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An overview on the experimental and mathematical modelings of angiogenesis and vasculogenesis

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ABSTRACT

Angiogenesis and vasculogenesis as a critical and multiscale process has attracted wide attention in tissue engineering. Therefore, various models have been developed to understand how endothelial cells assemble into vessels and what factors are able to regulate it. These models can be classified in three major groups: *in vivo*, *in vitro* and mathematical models. *In vivo* models have been used to provide a more detailed understanding of angiogenesis mechanism and its regulators in tissue engineering research. By using in vitro models several methods have been designed to stimulate angiogenesis and vasculogenesis in synthetic constructs via the modification of biomaterial properties and microfabrication techniques. In order to save time and cost, mathematical and computational models have been introduced to study the effect of many parameters on angiogenesis in physiological and pathological conditions. This review will summarize various type of *in vitro* and mathematical models which have been designed to investigate angiogenesis in different states.

Keywords: *angiogenesis, vasculogenesis, mathematical modelings, computational models.*

1. INTRODUCTION

Angiogenesis is defined as a process in which the organism forms new blood vessels from pre-existing ones. Angiogenesis is an important process for normal physiologic functions of the body such as wound healing and pathological condition like tumorosis. Many attempts have been developed to study the different aspects of blood vessel formation such as sprouting of vessel, forming of lumen and vascular remodeling [1, 2].

In more precise view, it was reported that two kind of blood vessel formation process exist; vasculogenesis, which is assembling of blood vessels during embryonic development by angioblasts, or hemangioblasts, and angiogenesis or neovascularization, which is the branching and spreading of adjacent blood vessels. Angiogenesis can also occur by employment of endothelial progenitor cells (EPCs) from the bone marrow [3].

It was well known that structural or functional vessel irregularities contribute in many diseases because this extensive network feeds all tissues. For example some diseases like myocardial infarction and stroke appear when inadequate vessel maintenance or growth occur and cancer, inflammatory disorders, and eye disease happen due to excessive vascular growth or abnormal remodeling [3].

In order to fabricate an efficient vascular engineered tissue, it is necessary to increase insight into the molecular and cellular processes of angiogenesis and vasculogenesis. Therefore, this review, first provide overview of angiogenesis mechanism and its regulator factors. Then we focus on two type of models (in vitro and Mathematical models) which have been developed not only to study angiogenesis and vasculogenesis but also to save time and cost. In in vitro model section we highlight the new techniques in vascularization to induce angiogenesis to fabricate biomimetic and vascularized 3D engineered tissue constructs. In mathematical section we summarize different approaches of modeling, application of computational in tissue engineering, the obstacles which should deal with to develop reliable models (Figure 1).

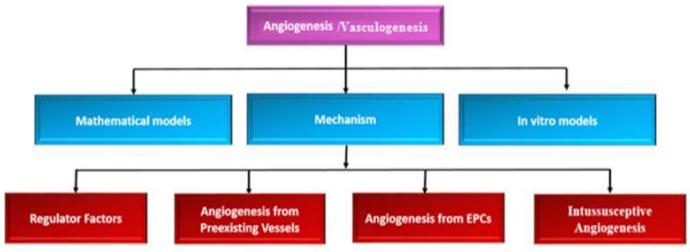

Figure 1. A schematic of the review content.

2. MOLECULAR MECHANISMS OF ANGIOGENESIS

Angiogenesis is an important process that influences physiologic processes such as wound healing, the vascularization of ischemic tissues, and pathologic processes like tumor formation and diabetic retinopathy. Therefore many attempts have been made to found out the mechanisms of angiogenesis, and to bring up the function of pro- or anti-angiogenic agents.

Generally, angiogenesis occurs in three ways: first from the extension and branching of adjoining blood vessels (sprouting angiogenesis) (Figure 2, A), second from splitting process in which factors of interstitial tissues attack existing vessels, developing transvascular tissue pillars that extend (intussusceptive angiogenesis) (Figure 2, B) and third, from drafting of endothelial progenitor cells (EPCs) from the bone marrow (Figure 2, C) [4].

2.1. Angiogenesis from Preexisting Vessels.

This kind of angiogenesis includes: (a) Vasodilation caused by VEGF and accordingly augment in permeability; (b)

disconnected endothelial cell's signaling by plasminogen activator, moreover enzymatic degradation of ECM. (c) Migration of Endothelial cells. (d) Proliferation of Endothelial cells. (e) Inhibition of growth and remodeling into capillary tubes, generally Maturation. (f) Using young endothelial cells (periendothelial cells) for construction of mature vessel tubulogenesis (EC tube formation).

