The ECM interacts with local cells to medicate various functions, such as proliferation, migration, and autophagy. ECM remodeling has a profound influence on the morphogenesis of cells and their functions [8]. Many pathological states, such as fibrosis or invasive cancer, are accompanied by dysregulation of the ECM structure, composition, abundance, and stiffness. It is imperative for researchers to obtain a thorough understanding of the way that ECM affects organ structure and function, and the way that ECM remodeling influences cell behavior and disease progression.

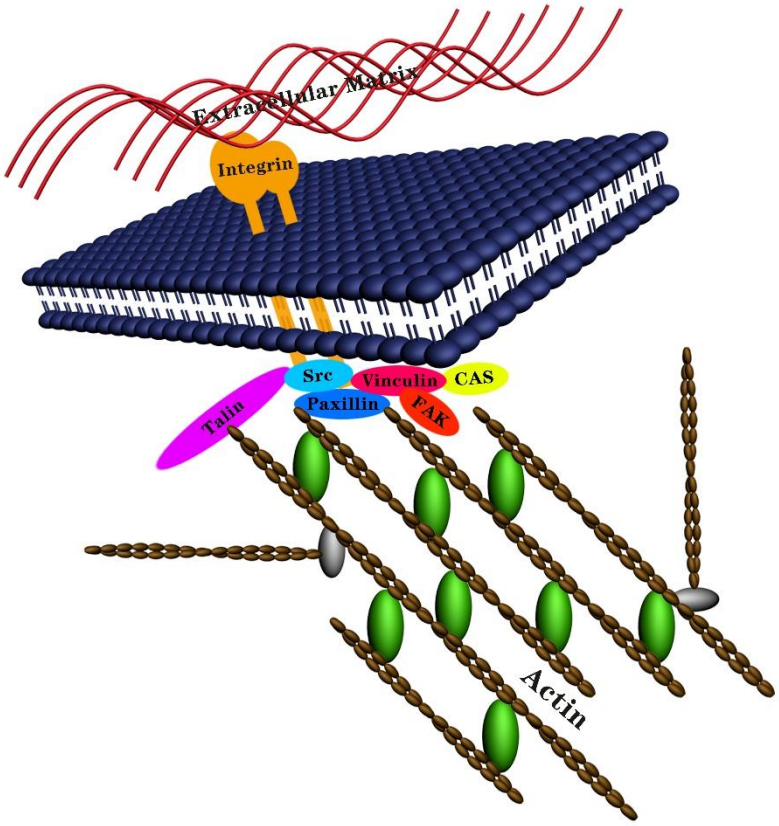

Fig. (1). FAs are ideal checkpoints which control inside-out and inwards transduction of biomechanical signals of the extracellular matrix.

The ECM of vessels is a network consisting of fibers and pores lined in an isotropic manner, ranging in size from the nanoscale (1–100 nm) to the submicron level (100–1000 nm) [9]. The local stiffness of the ECM has been reported to be between 5–140 kPa. The stiffness of the ECM plays a concerted role in vascular assembly and maintenance, even at the microvasculature level. In pathological conditions, ECM has been proved to have thickened in a number of tubes [10]. Moreover, ECs function is significantly affected by the elasticity of their local surrounding ECM, which provides matrix and stability for angiogenesis [11]. ECs have also been shown to secrete certain ECM components, which increase overall vessel rigidity in many pathological states [12]. Located basolaterally to all EC monolayers [13], the local ECM is a heterogeneous and complicated mixture of laminin; fibronectin; enactin; nidogen; collagen IV and V; the heparin-sulfate

CHAPTER 3

Microenvironment of Pathological Vascularization

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Abstract: Past studies have shown that many destructive diseases drive abnormal angiogenesis and progression, such as inflammatory diseases, cancers, rheumatoid arthritis, and atherosclerosis diseases. These diseases, which have a variety of consequences, show some common pathophysiological characteristics, among which the proliferation of endothelial cells, recruitment of immune cells, and high-expression of angiogenic factors play a key role. At the same time, local hypoxia, inflammation, senile, and local ischaemia cause adverse consequences such as abnormal vascularisation. Abnormal blood vessels usually include vascular structural abnormalities, abnormal endothelial cells, excessive vascular permeability, vascular dysfunction, *etc*. The pathological microenvironment is related to abnormal vascularisation and further aggravates the abnormality of vascularisation. Therefore, this review will be helpful for further study of vascularised tissue engineering.

