Vascularization of Biomaterials for Bone Tissue Engineering: Current Approaches and Major Challenges

Ehsan Jabbarzadeh[1,2], James Blanchette[1], Tarek Shazly[3], Ali Khademhosseini[4,5,6], Gulden Camci-Unal[4,5] and Cato T. Laurencin[7,8]*

[1]*Department of Chemical Engineering, University of South Carolina, SC 29208, USA;* [2]*Department of Orthopaedic Surgery, University of South Carolina, SC 29203, USA;* [3]*Department of Mechanical Engineering, University of South Carolina, SC 29208, USA;* [4]*Center for Biomedical Engineering, Department of Medicine, Brigham and Women's Hospital, Harvard Medical School, Cambridge, MA 02139, USA;* [5]*Harvard-MIT Division of Health Sciences and Technology, Massachusetts Institute of Technology, Cambridge, MA 02139, USA;* [6]*Wyss Institute for Biologically Inspired Engineering, Harvard University, Boston, MA 02115, USA;* [7]*Department of Orthopaedic Surgery, University of Connecticut Health Center, Farmington, CT 06030, USA;* [8]*Department of Chemical, Materials and Biomolecular Engineering, University of Connecticut, Storrs, CT 06269, USA*

Abstract: Tissue engineering uses various approaches to restore bone loss and heal critical-size defects resulting from trauma, infection, tumor resection or other musculoskeletal diseases. The success of bone tissue engineering strategies critically depends on the extent of blood vessel infiltration into the scaffolds. It has been demonstrated that blood vessel invasion from the host tissue into scaffolds is limited to a depth of several hundred micrometers. Limited vessel perfusion restricts the formation of bone in central regions of the scaffold, leads to loss of cell viability in this region and ultimately does not support healing of the defect. This review addresses the importance of vascularization in bone tissue engineering, discusses the key factors regulating the process of angiogenesis, and provides an overview of current approaches to direct blood vessel formation in biomaterials.

Keywords: Regenerative engineering, engineered biomaterials, angiogenesis, bone, hypoxia, microscale technology.

BONE TISSUE ENGINEERING

Current approaches to repair bone injuries, trauma and deformities widely use autografts and allografts [1-3]. Autografts are the gold standard for clinical bone repair as they are osteoinductive and do not pose a risk of disease transmission [4, 5]. The major drawbacks in using of autografts are the need for a secondary surgery, donor shortage and donor-site morbidity [6-9]. Although allografts can be used as alternatives, immune rejection and risk of disease transmission are considered as major limitations [4, 10-13]. The recent emergence of regenerative engineering has made it possible to develop viable grafts for bone repair. This approach is based on the use of specific cell types, growth factors, and three dimensional (3D) porous scaffolds, by themselves or in combination [14-16].

Scaffolds play an important role in providing a supporting extracellular matrix (ECM) onto which host cells, or transplanted cells can preserve their normal functions, grow, and differentiate [17-21]. To date, biodegradable polymers such as poly(lactic acid) (PLA), poly(glycolic acid) (PGA) and their copolymers poly(lactide-*co*-glycolide) (PLGA) as well as ceramics such as hydroxyapatite (HA) have received significant attention to fabricate scaffolds for musculoskeletal repair [22-31]. This is due to the capability of these polymers to fabricate scaffolds with high degree of biocompatibility, osteoconductivity and tunable mechanical properties. As an example, the osteoconductivity of PLGA scaffolds have been manifested by high levels of a bone marker, alkaline phosphatase (ALP) activity, by seeded osteoblasts [32]. The degradation of PLGA results in lactic and glycolic acids, both found naturally in the body [33]. To this end, a number of fabrication methods have been developed to synthesize PLGA into scaffolds with appropriate structure and mechanics. To name a few, we can refer to solvent casting particulate leaching, microsphere sintering, phase inversion, and prototyping techniques [19, 34].

Growth factors can be incorporated into scaffolds to direct cell proliferation, migration, and differentiation. A significant number of studies have focused on selectively incorporating osteoinductive growth factors in the scaffolds [35-38]. From the group of candidate factors, bone morphogenetic proteins (BMPs) have received significant attention in bone tissue engineering applications. BMP's 2 through 7 have been shown to elicit the healing of bone defects in a variety of *in vivo* models [39-46].

Cells seeded on scaffolds may participate in neotissue formation and release growth factors that signal host cells to migrate into the scaffolds and differentiate into specific lineages. The tissue engineering community has used various sources of cells (e.g., osteoblasts) to recreate the building blocks of natural bone [47-50]. For example, stem cells are

*Address correspondence to this author at the Department of Orthopaedic Surgery, Department of Chemical, Materials and Biomolecular Engineering, Connecticut Institute for Clinical and Translational Science, Institute for Regenerative Engineering, The University of Connecticut, Farmington, CT 06030-3800; Tel: (860) 679-2594; Fax: (860) 679-1255;
E-mail: Laurencin@uchc.edu

2211-5536/12 $58.00+.00

uniquely positioned as the most promising cell source for tissue engineering strategies due to their capability of self-renewal as well as the ability to differentiate into diverse cell types [51-57]. Control over stem cell self-renewal, differentiation and maintenance of stable cell lineage commitment is crucial for harnessing the cell plasticity and realizing its utility in regenerative medicine. The widespread research and clinical use of stem cell-derived tissues can be promoted by improved understanding of the cellular and molecular mechanisms of stem cell differentiation.