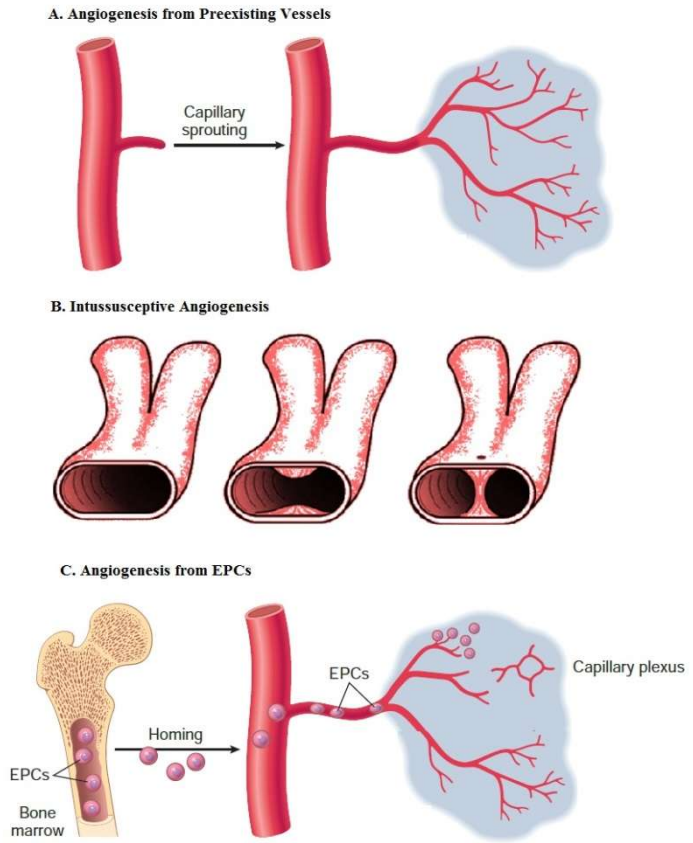

Figure 2. The basic morphological events for three types of angiogenesis. (Modified from Conway EM et al: Molecular mechanisms of blood vessel growth [5].

An Endothelial sprout process through the ECM includes release of an angiogenic stimulus like VEGF-A ([6, 7]) [19-20]. This process named Filopodia release abundant amount of enzymes which digest developing steps toward making sprout in ECM ((Small, Stradal *et al.* 2002, Van Hinsbergh and Koolwijk 2008)). The Filopodia in a cell subsidizesVEGF-A receptors, sense their concentration and allign them due to their gradient. When the substratum harbours enough number of Fiopodia on a tip cell, intraction between actin filament and Filopodia pull the cell toward the VGEF-A. simontanously, stalk cells that follow tip cells make capillary sprout elongate. Vacuoles combine and form a lumen by stalk cells. These stalk cells become the trunk of the freshly made capillary. When more than one tip cell of a capillary reach to the VEGF-A's release source, they fuse togheter and constitute a continuous lumen. VEGF-A concentration levels will back to the normal when enough oxigen fed to the local tissue. Maturation and stablization of the new capillary needs some sort of nechanical signals and shocks (Chien 2007).

Following researchs in the field of creating viable blood vessels can imply the main reasons which made angiogenesis by VEGF-A and gene therapy methods non optimized.

2.2. Intussusceptive Angiogenesis.

Intussusceptive or splitting angiogenesis is about expansion of vessel wall into lumen on account of splitting a single vessel in two. Splitting angiogenesis is known as a fast and efficient mechanism compared by sprouting angiogenesis. This type of angiogenesis happens throughout life but plays a significant role in vascular development at very first steps specially when growth is fast and resources are limited ((Kurz, Burri *et al.* 2003, Jain 2005). Although, splitting angiogenesis occurs just when there is already capillaries.

Sign of occurrence of this type of angiogenesis is due to presence of transcapillary tissue stands (Figure 4). Distinction of tissue stands require SEM microscopy or TEM. Intussusceptive angiogenesis was observed in postnatal lungs tissue, vascular baskets around glands, intestinal mucosa, kidney, skeletal muscles, heart and even brain tissue. This type of angiogenesis also has role in creation of vein bifurcation (Kurz, Burri *et al.* 2003, Carmeliet and Tessier-Lavigne 2005, Chien 2007).

In the other hand, sprouting angiogenesis is more controllable compared to intussusceptive angiogenesis because of its older age ([8]). The measurement methods involve in the splitting angiogenesis make it difficult to understand this mechanism more precise. However, by the precious of chick chorioallantonic membrane (CAM) and monitoring the application of VEGF-A in this system, there is little doubt that many factors and parameters involved in splitting angiogenesis.

2.3.Angiogenesis from Endothelial Precursor Cells (EPCs).

Source of WPCs can be bone marrow inside the tissue to commence angiogenesis. The exact mechanism is unknown. Generally these cells express some kind of markers as well as VEGFR-2, and vascular endothelial-cadherin (VE-cadherin). EPCs may participate in re-endothelizatin of vascular implants and neovascularization of ischemic organs, cutaneous wounds, and tumors. Increasing in the number of circulating EPCs in patients somehow shows the role of EPC in vascular functions.

2.4. Angiogenesis regulating factors.

Generally, VEGF is the most important growth factor in adult tissues during wound healing, tumors, chronic inflammation angiogenesis [1, 4].

VEGF is secreted by many mesenchymal and stromal cells. Among all kind of VEGF receptors, VEGF-2, a tyrosine kinase receptor, is the most important one. VEGF induces the migration of EPCs in the bone marrow, and cause increase in proliferation and differentiation of cells at angiogenesis region [9].

Endothelial cell proliferation, differentiation and migration can also be motivated by FGF-2 from the many VEGF effects and variety of the shocking mechanism that regulate its expression. Endothelial cells develop into a perfect pattern of vessels on account of a vasculogenesis modulation named Notch pathway. In summary, Notch pathway control the level of responsive VEGF by the hand of releasing some kind of receptors and ligands like Jagged1, Jagged2, Delta-like ligands and transmembrane receptors [10-12] (Figure 3).

Regardless of formation process of capillaries, new ones need to be stabilized. So they need recruitment of pericytes and periendothelial cells and deposition of ECM proteins. Biomolecules like angiopoietins 1 a 2 (Ang1 and Ang2), PDGF, and TGF-b participate in the stabilization process [13].