Keywords: Angiogenesis, Ageing, Inflammation, Ischemia and Hypoxia, Pathological Vascularization.

1. INTRODUCTION

Angiogenesis, the formation of new blood vessels from pre-existing vessels, is necessary for various physiological processes. During evolution, blood vessels emerge, and transport oxygen and nutrients to the distant tissues and organs [1]. Undoubtedly, these blood vessels are essential for the growth of tissues in embryos and repair of injured tissues in adults. However, the imbalance of vascular growth leads to many diseases. Similarly, the pathological microenvironment of these diseases also accelerates the formation of abnormal blood vessels [2, 3]. As a matter of fact, pathological angiogenesis is a marker of malignant tumours, and various metastatic, ischaemic, and inflammatory diseases [4]. The pathological microenvironment of vessels is a dynamic network, which is composed of many factors.

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Microenvironment has an increasingly significant effect on angiogenesis under the conditions of diseases. On the other hand, angiogenesis is a marker of growth, invasion, and metastasis of malignant diseases. Increasing studies have shown that the microenvironment of diseases is closely related to pathological angiogenesis. In this review, the effects of the microenvironment of tumours, and various metastatic, ischaemic, and inflammatory diseases on angiogenesis are analysed in detail to provide a broader idea for angiogenesis research, especially for understanding angiogenesis in tissue engineering.

2. PATHOLOGICAL CONDITIONS LEADING TO ADVERSE VASCULARISATION

Pathological angiogenesis is a sign of cancers and various ischaemic and inflammatory diseases [4], as shown in Fig. (**1**). By exploring the formation of abnormal blood vessels, their mode of action, the key molecules, and microenvironments involved, and related studies, it would help us understand the occurrence and development of cancers, and ischaemic, and inflammatory diseases [5, 6]. During evolution, blood vessels emerge and then transport oxygen and nutrients to distant tissues [7, 8]. Undeniably, these blood vessels are essential for sustaining the growth of normal and injured tissues. However, abnormal, excessive, and insufficient vascular growth is usually involved in the pathogenesis of many diseases [2]. After birth, angiogenesis continues to play a significant role in organ growth. While the growth of blood vessels is stationary in adulthood, angiogenesis occurs only during pregnancy [1]. Nevertheless, endothelial cells manifest their remarkable divisive capacity in the face of pathological stimulation, such as vascular hypoxia and lymphangitis [9]. During healing and repair of wound, angiogenesis is reactivated. However, in several other diseases, excessive stimulus can break out the balance between stimulants and inhibitors, which lead to abnormal angiogenesis [10]. The most common conditions for activation of abnormal angiogenesis are inflammatory diseases and malignant tumours, however, other diseases can also have an impact, such as asthma, obesity, cirrhosis of the liver, diabetes, endometriosis, AIDS, multiple sclerosis, autoimmune diseases, and bacterial infections [1, 11].

2.1. Inflammation-Related Diseases and Angiogenesis

Angiogenesis participate in the occurrence and progression of many destructive diseases, such as cancers, atherosclerosis, inflammatory bowel disease [IBD], and rheumatoid arthritis [12]. Angiogenesis is regulated by different types of cells in its microenvironment, such as immune cells [13 - 16]. During inflammation, both endothelial and parietal cells are involved in the migration of white blood cells to

inflammatory centres [17, 18]. Immune cells participate in wound healing by controlling the formation of new blood vessels. Emerging research has shown that these cells are involved in promoting angiogenesis and vascular remodelling in ischaemic injury, cancers, and wound healing [19, 20]. These immune cells promote angiogenesis under the strong influence of their microenvironment, especially the inflammatory microenvironment [21].