BONE IS A VASCULARIZED TISSUE

Like most other tissues, bone is highly vascularized and relies on blood vessels to supply nutrients such as oxygen and to facilitate removal of metabolic waste products. In the native bone tissues, constituent cells are present in a 100-200 μm proximity to blood vessels and capillaries to ensure adequate transport of oxygen, nutrients and waste products [58, 59]. The critical role of vascularization in bone development, growth and repair has been well established [60-68] and is hypothesized to be the initiating factor in new bone formation [69]. While this claim is yet to be verified, there is substantial evidence that angiogenesis (formation of new blood vessels from pre-existing ones) is a critical process during endochondral ossification [70, 71]. In addition, the disruption of angiogenesis has been shown to inhibit the repair of femoral fractures and cortical bone defects in animal models [72-74].

In bone tissue engineering applications using porous materials, it is desirable that scaffolds allow for early internal development of functional blood vessels. Neovascularization within the scaffolds reduces the impediment to biochemical transport relative to diffusion through the otherwise present fibrous capsule characteristics of the classical foreign body response (FBR) [75, 76]. Mitigating the FBR in this way allows for optimal nutrient/waste transport for engineered tissues that are grown in porous scaffolds and has the potential to promote rapid, controllable dosing in drug delivery and immuno-isolated gene and cell based implant therapies [77].

To fully realize the promise of bone tissue engineering therapies, it is vital that we explore strategies to control the neovasculature development. Accomplishing this goal requires a thorough understanding of the mechanisms by which various signals affect the formation of blood vessels within biomaterials. A number of parameters affect vascular growth in porous scaffolds including: (i) porous architecture (pore size and porosity), (ii) transport of growth factors within the scaffolds (diffusion and concentration gradients), and (iii) cell response to growth factors (chemotaxis and chemokinesis) [78]. The following is an overview of the significant efforts that have been devoted to characterize and control the mechanisms that govern angiogenesis, as well as the major approaches to engineer vascularized biomaterials.

THE BIOLOGY OF ANGIOGENESIS

Blood vessel formation can be classified as angiogenesis or vasculogenesis, the former referring to formation of vessels from the pre-existing vasculature and the latter to the initial formation of vessels during embryonic development. These processes are cumulatively referred to as neovascularization and involve complex interactions among a wide variety of regulatory factors and different cell types. Angiogenesis is a highly regulated and essential component of some physiological processes, such as wound healing, but if unregulated can lead or contribute to a variety of disease states. Due to its central role in tumor development, the various stages of angiogenesis have been studied most extensively in relation to cancer. A large number of studies have linked angiogenesis with tumor progression and a worsening of patient prognosis [79-81]. These studies have motivated the development and testing of a range of anti-angiogenic compounds in an effort to improve patient survival and quality of life for a range of tumor types. The importance of angiogenesis in tumor development was first recognized by the pioneering work of Judah Folkman in the 1970s. While initially controversial, examination of growth of tumors demonstrated the critical role that angiogenesis plays in the proliferation and dissemination of cancer cells. Once a small tumor mass becomes highly vascularized, nutrients normally delivered to healthy tissue will be consumed by dividing cancer cells and contribute to tumor growth. In such a case, cancer cells will displace healthy cells until the tumor reaches a diffusion-limited maximal size. While cancer cells will typically not initiate apoptosis in a low nutrient environment, they do require the basics of cell function like oxygen, glucose and amino acids.

Tumor cells will therefore continue dividing because they do so without regard to nutrient supply, but many will also perish because of insufficient nutrition. The tumor cells at the outer edge of a mass can readily access nutrients and therefore thrive, while internal cells die and create a necrotic tumor core. A steady state tumor size will emerge over time, which is a self-equilibration of cell growth and death given the current connection with the circulatory system. This diffusion-limited maximal size of most tumors is around 2 mm [82]. To grow beyond this size, the tumor must induce the formation of blood vessels to provide the nutrients necessary to fuel its continued expansion.

Whether triggered by a nutrient-starved tumor mass or an implanted tissue engineered scaffold, angiogenesis is a process proven to be the result of numerous interactions, among regulators, mediators and stimulatory molecules. These molecules regulate the proliferative and invasive activity of the endothelial cells that line blood vessels. Some of the most prominent angiogenesis stimulatory molecules include vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF), platelet-derived growth factor (PDGF) and certain matrix metalloproteinases (MMPs). Table **1** lists a number of stimulatory molecules and their role in angiogenesis. Some endogenous angiogenesis inhibitors are the interferon family (α, β and γ), thrombospondin-1 and -2, certain tissue inhibitors of matrix metalloproteinases and protein fragments such as angiostatin and endostatin.