Materials or conditions that motivate VEGF expression, include certain cytokines and growth factors (e.g., TGF-b, PDGF, TGF-a) and obviously, tissue hypoxia, can affect physiologic and pathologic angiogenesis.

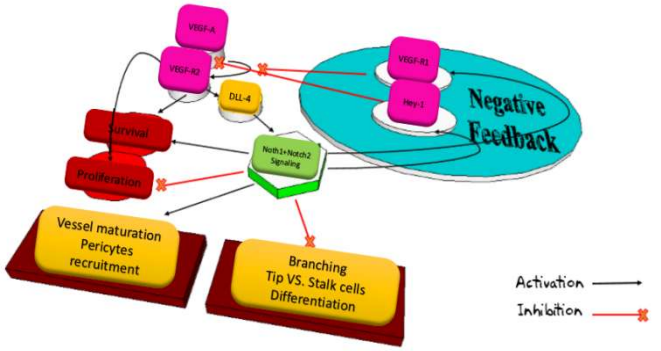

Figure 3. Notch pathway role in angiogenesis and control the level of VEGF.

One of the most important factors in angiogenesis process is directed migration of endothelial cells which are required for the creation of new blood vessels. This process can be controlled by several categories of proteins, consist of: (1) intergins, which are prominent for the formation and maintenance of freshly formed vessels. (2) matricellular proteins, which categorized in two main sub branches: (a) chemical factors, (b) Mechanical factors.

However one of the key regulating strategies for angiogenesis is using metabolic factors, another effective way to control this process is using engineered mechanical factors. According to the literature, it seems all the basic knowledge of metabolic regulating strategy is same in many situations. So it does make sense if we focus on the mechanosesory mechanisms to reach a better understanding of them in the aim of design unique therapeutic interventions to control angiogenesis.

The mechanical forces applying on the walls of blood vessels because of blood flow cause a cyclical mechanical strain on the walls of arteries and arterioles (note this fact blood pressure is pulsatile) and a constant strain on veins in which the blood pressure is not pulsatile. This tangential force causing morphological changes to endothelial cells. The walls of arteries and veins can also be stretched circumferentially that conclude from vasodilation and compressed circumferentially as a result of expand in blood vessels [14].

Mechanism that makes endothelial cells able to sense shear stress depends on molecular elements like ECM, cell-ECM adhesion, cell-cell adhesion complexes, membrane components, and cytoskeletal filaments. Furthermore, recent studies shows that epithelial sodium channels may also effect on the sensitivity of endothelial cells to sense the shear stress [15].

Generally, the mechanism of how shear stress can induces angiogenesis is poorly understood and there is a promising area of interest to pursue studies[16]. For instance, it is known that mechanical stress in blood flow results more intussusceptive growth [17, 18].

In some complicated tissues like heart, skeletal muscle, and brain in which the vasculature has primary a nutritive function, neither blood flow nor mechanical factors can regulate angiogenesis on their own. Because blood flow itself is regulated by metabolic factors in these tissues. So the effect of blood flow in this cases is accessory not regulative on angiogenesis. But there is a possibility to find mechanical factors effect as a controlled variable in a negative feedback regulation of vascular growth.

A key component of angiogenesis is the motility and directed migration of endothelial cells, required for the formation of new blood vessels. These processes are controlled by several classes of proteins, including (1) integrins, especially avb3, which is critical for the formation and maintenance of newly formed blood vessels, (2) matricellular proteins, including thrombospondin 1, SPARC, and tenascin C, which destabilize: (i) Chemical factors; (ii) Mechanical factors.

Although the overall regulation of angiogenesis is dominated by metabolic factors in most tissues of the body, mechanical factors also play crucial roles in virtually every aspect of the angiogenic process. Migration of endothelial cells, tube formation (tubulogenesis), and pericyte/smooth muscle cell migration to newly formed endothelial sprouts are critical steps in the angiogenic process that depend upon mechanosensory mechanisms. These mechanosensory mechanisms need to be better understood because they represent control points in the angiogenic process that are not likely to be growth factor specific. In other words, regardless of the growth factor(s) that stimulate angiogenesis, the fundamental steps required to build new capillaries are essentially the same. A better understanding of the mechanosensory mechanisms could therefore provide the basis for unique therapeutic interventions to control angiogenesis.

The walls of blood vessels are subjected to mechanical forces caused by blood flow, vasodilation, and blood pressure. Blood pressure causes a cyclical mechanical strain on the walls of arteries and arterioles (where blood pressure is pulsatile) and a constant strain in capillaries and veins where blood pressure is usually nonpulsatile. Because flowing blood exhibits a viscous effect, it tends to “stick” to the endothelium creating a shear stress that is proportional to the product of fluid viscosity and the velocity gradient between adjacent layers of the flowing blood Endothelial cells in all blood vessels are exposed to shear stress, which is a force that acts tangential to the endothelial cell surface causing morphological changes to endothelial cells. The walls of arteries and veins can also be stretched circumferentially as a result of vasodilation and compressed circumferentially as a result of vasoconstriction [14].

Physical forces caused by blood flow and blood pressure act on the walls of blood vessels. Flowing blood generates shear stress tangential to the endothelial cell surface. Circumferential stretch is caused by the action of blood pressure [14].