2.1.1. Inflammation-Related Diseases and Angiogenesis

Moderate inflammation is important for maintaining homeostasis [22], but severe and persistent inflammation leads to some chronic inflammatory diseases, such as cancers [21], atherosclerosis [23], IBD, and rheumatoid arthritis [24]. Hypoxia-inducible factors [HIFs] are produced by inflammatory tissues, which are finished by activating endothelial cells, macrophages, and fibroblasts due to local hypoxia [25 - 27]. Long-term infiltration of these factors leads to adhesive degradation of endothelial cells and microvascular instability [28, 29]. On the other hand, it is beneficial to the proliferation and migration of endothelial cells and angiogenesis [30, 31].

Although these diseases develop differently and have different clinical manifestations, they have the same pathophysiological peculiarity, among which enhanced vascular permeability, macrophage polarization, and monocyte recruitment play essential roles [12]. Tumour-associated macrophages [TAM] excrete some angiogenic growth factors and proteases, which include vascular endothelial growth factor [VEGF], to promote angiogenesis and further migration and infiltration of tumours [32 - 34]. Atherosclerotic plaque can be formed by some proteolytic enzymes, which are secreted by macrophages, including matrix metalloproteninases [MMPs] [35]. Similar to angiogenesis of tumours, neovascularisation is mediated by macrophage-derived angiogenic factors in atherosclerotic plaques [36]. Other inflammatory diseases function similarly. Local hypoxia and increased vascular permeability caused by inflammation lead to new angiogenesis. During this period, the related pro-inflammatory factors and angiogenic factors secreted by macrophages play a key role.

2.1.2. Pathological Mechanism of Inflammatory Angiogenesis

First, aggregation of immune cells plays an important role in the process of inflammatory angiogenesis. Immunocytes associated with angiogenesis include natural killer cells, neutrophils, dendritic cells, eosinophils, mast cells, macrophages, and T cells [37]. It has been reported that angiogenesis is induced by macrophages in wounds and tumours, which stimulate ischaemia-induced

CHAPTER 4

Vascularization in Co-Culture Systems

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Abstract: Since endothelial cells are not able to create capillaries by themselves, proangiogenic factors are indispensable for endothelial cells to migrate and form microcapillaries. Thus, exogenous proangiogenic compounds are needed to improve the formation of microcapillary-like structures. Multiple forms of cell-cell interactions could result in the production of essential proangiogenic factors in co-culture systems. Many studies have examined that the co-culture systems of endothelial cells and other cell types, such as osteoblasts or mesenchymal stem cells (MSCs), can facilitate the formation of capillary-like structures. The focus of this chapter is threefold: (1) Informing the biological function of vascularization in the physiological environment. (2) Introducing typical co-culture system models for vascularization. (3) Identifying the proangiogenic factors that play crucial roles in the formation of capillary-like structures.

Keywords: Biomechanical Stimulation, Bone Tissue Engineering, Cardiac Regeneration, Cell-ECM Adhesion, Cellular Interactions, Co-culture, Cell-cell Adhesion, Direct co-culture, Endothelial Cells, Extracellular Matrix, Indirect co-culture, Media, Mesenchymal Stem Cells, Osteoblast, Oxygen Environment, Scaffolds, Seeding Methods, Skin Regeneration, Soluble Factors, Vascularization.

1. VASCULARIZATION IN PHYSIOLOGICAL AND PATHOLOGICAL CONDITIONS

1.1. Vasculogenesis and Angiogenesis in Healing and Tissue Regeneration

Vascular networks consist of two fundamental mechanisms: vasculogenesis and angiogenesis. The former is the vascular regeneration and the formation of capillary plexus *via* endothelial progenitor cells (EPCs). The latter indicates the generation of new vessels from the preexisting vascular network, involving capillary sprouting and remodeling.