The formation of a new vessel from the pre-existing vasculature is characterized by a number of sequential events. Prior to neovascularization, endothelial cells exist in a near quiescent state with only about 1 in every 10,000 (0.01%) undergoing division at a given time [83]. The turnover rate of endothelial cells increases up to 50-fold during the forma-

Table 1. Endogenous Angiogenesis Stimulators

Acidic fibroblast growth factor (FGF-1)	Potent general mitogen and motogen for endothelial cells
Basic fibroblast growth factor (FGF-2)	One of the most potent mitogens and motogens for endothelial cells. Can stimulate VEGF secretion and these factors often work synergistically
Epidermal growth factor	Stimulates proliferation and increases motility of endothelial cells
Hepatocyte growth factor	Secreted by mesenchymal-derived cells, a mitogen and motogen for endothelial cells and inducer of VEGF
Platelet-derived growth factor family	Stimulates proliferation and motility of endothelial cells. Involved in recruitment of pericytes to vascular sprouts and maturation
Transforming growth factor α	Mitogen for endothelial cells, stimulates angiogenesis
Transforming growth factor β	Stimulates production and activation of MMP-2, but also shown to inhibit endothelial proliferation *in-vivo*
Vascular endothelial growth factor (VEGF) family	Mitogen, motogen and survival factor for endothelial cells. Can guide migration of tip cells and growth of vascular sprout
Angiogenin	Angiogenic enzyme which promotes vessel formation
Angiopoietins	Secreted angiogenic factors that bind VEGF receptor (Tie-2) and stimulate endothelial cell survival. These factors are also involved in vascular maturation and can be classified as angiostatic when they promote adoption of the quiescent phenotype from endothelial cells
Interleukin-6	Pro-angiogenic factor expressed transiently by capillary network around follicles
Interleukin-8 (CXCL8)	Mitogen for endothelial cells secreted by cancer cells
Matrix metalloproteinases (MMPs)	Remodel the surrounding matrix to release sequestered angiogenic factors, produce angiogenic (and angiostatic) matrix fragments
Tumor necrosis factor α	Pro-inflammatory cytokine shown to stimulate angiogenesis

tion of a new vascular sprout [84]. Endothelial cells can be classified as tip cells or stalk cells based on their location on the growing vascular sprout. These cell types are distinguished by a sharp contrast in notch activity. Formation of the vascular sprout requires the remodeling of the ECM surrounding the vessel from which this new branch emerges as well as disengagement of surrounding cells like pericytes. MMPs are responsible for degrading the ECM and as the endothelial cells proliferate and migrate along the gradient of angiostimulatory molecules, continued ECM turnover can release sequestered factors, which further accelerate this process. The tip and stalk cells provide the framework for the new vascular network [85, 86]. Endothelial cell sprouts organize into tubular structures and connect (anastomose) with each other as well as the existing vascular network.

Formation of new vessels during physiological angiogenesis is self-limited due to the production and release of angioinhibitory molecules (Table **2**). The equilibrium that normally exists between stimuli and inhibitors for angiogenesis is thought to be unbalanced during tumor cell-initiated neovascularization. While new vessels enable the tumor to grow by redefining the diffusion-limited maximal size, lack of vascularization curtails growth and favors tumor stabilization [82]. Some tumor masses never grow beyond this point, as they are incapable of recruiting new vessels. Acquisition of the angiostimulatory phenotype, also called the "angiogenic switch", is thought to result from a local imbalance between positive and negative regulators of angiogenesis. Efforts to drive angiogenesis in regenerative medicine strategies can use this process as a model with attention paid to encouraging the necessary remodeling and vascular maturation that are often absent in tumor vasculature.

STRATEGIES TO PROMOTE VASCULARIZATION OF BIOMATERIALS

Relying on infiltration of vessels into the scaffold after implantation has not been successful to date. Thus, off-the-shelf tissue engineering products are limited to thin or avascular tissues (e.g. skin and cartilage) [87, 88]. Blood vessels from the surrounding host tissue initially invade a superficial distance in the constructs [89-91]. Importantly, vessel infiltration beyond this depth for larger scaffolds occurs too slowly to support newly formed tissue or cells seeded on these constructs [92-95]. In addition, the newly formed capillaries formed are transient in nature [96]. Therefore, the development of approaches to enhance vascularization is essential for a successful clinical outcome of bone tissue engineering strategies. The tissue engineering community has designed different strategies to accelerate the infiltration of host vasculature and to encourage a greater depth of network formation into the scaffold (Fig. **1**). In the following, we review these approaches, their principles and drawbacks.

Angiogenic Growth Factor Delivery

Soluble growth factors regulate cell proliferation, migration, and cell-cell interactions during angiogenesis. Some of the known growth factors are VEGF, bFGF, PDFG and

Table 2. Endogenous Inhibitors of Angiogenesis

Angiopioetins	Combined angiogenic and angiostatic behavior as discussed in Table **1**
Interferon-α and β	Block production of pro-angiogenic molecules
Interferon-γ	Induced by IL-12, blocks induction of angiogenesis by FGF, induces production of IP-10
Interferon-γ-inducible protein (IP-10) (CXCL10)	A CXC chemokine induced by interferon-γ, active anti-angiogenic moiety
Interleukin 10	Stimulates TIMP-1 expression, inhibits secretion of MMP-2
Interleukin 12	Inhibits angiogenesis *in vivo* by stimulating interferon-γ to induce IP-10, activates natural killer cell cytotoxicity of endothelial cells
Platelet factor-4 (CXCL4)	Member of CXC chemokine family that inhibits endothelial cell proliferation and migration
Soluble VEGF receptors	Soluble form of VEGF receptors are capable of binding VEGF molecules in the circulation and reducing net function
Thrombospondin-1	Endogenous inhibitor of angiogenesis down regulated with p53 mutation
Tissue inhibitor of matrix metalloproteinase-1, -2 (TIMP-1,-2)	Able to block activity of some MMPs, decrease induction of endothelial cell migration and invasion
Angiostatin	Plasminogen fragment containing kringle domains that inhibit angiogenesis
Endostatin	Fragment of type XVIII collagen that inhibits endothelial cell proliferation and angiogenesis
Prolactin fragment	Inhibits endothelial cell proliferation
Secreted protein, acidic and rich in cysteine (SPARC)	Inhibits cell attachment and spreading, fragments shown to have anti-angiogenic activity