Molecular elements play an important role in sensing shear stress in endothelial cells. These molecular elements include extracellular matrix (ECM), cell–ECM adhesion, cell–cell adhesion complexes, membrane components (ion channels, caveolae, surface receptors), and cytoskeletal filaments .In addition, recent studies have suggested that epithelial sodium channels (ENaCs) may also play a role in sensing shear stress in multiple cell types . Shear stress applied the luminal surface of endothelial cells is thought to be transmitted throughout the cell as well as to cell junctions and cellular adhesions to the ECM [15].

Thoma’s early observations in chick embryos that blood vessels with higher velocities of blood flow (higher shear stress) became larger whereas those with slower velocity atrophy have

been substantiated in many laboratories in various animal preparations .Mechanical factors associated with blood flow are thought to stimulate capillary development by intussusceptive angiogenesis. In capillaries, intussusception refers to the splitting of single capillaries into two capillaries. Endothelial cells activated by shear stress extend intraluminally forming two endothelial tubes through which blood can flow. Experimental proof for shear stress-induced angiogenesis has been achieved by chronic administration of vasodilators, primarily the α-adrenergic blocker, prazosin. Prolonged treatment with prazosin can increase muscle blood flow about threefold and stimulate angiogenesis. Prazosin-induced angiogenesis could be VEGF-A-dependent .Also, shear stress can activate the VEGFR2 pathway independent of VEGF-A. Other vasodilators such as adenosine and dipyridamole (which increases adenosine levels in tissues) can also increase shear stress and stimulate angiogenesis; however, adenosine has multiple angiogenic actions independent of shear stress. Overall, the mechanism of shear stress-induced angiogenesis is poorly understood [16].

Neither blood flow nor mechanical factors associated with blood flow can actually regulate angiogenesis in heart, skeletal muscle, brain, and other tissues in which the vasculature has primary a nutritive function. Why? Because blood flow itself is regulated by metabolic factors in these tissues. For this reason, the proangiogenic actions of shear stress are thought to facilitate, but not regulate the angiogenesis. Likewise, those steps in the angiogenic process that require mechanosensation of physical stimuli serve to implement angiogenesis under the umbrella of metabolic regulation. There are, however, instances in which flow itself can be considered a controlled variable in the negative feedback regulation of vascular growth. For example, lymphangiogenesis occurs when the rate of fluid loss from blood capillaries exceeds the fluid removal capacity of resident lymph vessels. It is also possible that the flow of interstitial fluid in the interstitial spaces of the kidneys plays a role in controlling angiogenesis in the peritubular capillary bed. This latter possibility will be addressed in future editions.

3. *IN VITRO* MODELS FOR ANGIOGENESIS AND VASCULOGENESI STUDY

In vitro models provide the advantages of low costs and the ease of trying the influences of regulating factors on cell behavior related to angiogenesis. Thus, numerous attempts have been developed to study angiogenic mechanisms in cultured endometrial tissue.

Folkman and Haudenschild [19] observed angiogenesis in vitro two decades ago. They cultured capillary endothelial cells and observed the spontaneous organization of these cells into capillary-like structures. Phase contrast microscopy and TEM analysis confirmed the presence of a lumen within these structures. This study of angiogenesis was considered as a basis for the definition of in vitro endothelial angiogenesis and so many attempts have been designed and referred to the presence of a lumen in the capillary structure as a standard for the confirmation of an in vitro model. From a physiological point of view, an ideal in vitro model should be rapid, easy to use and quantify, reproducible and consider all the representative steps of in vivo angiogenesis such as detachment of endothelial cells from the vascular wall, final tubular morphogenesis, maturation. In vitro models can be divided in two groups according to the reorganizing way of the cells, two-dimensional (when the cells form lumen on the surface of the substrate) and three-dimensional (when the cells develop tubal structures in the surrounding matrix) assays. Furthermore, several *in vitro* systems have been developed to study the cellular events of vasculogenesis. By using cell culture derived from embryo mesodermal and embryonic stem (ES) cell differentiation assays, it was possible to investigate vasculogenesis process approximately as it occurs in the embryo *in vivo*. It was reported that adherent cultures of dissociated cells from quail blastodiscs generated both hematopoietic and endothelial cells that acummolated into characteristic blood islands and produced tubal structures in long-term culture [20]. The murine ES cell-derived embryoid body (EB) formation assay may can be considered as full of promise due to formation of primitive vascular plexus. This study provided an attractive tool for dissecting the mechanisms involved in the vasculogenesis process.

Because formation of three-dimensional vascularized tissue is a major challenge hindering the widespread clinical application of tissue engineering. In this section we summarize different in vitro studies which have been developed to induce angiogenesis and vasculogenesis. In addition, the ability to increase and control vascularization of tissues provides this opportunity to advance the clinical utility of therapeutic angiogenesis such as in wound healing of tissues, myocardial ischemia and peripheral arterial ischemia [21].

3.1. Stimulation of vascularization in vitro models.

Although many studies have developed to induce vascularization engineered tissues over the last few decades, establishing an efficient three-dimensional vascularized tissues has still remained a real challenge in tissue engineering. Recent advances by using microfabrication techniques in the field of tissue engineering have greatly accelerated the progress to fabricate vascularized tissue constructs. Various techniques which have been used to induce vascularization within tissues which can be broadly categorized into two distinct groups, including prevascularization-based methods and vasculogenesis and angiogenesis-based techniques. Table 1 provides an overview of these techniques for vascularization in 3D hydrogels.

3.2. Stimulation of vascularization by Prevascularization-based techniques.

Prevascularization methods such as (i) molding methods, (ii) layering methods, and (iii) hybrid methods of engineered tissue constructs have attracted tremendous attention due to the possibility of providing immediate perfusion of the constructs, improving cell proliferation, delivering of oxygen and nutrients and removing of metabolic wastes in a continuous manner.