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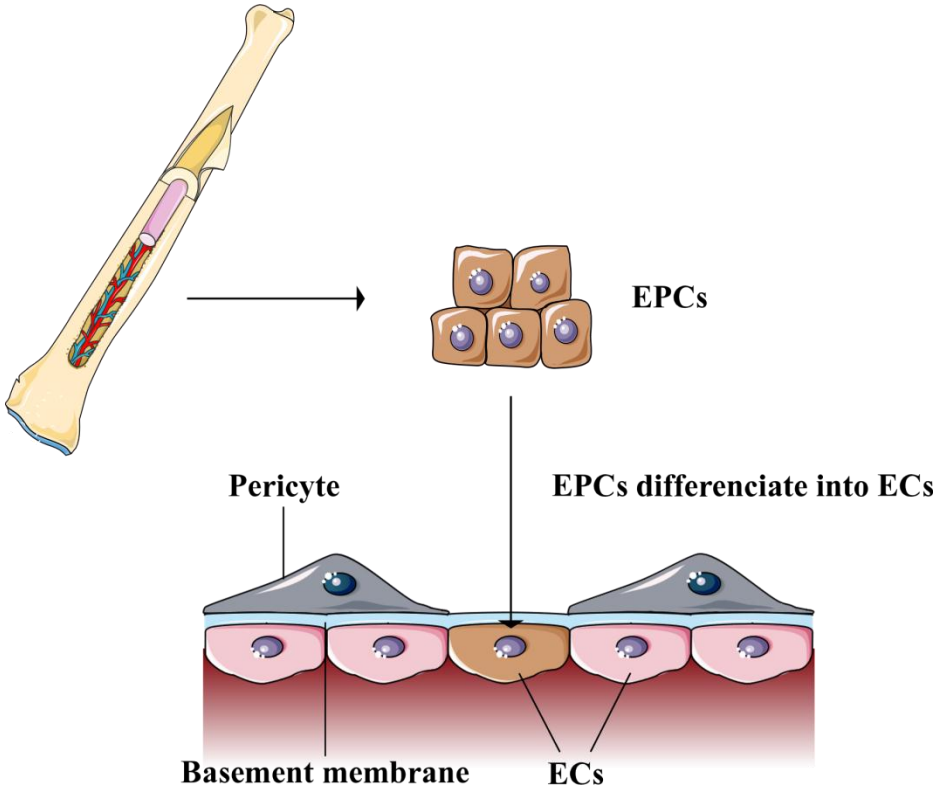

Fig. (1). Vasculogenesis.

Vascular systems play crucial roles in an abundance of biological processes, including metabolism [1], development [2], immunity [3], healing [4], and regeneration, as well as the progression of many diseases. Transporting oxygen and nutrients and removing metabolic waste, vessels are essential for growth and development. They promote the circulation of immune cells and rapidly deliver them to surrounding tissues when necessary. The versatility of vascular networks stems from the meshwork and the multiple cell types that make up blood vessels. Vessels are composed of endothelial lining surrounded by perivascular cells (PVCs) (smooth muscle cells and pericytes) and extracellular matrix (ECM) [5]. What is more, ECM is of paramount importance for normal vascular function.

Vascular networks consist of different forms of vessels: arteries, arterioles, capillaries, venules, and veins. Blood rushes from the heart into main arteries, then branches out into small arteries and flows in small arteries and capillaries. In the process of returning to the heart, blood flows through capillaries, tiny veins (venules), veins, and cavity veins. Capillaries connect small arteries and venules. The permeability of the capillary walls allows oxygen delivery and metabolic

exchange. Therefore, the function of transforming nutrition and oxygen to tissues is performed mostly by microvascular networks, which encompasses small arteries, capillaries, and small veins [6].