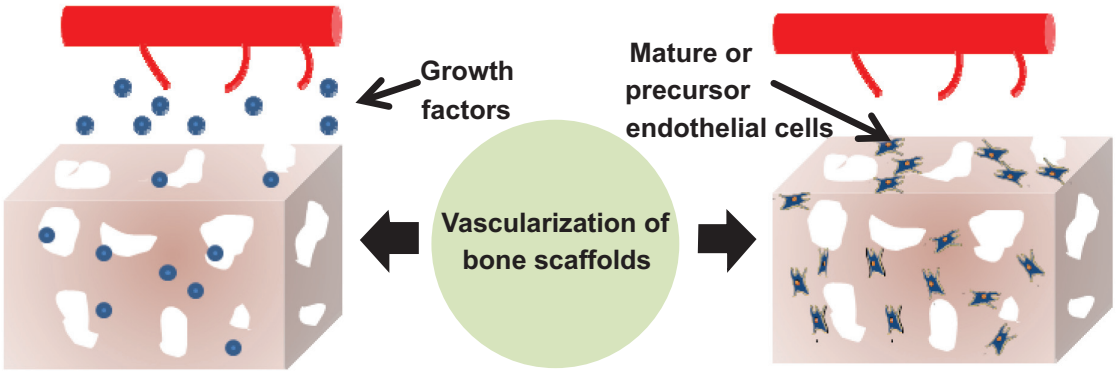

Fig. (1). Schematic drawing of growth factor delivery and cell transplantation strategies to induce vascular growth in scaffolds for bone tissue engineering.

transforming growth factor-*β* (TGF-*β*). VEGF is considered as the most potent angiogenic growth factor that induces endothelial cell migration and proliferation [97-99]. Similar to VEGF, bFGF is synthesized by a number of cell types and is shown to stimulate endothelial cell proliferation and migration [100]. PDGF is shown to influence newly formed vessel maturation by recruiting pericytes [101, 102]. TGF-*β* is a multifunctional growth factor that is produced by variety of cell types such as fibroblasts and endothelial cells [103].

Inspired by the mechanism of tumor angiogenesis, various groups have employed angiogenic growth factors to drive endothelial cells from host blood vessels to migrate into pore spaces of scaffolds. Through the use of growth factors such as VEGF, bFGF, and platelet-derived growth factor (PDGF alone or in combination new blood vessel formation can be accomplished [104-113]. Growth factors encapsulated within a polymeric matrix or adsorbed to the surface of scaffolds have been demonstrated to establish a localized system capable of sustained protein delivery towards enhancement of vascular infiltration [114-118]. Direct growth factor delivery approaches however, face major drawbacks including the short half-life and toxicity of growth factors. In addition, the limited control over distribution of factors can lead to neovascularization at undesired sites, rheumatoid arthritis and tumor growth [119, 120].

An alternative strategy to the delivery angiogenic growth factors within porous biomaterials is *ex-vivo* gene therapy. This strategy is based on the use of genetically modified cells to impart the natural release of bioactive proteins at physiological doses. Although gene therapy approaches have significant promise to improve biomaterial vascularization and ultimate fracture healing [121-125], the host immune response to the viral vectors limits their application only to immune compromised animal models. Recent efforts by various groups on characterization of the biology of immune response to viruses and modification of the genome of vectors may help realize the full potential of gene therapy [126-129].

Cell Transplantation

Another strategy to promote vascular formation in biomaterials involves the incorporation of mature or progenitor endothelial cells and smooth muscle cells into scaffolds to serve as precursors of a vascular network. Endothelial cells form the lumen of blood vessels, constitute a barrier for nutrient transport and provide a non-thrombogenic surface for blood contact, playing a vital role in normal vascular function as well as angiogenesis [130-132]. Smooth muscle cells confer mechanical strength to the vessel directly and through production of ECM, and control vessel pressure and tone. The cell transplantation approach is based on the hypothesis that transplanted endothelial cells can anastomose with invading host cells and form a functional vascular network within the scaffolds. A clear advantage of this approach is the reduction of the time necessary for the formation of new blood vessels [133-141].

The striking results of recent *in vitro* and *in vivo* endothelial cell transplantation studies have inspired the development and application of this strategy. Endothelial cells can organize into capillary-like structures containing lumen on collagen-based matrices [133, 134]. It was further demonstrated that these capillaries can integrate with the host vasculature and become functional perfused blood vessels [135]. As an example, Tremblay *et al*. demonstrated that the endothelial cells cultured in skin grafts can anastomose to the host vascular system within 4 days, whereas vascularization of a non-vascularized graft took as much as 14 days [142]. Remarkably, the injection of endothelial progenitor cells into acute ischemic sites was shown to result in neovascularization in a few weeks and augmented the surviving areas.