One of the molding methods to fabricate microfluidic hydrogels is to use microneedles. These microneedles are placed inside a chamber and are locked in place by adding hydrogel precursors. Open channels are then created after the removal of these microneedles.

Table 1. An overview of the techniques to induce vascularization in the hydrogels

	Methods	Advantages	Disadvantages	Ref.
Prevacularization	Molding based on microneedle	Easy Inexpensive	Unsuitable for the formation of complex networks of interconnected channels. Need of manual handing	[22, 24]
	Molding method using dissolvable network	Easy to deal with Inexpensive Scalable	Use of organic solvents	
	layering method			[25]
	Bio-printing	Precise cell placement High repeatability Possibility of fabricating arbitrary complex constructs Automated Scalable	Require specific equipment	[28] [27]
Vasculogenesis and angiogenesis-based techniques	Photolithography			[36]
	Soft lithography	the potential for developing complex structures, simple convenient for patterning on diverse substrates over a wide range of scale in 2D planes High repeatability Accurate control at the microscale	Cumbersome for the need of multi-iterative procedures	[32, 33]
	Using gradient of growth factors	mimicking the in vivo microenvironment of angiogenesis, maintaining the angiogenic factor activities for a prolonged period of time		[44] [45]
	Using co-culture of different cells			[51] [52]

For example, Sakaguchi reported a new strategy based on microneedle molding to maintain cell viability in co-cultured cell sheet of neonatal cardiomyosites and endothelial cells. They incubated triple-layer cardiac cell sheets on the microfluidic collagen surface and showed endothelial cells migrated to vascularize, and connect with the microchannels in the collagen gel and new capillaries developed. Then other triple-layer cell sheets were added repeatedly resulting a 3D construct with simultaneous beating [22]. Although this is a simple and readily available technique to construct microengineered hydrogels, it has its own limitations, such as manual handling of the molding process, interconnecting neighboring channels and fabricating only simple straight architecture [23]. The combination of porous hydrogels with microneedles provides additional interconnections, which induce angiogenesis and diffusion coefficient and lead to increased cell viability. Madden *et al.* applied micro molding technique to form parallel channels in the porous hydrogels. This arrangement stablished cardiomyocyte bundles, increased angiogenesis and supported cell proliferation which reached adult heart densities[24].

Moreover, Chen *et al.* used a carbohydrate sacrificial material combined with 3D printing and cell infusions via rapid prototyping technique to create vascular networks. They developed a three-dimensional carbohydrate-based backbone was printed using 3D printing technology and then added hydrogels with encapsulated. Therefore a perfusable vascular network architecture was formed which was surrounded by ECM mimetic hydrogels. After gelation the carbohydrate glass network easily was dissolved in water leaving behind a cylindrical channel network. The channels then were infused and seeded with HUVECs and finally endothelialized channels of HUVEC sprouts was seen.

In the layering methods, a network of interconnected channels is developed by stacking the layers and grafting of preformed planer hydrogel slabs. The slabs first are patterned by using micropatterning techniques like photomask lithography or micromolding so that when they stacked together a three dimensional network of channels is created. In order to obtain a tightly sealed interconnected perfusable network of channels, the neighbor layers are linked together irreversibly via partial melting or fusion of hydrogel at the interfaces. For example, Zheng *et al.* [25] developed a network of tubular structure by using the layering methods. They used a silicone mold to pattern PDMS stamps and cast collagen gel. Then they bonded thin layers of

patterned hydrogels over a flat hydrogel layer and formed a microvascular network. In this study a smooth and functional endothelium layer without any leakage was created which also promoted angiogenesis.

In bioprinting method living cells and biomaterials in a three dimensional space are deposed to fabricate a biomimetic construction by using computer-aided design. This technique is very suitable to form complex structures of channels within hydrogels [26]. Another advantage of bioprinting is that cells are able to find themselves in an appropriate microenvironment not only from the point of structural view for support but also from point of biological view for increasing adhesive cellular contacts which provide molecular signaling [27]. Lee *et al.* [28] fabricated a stable, human-scale tissue constructs of any shape by using a computer model clinical imaging data of the anatomical defect and translating the model into a program that bridles the motions of the printer nozzles, which distribute cells to distinct locations. They showed the combining of microchannels into the tissue constructs improved diffusion of nutrients to printed cells and dominated the diffusion limit of 100–200 μm for cell survival in engineered tissues.

3.3. Stimulation of vascularization by using micropatterning technologies.

According to the mechanisms of vasculogenesis and angiogenesis, numerous studies have been developed to improve vascularization in three dimensional hydrogels in a controlled and regulated manner. As mentioned earlier, in vasculogenesis process new blood vessels are formed from EPCs through the formation of vascular plexus and in angiogenesis process groups of ECs from pre-existing vessels sprout, migrate and organize to create new tubular structures eventually forming blood vessels. Therefore in various approaches, both of biophysical and biochemical cues have been used to improve the neovascularization in engineered tissue constructs by fabricating a biomimetic microenvironments for the cells. These approaches can be classified four categories including (1) micropatterning such as photolithography, micromolding, photolithography (2) the use of biomaterials including molecules with angiogennic effects, (3) using of gradient of growth factors and (4) using co-culture of multiple cell types to control cell-cell interactions.