1.1.1. Vasculogenesis

Vasculogenesis is the initial formation of vessels by cells differentiating into endothelial cells *in situ*. The process is only related to embryonic life previously, while recent studies have reported that vasculogenesis also occurs in adult tissues. The process involves angioblasts and endothelial progenitor cells.

Angioblasts, which are considered to be precursors of embryonic endothelial cells, are recruited during embryonic and fetal growth. They migrate, separate, and finally assemble into vessels, simultaneously differentiating into mature endothelial cells. Phenotypes of angioblasts change during the cardiovascular, aortic, and venous formation of embryos among multiple species [7].

EPCs usually exist in post-natal vasculogenesis, including healing, ischemia, atherosclerosis, myocardial infarction, and tumor growth. Fig. (**1**) shows the involvement of EPCs in vasculogenesis. During permanent wound healing and inflammatory reactions, such as obesity, atherosclerosis, hypercholesterolemia, and diabetes, the vasculature is distorted and confused—the phenomenon associated with a lower quantity of cEPCs(circulating endothelial progenitor cells). Vascular dysfunction manifests as a lower response to growth factors/chemokines [8]. Besides, the addition of EPCs to the injured vessel wall leads to vascular re-endothelialization. Another way to achieve re-endothelialization is colonization and re-endothelialization of cEPCs in the implanted biomaterials, which is also a hope for future therapy [9].

Neovascularization is essential to transport proteins and cells to injury sites and plays a critical role in tissue repair, growth, and development.

1.1.2. Angiogenesis

Angiogenesis refers to the formation of microvessels from existing blood vessels and capillaries through elongation, inosculation, intussusception, or sprouting.

Inosculation is vital for establishing a connection between the transplanted vascular network and the host microcirculation network. Intussusception involves bifurcation of vessels, which remodels the existing vasculature by the protrusion and fusion of opposing vessel walls in the lumen (See Fig. **2**). Compared with sprouting, intussusception angiogenesis is considerably swift in the expanded

CHAPTER 5

Angiogenesis and Materials

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Abstract: In the process of tissue engineering vascularization, as one of the three major factors of tissue engineering, scaffold materials play a vital role in the various processes of vascularization. As a starting point, this chapter first introduces the reader to several strategies for scaffolding materials to promote vascularization. It basically describes the reasons for the use of scaffold materials in vascularization and the necessity of scaffold materials in tissue engineering vascularization. Then we will focus on how to properly use the scaffold material. The design of the scaffold material itself is a key factor in the function of the material, and the scaffold material with ideal biological properties can make the process of vascularization more effective. Factors such as the topology of the material and the physical and chemical properties of the material affect the success rate of vascularization to varying degrees. We hope that readers can obtain the basic knowledge and principles of stent design from this chapter. Finally, a number of fresh ideas have emerged for the design of tissue engineered vascular materials, such as new material handling methods, new ways of combining cells, and so on, which have improved the vascularization process to varying degrees. Scaffold materials have shown attractive prospects and great possibilities in vascular tissue engineering. Previous studies have found many materials associated with vascularization, but there are also many problems to be solved. With the development of materials science and engineering, it is believed that there will be new vascular stent materials with better performance and more suitable for vascularization in the future.

Keywords: Angiogenesis, Degradation Modes of Scaffold, Materials, Tissue Engineering Vascularization.

1. INTRODUCTION

With the development of tissue engineering technology, repairing large-area bone defects using tissue-engineered bone has become a widely used approach. The scaffold materials with good three-dimensional structures can promote cell growth and proliferation and tissue ingrowth, and a variety of materials can reach a combined effect to meet the clinical demand.

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In addition, it is important to actively seek new material preparation technology and improve the existing methods, in order to create an excellent scaffold. However, vascularization is still a major challenge for bone tissue engineering.