Mesenchymal stem cells (MSCs) have also been used in cell transplantation approaches to vascularize tissues. Under appropriate conditions, MSCs can differentiate along endothelial and smooth muscle cell lineage [51-57, 143-150]. These cells can integrate with developing vasculature and new bone within the scaffolds, in a manner closely mimicking the normal development [151-157]. MSCs can also respond to microenvironmental changes and adjust their release profile accordingly. Once vascular cells have been recruited to the site, MSCs have the ability to integrate either into the forming vessels or the surrounding bone. This eliminates the need for clearance of the delivery vehicle as is seen with synthetic systems. Genetic modification of cells can increase the secretion rate of one or more agents but comes with the challenges and safety concerns associated with genetic engineering. Moreover, abnormal secretion of agents such as VEGF or bFGF could impede vascular maturation.

Cell-based strategies have a number of advantages, which include release of multiple factors controlled by environmental conditions, no requirement for large reservoirs of agents that could rupture prematurely and the ability of the delivery system to integrate with the developing tissue. A major challenge facing cell delivery strategies is the low viability of the transplanted cells. The limited anastomosis and integration of transplanted cells with host circulation jeopardizes successful results. In addition, endothelial cell transplantation approaches possess a limited control over blood vessel maturation in scaffolds.

Hypoxic-Conditioning to Drive Physiologic Release of Angiogenic Growth Factors

Cells used for tissue engineering are isolated from sites in the body where oxygen tensions are far below the 20% oxygen level used in cell culture studies. While cells in the lining of the lung and other highly perfused organs (such as the liver, heart and kidneys) exist in oxygen tensions similar to a 4-14% oxygen environment, many other cell types exist in the "hypoxic" range (less than 5%). Many of the *in vitro* studies in bone tissue engineering are conducted in oxygen environments, which do not accurately represent the *in vivo* conditions following implantation. A bone defect site requiring a scaffold to promote healing has a low oxygen tension (0-3% typically) due to damage to the local vasculature that accompanies bone loss [107, 158]. These conditions can have a negative impact on cell function but are also important in guiding cell phenotype. Low oxygen (hypoxic) signaling is critical in embryonic development and oxygen should be treated not only as a nutrient but also a potent signaling molecule [159-161].

Angiogenesis in ischemic tissue is also a process driven by hypoxia and more specifically, hypoxia-inducible factors (HIFs). It has been estimated that 5% or more of our genes are regulated by HIF-1. VEGF, bFGF, angiopoietin 2 (Ang-2), TGF-β and PDGF are examples of angiogenic molecules whose expression is increased by HIF-1 activity [162-164]. Hypoxia and HIF-1 are also implicated in the recruitment of circulating angiogenic cells, critical in vascular remodeling [165-167]. HIF-1-deficient mouse embryos fail to develop a vascular network and this genetic modification is embryonic lethal at midgestation.

Much of the prior research on the role of hypoxia and HIF-1 in driving vascular growth is in relation to cancer. As tumors expand past the limits of the local nutrient supply, angiogenic factors are secreted and additional vessels are recruited to the site fueling further expansion of the tumor. Interplay of multiple factors is essential for capillary development *in-vivo* and represents a strategy superior to those where a single agent is released to drive this process [168, 169]. VEGF has been a popular choice to drive vascular ingrowth into scaffolds but it does not work alone. VEGF can drive endothelial cell proliferation but it will also inhibit vascular maturation so at best its delivery needs to be closely regulated. Co-culture of endothelial and smooth muscles cells leads to reduced responsiveness to VEGF by the endothelial cells [170]. However, combinations of VEGF with

other factors such as Ang-2 do stimulate capillary sprouting in co-culture systems. It is therefore not surprising that relying on controlled release of a single factor does not produce sufficient vascular development and responsive delivery of multiple agents is a more promising approach. Hypoxia (and specifically HIF-1) is shown to trigger secretion of a cocktail of angiogenic factors. This allows us to create an environment within the scaffold that more accurately mimics the one seen during physiological angiogenesis [171]. A schematic representation of this process is shown in Fig. (**2**).

Microscale Technologies for Vascularization of Biomaterials

Studies have shown that it is challenging to create the natural complexity and spatial organization in engineered biomaterials especially when aiming to mimic highly vascularized tissues, such as, bone, liver or heart [172]. To address this constraint, microscale technologies, such as microfluidics, soft lithography, bioprinting and photolithography have been used to generate microvascular geometries in biomimetic constructs [58, 173-175]. Among these techniques, microfluidics is a widely used technique to generate vessel-like structures. Microfluidic channels with capillary patterns have been constructed utilizing hydrogel-based materials [176-178]. For example, 3D vascular tissues were mimicked by a microfluidic set-up, which enabled deposition of different types of vascular cells in each layer [173]. Additionally, perfusable channels were fabricated within cell-encapsulated hydrogels to form microvasculatures in engineering biomaterials [177, 179].