The micropatterning methods are simple and suitable to pattern complex structures of cell-laden hydrogels and elastomers on various substrates. Although these methods are useful for detailed investigation of cell behavior under diverse microarchitectural cues, they are limited to two dimensional substrates, and as they cannot be useful for development of three dimensional perfusable vascular structures.

Soft lithography is another microfabrication technique which has applications in manufacturing microfluidic hydrogels and templates for cell patterning. This method was known as the most robust method to fabricate polymer-based microdevices. Generally in this technique a hydrogel which can contain cells or not (e.g., agarose) is poured onto a polymeric substrate then it is allowed to gel at room temperature and ambient pressure then the gel is peeled off the substrate and put on a flat layer gel [29].

Although soft lithography is remarkably accurate and highly reproducible, it requires that the scaffold be continuous and free-standing structures cannot be fabricated through this method Easy accessibility, simplicity, cost effectiveness and time efficiency are the advantages of this method however it has limitations with ionically and chemically crosslinked hydrogels. Liau *et al.*, applied a versatile soft lithography method to synthesis 3D cardiac tissue with controllable size and architecture. In this work, 3D cell alignment cues for stem cells were introduced in fibrin hydrogels. Cardiovascular progenitors (CVPs) were differentiated into different cells including cardiomyocytes, endothelial cells and smooth muscle, and autonomously supported the formation of functional cardiac tissues. The engineered tissues indicated the organizational and functional properties of native neonatal myocardium such as density, alignment, electromechanical characterizations, conduction velocity between and significant contractile forces [30].

Bian *et al.*, utilized soft lithography to pattern 3D hydrogel environment and cultured neonatal rat cardiomyocytes within the patches. These cardiac tissue patches contained dense cardiomyocyte with highly alignment and electromechanicall connections among cells. Engineered 3D cardiac tissues maturation functionally and structurally. The morphometric assessment revealed that cells proliferated densely and uniformly in the 3D patches and were aligned in the photomask and DTMRI vector directions with minimal deviation [31]. There has been a rapid development in the area of micropatterning technologies for applications in promoting the formation of vascular networks in engineered tissue constructs [32, 33].

Photolithography is a technique in which usually a photosensitive polymeric solution such as methacrylated gelatin of hydrogel is exposed to the light to be cross linked. This light with a certain wave length passes through a photomask, crosslinking process is started by the free radical-based chemical reactions between the photo-initiator and the polymeric solution. Then only defined sections of hydrogel which exposed to the light are cross linked while the leftovers of the material remains uncrosslinked and finally is washed out, creating a hydrogel structure with certain micropattern on the substrate [33, 34]. This method can applied as a tool to develop blood vessel-like structures for organized vasculatures in tissue engineering. For example, Nikkhah *et al.* used photolithography to fabricate a three dimensional endothelial cord-like structure of micropatterned GelMA hydrogels [35]. They changed the geometrical features of patterned hydrogel and observed that the behavior of endothelial cells varied. In addition, Lin *et al.* investigated the application of this technique to generate vascular networks of GelMA by using endothelial colony-forming cells (ECFCs) which was derived from human blood [36].

3.4. Stimulation of vascularization by using functionalized biomaterials.

It has been reported that using biomaterials combined with various angiogenic biomolecular cues can promote the formation of vascular network [37]. Different behavior of cells such as attachment, proliferation, migration and other features such as penetration, organization and matrix remodeling can be guided by functionalizing the scaffold materials with growth factors [38]. For example, immobilizing angiogenic growth factors (GFs), ECM proteins, peptides, or other biomacromolecules within the scaffolds improves vascularization and formation of vascular networks.

In one study, it was exhibited that covalent immobilization of VEGF in collagen hydrogel improved angiogenesis compared to collagen hydrogel without any VEGF. In this project, VEGF and angiopoietin-1 (ANG1) to collagen via EDC crosslinking increased higher tube formation by endothelial cells compared to the collagen scaffolds with individual components [39]. In addition, heparin has also been immobilized in the scaffold and then interacted with VEGF and resulted in prolonged VEGF release and leading improvement in angiogenesis indirectly [40].

Moreover, immobilizing biomaterials can be performed by nonchemical reaction instead of chemical immobilization. For instance, by addition of enzyme sensitive moieties or using non-covalent-interaction can mediate immobilization and release rate of growth factors [41]. Mie *et al.* for example, used artificial ECM molecules to immobilize them non-covalently and then interact with several growth factors and indicated this process enhance angiogenesis [42]. This method has important advantages such as imitating the in vivo microenvironment of angiogenesis, retaining the functionality of angiogenic factor for a prolonged period of time. This long time of activity protects the compounds against fast metabolism through routes such as endocytosis and encapsulating and delivering angiogenic compounds in a temporally and controlled manner [43].

3.5. Stimulation of vascularization by using gradient of growth factors.

The use of concentration-gradients of common angiogenic growth factors mostly in microfluidic systems is a great method to improve vascular structures in a cell laden or cell-seeded hydrogel. In this technique, a concentration gradient of growth factors is created in the neighboring endothelial cells, then endothelia cells sense this gradient and migrate toward the region of high GF concentration. They align themselves into well-organized structures afterwards and promote the capillary-like tubular structure formation [44]. Several growth factors have been used to induce vasculogenesis in engineered tissue including VEGFs, the fibroblast growth factors (FGFs), angiopoietins, the transforming growth factors (TGFs), and the platelet derived growth factors (PDGFs) [44]. In order to create a sustained release during prolong time, the growth factors have been combined with the scaffolds and so local concentration gradients was formed through microfluidic channels. In another study, VEGF was loaded on scaffolds of PLGA and doubled capillary structures compared to the control scaffolds without VEGF [45]. Perets *et al.* [46] developed a hybrid scaffold system of basic FGF (bFGF) encapsulated PLGA microspheres within alginate gel and performed in vivo analysis. They observed that after 21 days of implantation, a significant number of vessels was created by using bFGF compared to the scaffolds without bFGF.