2. TISSUE ENGINEERING BIOLOGICAL SCAFFOLD MATERIALS

The ideal healthy blood vessels in tissue engineering should have the following conditions: They should possess the ability to or simulate *in vivo* conditions. There are three layers of vascular wall structure, namely adventitia, media, and intima. They should exhibit biocompatibility, that is to say, it is not easy to produce thrombus and immune rejection. At the same time, it has similar biological characteristics with normal blood vessels, such as relaxation response to drug stimulation and needs to possess the mechanics exhibited by blood vessels. Third, it should also exhibit viscoelasticity and can withstand certain pressure. Scaffold materials are indispensable for achieving good vascularization processes.

With the continuous development of tissue engineering, repairing large-area bone defects using tissue-engineered bone has become a widely used approach. The scaffold materials with good three-dimensional structures can promote cell growth and proliferation, tissue growth, osteogenesis, and vascularization. Each scaffold has its own inadequacies; therefore, the combination of a variety of materials can achieve a combined effect to meet the clinical demand. In addition, it is important to actively seek new material preparation technology and improve the existing methods, in order to create excellent scaffolds [1 - 4].

2.1. The Design Concept of Ideal Biological Scaffold Material

Vascular stent materials play an indispensable role in vascular tissue engineering. The desired support for the growth of cells *in vitro*, which can provide the organization of blood vessels, certain mechanical strength and mechanical properties, seed cell growth. The ideal stent should have the following properties: (1) Controllable rate of biodegradation; (2) low immunogenicity, which does not cause an inflammatory response; (3) good biological properties; (4) good mechanical and physiological properties; (5) suitable porous structure; and (6) they should be easy to process and sterilize [1]. In short, it is necessary to provide specific three-dimensional (tubular) scaffolds for the construction of tissue-engineered blood vessels, which can be used as matrix materials for the implantation of vascular cells, so that the inoculated cells can be positioned, attached, and localized growth and proliferation can be promoted. Meanwhile, the materials can arrange the cells in the space of scaffolds, differentiate with specific functions, and synthesize appropriate extracellular matrix, following which tissue-

engineered blood vessels can be transplanted. *in vivo* stent materials should also have strong learning support and anti-blood pressure functions [2, 5 - 7].

2.2. Classification and Basic Concept of Biological Scaffold Materials

Currently used vascular stent materials include natural biological stent materials, synthetic biodegradable polymer materials, composite materials, and nanomaterials (Table **1**).

Table 1. Comparison of various scaffolds materials.

Material Classification	Natural Scaffold Materials	Synthetic Biomaterials	Nanomaterials
Material composition	Macromolecular materials such as collagen, alginate, *etc*.; acellular tissue matrix materials such as acellular dermal matrix, acellular vascular matrix, acellular bladder matrix, *etc.*	Non degradable polymer materials: Polyester and expanded polytetrafluoroethylene; degradable polymer materials: Poly (β - hydroxybutyric acid), poly (aminic acid), polycarbonate, polyurethane, poly (lactic acid) (poly (L-lactic acid, poly (dextran lactic acid, poly (lactic acid)) poly (glycolic acid) (combination of the two, poly (lactic acid and poly (glycolic acid))	Constructed by electrospinning, self-assembly, phase separation, *etc.*
Advantages	It comes from organism, with high biocompatibility, compliance and low immune rejection	Adjustable microstructure, surface morphology, mechanical properties and degradation rate Degradable cycle, eventually converted to water and carbon dioxide, easy to process	Provide a controllable environment for cell proliferation and directional differentiation, and significantly improve antithrombotic function
Disadvantages	Lack of ability to control extracellular matrix deposition and construction, with the potential risk of transmission of related animal origin pathogens	The synthesis technology is high and expensive, lacking special biological signal or functional group	The synthesis technology is high and expensive, lacking special biological signal or functional group

Natural biological scaffolds are derived from organisms and can be divided into macromolecular unstructured materials and acellular matrix scaffolds. The former includes chitosan, alginate, collagen, gelatin, and hyaluronic acid while the latter includes acellular dermal matrix, acellular small intestinal submucosa, SIS, and acellular vascular matrix. This kind of biomaterial has a strong affinity toward

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