Soft lithography has been used to create vascular networks in biopolymers, such as bifurcating channels or capillaries, making use of elastomeric molds. For example, microvascular patterns from poly(glycerol sebacate) (PGS) were generated by creating the replica from silicon wafers [180]. The PGS scaffold was then assembled into a microfluidic system and cultured with endothelial cells under physiological flow conditions up to 14 days.

The formation of vascular structures can also be achieved by photolithoghrapic strategies based on modular assembly principles. In one study, sequential assembly of photopatterned microgels has been carried out to obtain cylindrical microvessels with single or branched lumens [58]. Another study has demonstrated the alignment of endothelial cells in hydrogel channels by means of photolithography for vascularization purposes [181].

Researchers have recently introduced computer-aided rapid prototyping approaches to print vascularized tissues and organs for regenerative medicine [175]. These strategies have been used to develop microvascular networks in 3D hydrogels (Fig. **3**) [175]. Single cells or cell aggregates are suspended in hydrogels droplets and then placed on predefined positions to generate the shape of the target tissue. In an attempt to create endothelial cell-loaded hydrogel tubes, computer-aided bioprinting strategies were utilized [182, 183].

In addition to development of vascular microarchitectures in bioengineered constructs, formation of an endothelial monolayer inside the luminal region is critical for obtaining functional vessels [180]. For example, monolayers of

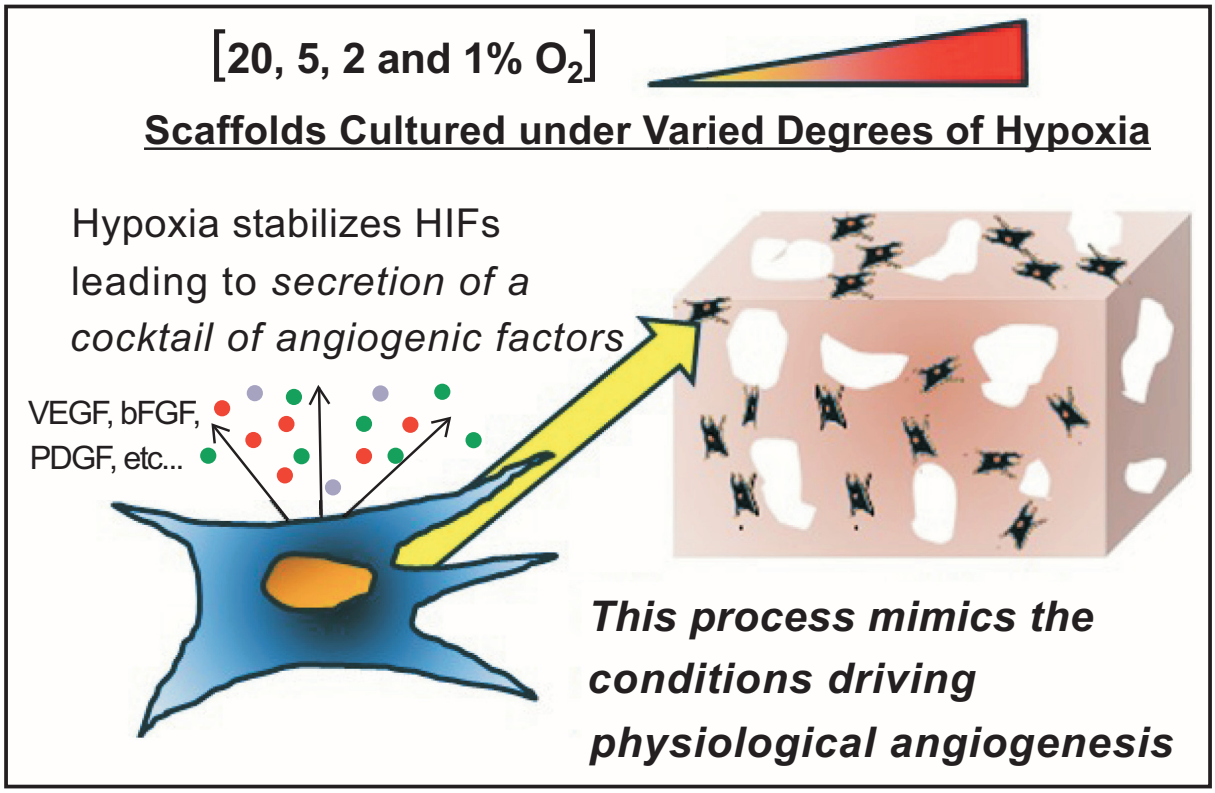

Fig. (2). Schematic drawing of the use of hypoxia to drive physiological angiogenesis in tissue engineered scaffolds.