It was shown that both single growth factor and several kind of them can be applied simultaneously. However, recent studies has disclosed that the combination of multiple growth factor types increases the formation of endothelial cells lumen and their stabilization. Hao *et al.* [47], for instance designed an hydrogel system of alginate to delivery of both VEGF and PDGF simultaneously and observed after four weeks of hydrogel injection into a rat myocardial infarction model, the scaffolds with both factors resulted in a more amount of vessel density of about in comparison of the scaffolds with either VEGF or PDGF. This study suggested that the serial delivery of VEGF and PDGF may be able to facilitate the formation of capillary vessels[47]. Moreover, in another study the individual and combined influences of VEGF and FGF2 on endothelial lumen formation was evaluated [48]. The results approved the synergistic impact of several growth factors by indicating that the system loaded with a combination of growth factors reduplicated the formation of mature blood vessels compared to the system with a single growth factor. Hopkins *et al.* proposed that, important elements to using growth factors efficiently for the formation vascular structures include: controlled rate of growth factor release, correct blend of growth factors, right dosage and suitable exposure time [49].

3.6. Stimulation of vascularization by using co-culture of different cells.

It was reported that co-culturing different proper cell types such as fibroblasts, SMCs and various stem cells under suitable microenvironmental cues can promote spontaneous alignment of the vascular cells and form vascular networks. There are many studies which have used this technique to promote vascularization through angiogenesis. These studies have shown that in engineered scaffolds containing endothelial cells or their progenitor cells capillary/microvascular tubules were created.

For example, in one study co-culturing of HUVECs, dermal fibroblasts, and keratinocytes on 3D porous chitosan-collagen scaffolds has been performed to engineer vascularized human skin. Capillary tubal-like structures have been observed after 15 days of culturing, but in the monoculture system was not seen. It was reported that the cell-matrix interactions and the cell-cell interactions of HUVECs with fibroblasts influenced the formation of three dimensional tubal structures [50]. Furthermore, the fibroblasts could produce large amounts of ECM they were co-cultured with VEGF producing keratinocytes.

Sudo *et al.* studied the effect of cell co-culturing [51] in a microfluidic system. They patterned two parallel channels on a PDMS platform and used collagen gel to investigate vasculogenesis in the co-culturing system of primary rat hepatocytes and endothelial cells. They reported that three dimensional vascular networks was formed and expanded across the middle of hydrogel while in monoculture system only two dimensional sheet structures were observed [51]. In addition, Yeon *et al.* [52] investigated the application of co-culturing HUVECs and fibroblasts cells in the development and characterization of perfusable tubal structures in a microfluidic device which had two connected channels contained fibrin gel by that eight small channels linked them together.

Many studies have highlighted the importance of using autologous stem cell sources to enhance vascularization in engineered tissues. In these researches various grafts with bone marrow cells and endothelial progenitor cells were cultured and the creation of tissue-engineered vascular grafts with good long-term function have been observed [53, 54].

4. MATHEMATICAL MODELS FOR ANGIOGENESIS AND VASCULOGENESIS

It is very difficult job to study the effects of all related factors simultaneously experimentally. In addition, experiments are costly and time-consuming and synthesis protocols mostly do not provide for independent study of specific elements. Therefore, many of mathematical models have been used to investigate different influence of various parameter on angiogenesis [55] [56, 57]. For instance, models have been developed to study how the effect of growth factors and receptors on endothelial cell proliferation, or their assembling into individual vessels. This computational models relate to angiogenesis in the context of physiological and pathological states. These numerical models can be categorized according to their spatial scale(s) to encompass. Thus, they are divided to a) single cell [55], b) multi cells [58], c) tissue scale [59]. others have at the whole tissue level [59]. Moreover, published models can be classified base on the time scales (in the order of minutes to weeks) that they were developed to simulate [60]. "Multi-scale" models, as a powerful tools also have designed to study both biomechanical and biochemical phenomena [61]. On the other hand, published models can be arranged in three groups according the technical approaches which they used. Table 2 represents this kind of categorizing of mathematical modeling of angiogenesis.

Table 2. The main approaches for modeling of angiogenesis.

Models	Properties	Ref.
continuum-based modeling	- Neighbor parts are similar to each other - Gradual variation exist across the system without discontinuities - Systems of differential equations that describe physical phenomena as being a continuous spread in space and/or time are solved. - Details of the constituents such as cell-level details and new capillary growth are ignored.	[62]
discrete approach	- The entities being modeled (e.g. endothelial cells) and their unique behaviors (e.g. proliferation) are explicitly represented. - Systems of differential equations at discrete locations (i.e. points on a 2-D grid) are solved. - Computational algorithms are applied.	[63, 64]
Stochastic modeling	- Probabilities are used to define biological events. - The randomness related to biological processes are clearly accommodate. - It is assumed that random variation and fluctuation in the system dominates the overall behavior of the system.	[58]

4.1. The applications of mathematical models.

The mathematical models are organized according to the central physiological or pathological settings tumor angiogenesis [62, 65], wound healing [66]). They also have been used in simulating tissue engineered constructs in which angiogenesis take placed [67], and in vitro vasculogenesis [55].