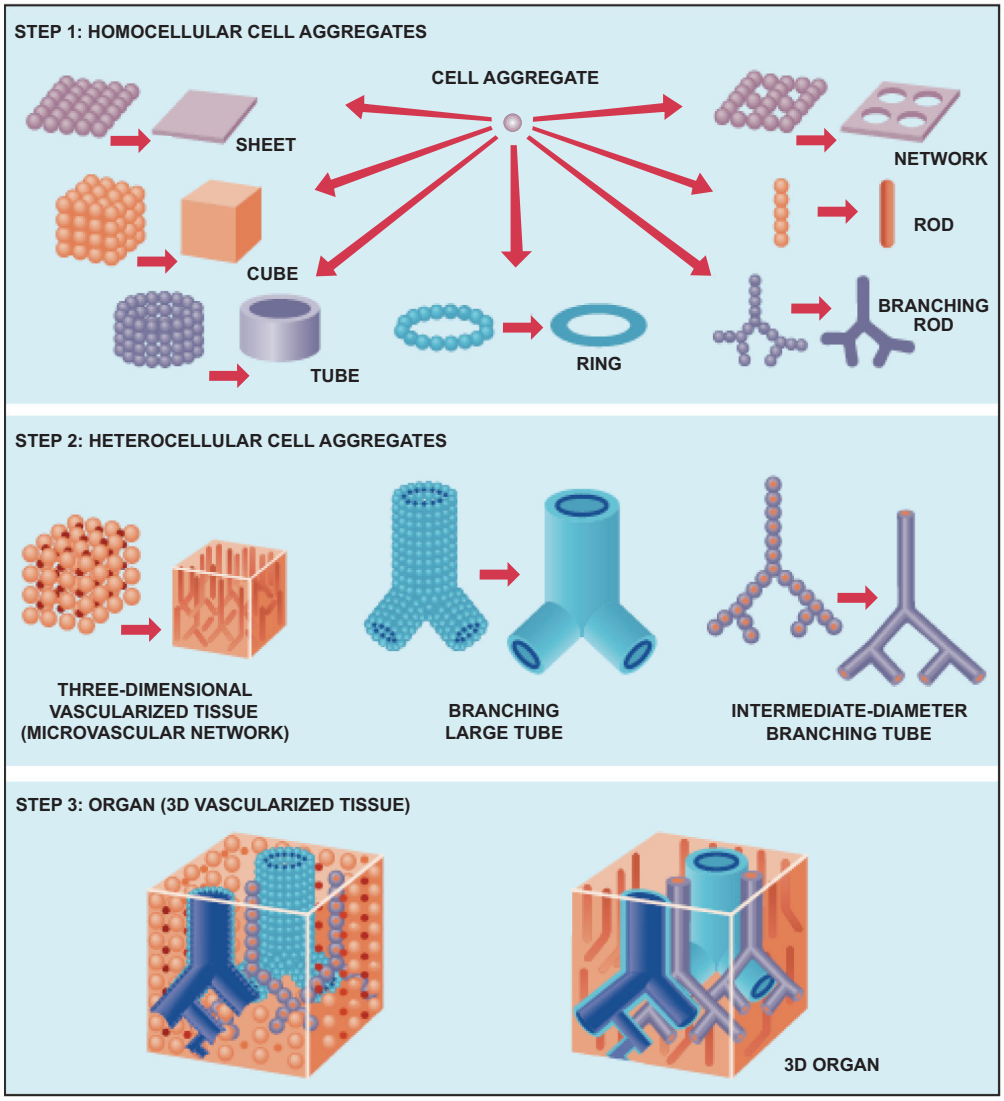

Fig. (3). Bioprinting of cellular aggregates in hydrogel-based materials. Reproduced by permission of Science and Medicine [175].

endothelial cells have been formed inside the lumen of a microvessel made of gelatin methacrylate (GelMA) hydrogel [184]. To accomplish this goal, self-assembled oligopeptide modified gold rods (600 um diameter) were seeded with endothelial cells and cultured until confluence was reached. Then hydrogel was deposited on the cells and exposed to ultra-violet (UV) light to induce crosslinking. An electrical potential was applied to remove the gold rods from cell-hydogel assembly. Finally, media was perfused through the conduit with a syringe pump and cultured up to 15 days with no channel deformation.

To sum up, microscale technologies have been successfully utilized to induce or regulate *in vitro* vascularization of biomimetic materials for tissue engineering applications. Recapitulation of the native complexity and organization can potentially be improved using microscale technologies by controlling size, shape and geometry of vascular networks in engineered tissues.

Mechanical Mediation of Angiogenesis within Biomaterials

Angiogenesis is mediated by the endothelial cell microenvironment, with cues derived from soluble growth factors, insoluble ECM molecules, and membrane receptor signaling pathways [185-187]. While significant progress has been made in delineating how each of these factors individually modulates cell phenotype, signal integration and cell fate in a comprehensive microenvironment are only partially understood. Capillary growth involves many interactions among neighboring cells, which may be assumed to share a highly similar microenvironment in terms of soluble mitogen availability. To enable capillary formation, cell subsets within the same tissue region must simultaneously undergo differentiation, proliferation, migration, and apoptosis. Because neighboring cells will be exposed to a nearly identical cocktail of soluble factors, the local ECM, specifically its mechanical interaction with adhered cells, has been explored as a deterministic cue for variable cell behavior [186, 188, 189].