For instance, Xu *et al.* [57] by coupling three elements including the growth of vessels, tumor growth and perfusion of blood, suggested a mathematical model to study tumor angiogenesis and the dynamic response of tumor cell to the microenvironmental variations. This study provides a comprehensive solution of blood perfusion variables by coupling the domains of intravascular, transcapillary and interstitial fluid flow and using the haemodynamic calculation. The authors also estimated vessel collapse process based on the wall shear stress criterion to give on vasculature remodelling. Their simulation indicated not only the process of tumor angiogenesis and its main features but also the spatial distribution of tumor cells within 24 days. An important advantage of this model was being independent of the initial tumor and networks which approve its using.

Numerical models of in vitro angiogenesis have also been introduced to investigate the interactions of endothelial cells and forming new vessels in culture. These models have provided an understanding angiogenesis process for tissue engineering researchers to fabricate microvascular networks *in vitro* constructs. In addition, numerical models provide rapid screening of designing suitable biomaterials with controllable properties such as porosity. For example, Sumo *et al.* [56] have designed a 3D agent-based model to study the influence of pore architecture of biomaterial platforms on angiogenesis. Thus 3D scaffold models with different pore architectures (e.g. homogeneous and heterogeneous) were defined (Figure 4, A, B, C) and software agents were used to represent endothelial cells, their interaction with each other and their micro-environment. leading to the invasion of blood vessels into the scaffold. Their results showed that large and interconnected pores are able to support angiogenesis extensively. In addition, Lemon *et al.* [68] suggested a simulation model to estimate the growth of blood vessels in a porous biomaterials. Their model simulated formation of a vascular network and the migration of capillaries within a virtual pore of the engineered scaffold produced by different methods (such as salt casting, solvent leaching) when it is implanted in host tissue (Figure 4, D). The authors considered an array of these pores by using rapid prototyping method (Figure 4, E). In addition, they explained the variation of the blood flow through the network

while growth proceeded and the impact of the different strategies of cell-seeding the pore on the extent of forming new vessels.

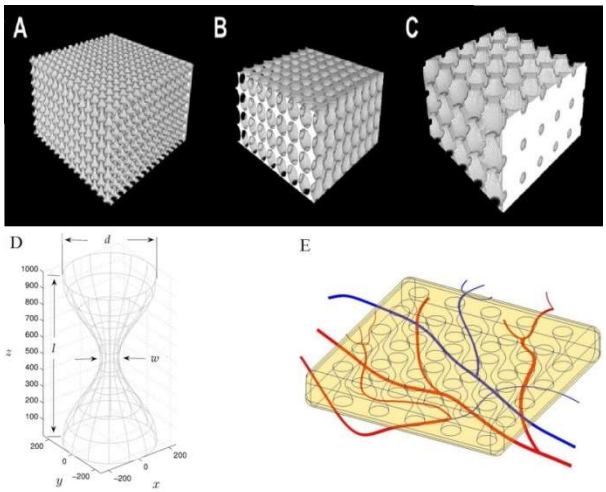

Figure 4. Non-uniform distribution of pores more closely approximates actual scaffold structures. A) a 275 micron uniform pore scaffold at 60% porosity, B) 275 micron ununiformed pores at 60% porosity and C) bioactive glass scaffold formed using a foaming technique [56]. D) A schematic diagram of the virtual pore and D) A schematic diagram of an array of virtual pores embedded in living tissue [68].

4.2. The main obstacles of mathematical modeling.

Although Computational models provide some advantages such as saving time and cost, they have certain challenges, caveats, and pitfalls that should be considered to produce a reliable meaningful and useful analysis. As first obstacle, it should be consider that angiogenesis is heterogeneous, non-linear, dynamic and multi scales which make it a complex process. Therefore, it is necessary to make assumptions which simplify angiogenesis for modeling so that the models are solvable and give reliable results. One of the main simplifications for angiogenesis modeling is to assume a simpler microenvironment. For example in some studies the models developed for two dimensional environments instead of real three dimensional. Another work to make a simplification in angiogenesis process is to consider only the effect of one important factors instead of the number of them [69]. The second obstacle is to identify of appropriate parameters and fixed or adjustable variables which quantitatively dictate the behavior of the model. Many of these parameters can be derived by experimental results. However, there is a significant challenge to measure some of these parameters by using in vivo current experimental methods, and so in vitro assays are applied to attain them. Therefore, in order to build a computational model for angiogenesis, both of experimental assays and mathematical techniques are used. Third problem is the validation of mathematical models. Because model validation is usually performed by comparison model results with experimental observed phenomena and measuring some parameters are very rigorous [70].

5. CONCLUSIONS

Vascularization is one of the most important challenges for the clinical application of tissue engineering. For generation of functional tissue engineered constructs, or regeneration of living tissues, vascularization should be considered. Therefore it is necessary to have a deep understanding of angiogenesis and vasculogenesis mechanism. In this review, an overview of the molecular mechanism of angiogenesis, the novel techniques available for vascularization of engineered tissue constructs was presented. It was mentioned that using microfabrication techniques and blending microfluidics into tissue engineering leads to generate vascularized biomaterials has been steadily gaining ground. In addition, different types of mathematical and computational modeling methods have been provided that have been employed in the study of angiogenesis and summarized an array of published models which investigate the impact of some elements on angiogenesis process.

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