In the course endothelial cell anchoring and exertion of contractile forces, deformations are induced in both the ECM and the cells. Cytoskeletal filaments, namely contractile microfilaments, mictotubules and intermediate filaments are implicated in the derivation and transmission of contractile forces as well as regulation of signaling pathways that result from cellular deformation [190]. The architecture and mechanical behavior of the cytoskeleton are typically described by a cellular tensegrity model [191-193], although some critics still question the applicability of this idealization [194]. Tensegrity assumes that component arrangement and preloading result in all filaments existing in a state of continuous tension, and has successfully explained mechanical transduction in cells [191]. Physical distortions from the baseline conformation of the cytoskeletal network provide a molecular mechanism for signaling pathway regulation. It is now well-understood that cytoskeletal deformation causes biochemical changes in constituent filaments that in turn promote immobilization of a variety of soluble molecules. When immobilized on cytoskeletal filaments, the bioactivity of soluble molecules, which regulate DNA synthesis, RNA synthesis, protein synthesis and glycolosis are altered providing a link between mechanically induced deformations in the cytoskeleton and molecular pathways that impact angiogenesis [187, 195-198].

To explain the highly localized ECM regulation of cell behavior in angiogenesis, the microvascular tissue, which is typically considered a continuum with respect to mechanical properties, must display significant mechanical variance in adjacent regions that are on the order of cell size. Vascular ECM continuously remodels under conditions of health and disease, with remodeling processes causing local changes in tissue structure, composition, and mechanical properties [199, 200]. In angiogenesis, ECM remodeling has been observed to occur with the necessary spatial fidelity to account for variable interaction with neighboring cells, with reported differences in basement membrane thickness in regions comprising and adjacent to a growing capillary sprout [185, 201]. Assuming identical composition and structure, geometrical changes alone in the ECM would influence the mechanical behavior of adhered cells, specifically the induced deformation throughout the cytoskeletal network. Highly localized ECM remodeling and a resulting variability in ECM-cell interactions may account for the observed difference in neighboring cell behavior. Although the complex interplay among the microenvironmental regulators of angiogenesis is yet to be fully resolved, it is clear that mechanical signal transduction plays a critical role affecting cellular behavior that is required for vessel formation.

CONCLUDING REMARKS

The great interest in the tissue engineering community in directed angiogenesis has led to a variety of approaches that utilize cells, growth factors and materials to enhance vascular formation and infiltration in scaffolds. In pursuit of vascularization of biomaterials, the complexity of cellular and molecular principles that guide vascular development in tissues should be studied in depth. Current vascularization paradigms fail to mimic the native physiology and they solely rely on newly formed capillaries to grow in and form mature blood vessels. Capillary networks induced by these strategies are not connected to arteries within the scaffolds, thus, will regress within a short time.

In order to overcome the limitations of traditional strategies, new approaches must recapitulate the physiological pattern of morphological cues that guide vascular development. The emergence of microtechnologies, such as nano/microfabrication, drug delivery, and stem cell culture and manipulation have provided potential platforms to integrate concepts from vascular biology and engineering to apply design principles extracted from nature to the construction of vascularization strategies. In this context, thorough understanding of how molecular signals control physiology, how cells communicate with each other and their environment, and how molecular machines are coupled to force signal transduction is warranted. It is evident that simply copying the static blood vessel shape and apparent endothelial cell functions may not result in the best design. A deep understanding of the transient features of biological structures that reside within the static morphology is essential. To this end, new paradigms should be organized around the fundamental theme that vascularization strategies are only realizable when we allow the angiogenesis process within porous biomaterials to be guided under epigenetic cues.

ACKNOWLEDGEMENTS

Declared none.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflicts of interest.

REFERENCES

[1] Biermann JS, Holt GE, Lewis VO, Schwartz HS, Yaszemski MJ. Metastatic bone disease: diagnosis, evaluation, and treatment. J Bone Joint Surg Am 2009; 91(6): 1518-30.

[2] Muscolo DL, Ayerza MA, Aponte-Tinao LA, Ranalletta M. Use of distal femoral osteoarticular allografts in limb salvage surgery. Surgical technique. J Bone Joint Surg Am 2006; 88(Suppl 1) Pt 2: 305-21.

[3] Johari A, Shingade V, Gajiwala AL, Shah V, D'Lima C. The use of irradiated allograft in a paediatric population: an Indian experience. Cell Tissue Bank 2007; 8(1): 13-22.

[4] Laurencin CT, Ambrosio AMA, Borden MD, Cooper JA. Tissue engineering: Orthopedic applications. Ann Rev Biomed Eng 1999; 1: 19-46.

[5] Kessler P, Thorwarth M, Bloch-Birkholz A, Nkenke E, Neukam FW. Harvesting of bone from the iliac crest--comparison of the anterior and posterior sites. Br J Oral Maxillofac Surg 2005; 43(1): 51-6.

[6] Silber JS, Anderson DG, Daffner SD, *et al.* Donor site morbidity after anterior iliac crest bone harvest for single-level anterior cervical discectomy and fusion. Spine (Phila Pa 1976) 2003; 28(2): 134-9.

[7] Zimmermann CE, Borner BI, Hasse A, Sieg P. Donor site morbidity after microvascular fibula transfer. Clin Oral Investig 2001; 5(4): 214-9.

[8] Nakajima T, Iizuka H, Tsutsumi S, Kayakabe M, Takagishi K. Evaluation of posterolateral spinal fusion using mesenchymal stem cells: differences with or without osteogenic differentiation. Spine (Phila Pa 1976) 2007; 32(22): 2432-6.

[9] Kretlow JD, Mikos AG. Review: mineralization of synthetic polymer scaffolds for bone tissue engineering. Tissue engineering. [Research Support, N.I.H., Extramural Review 2007; 13(5): 927-38.

[10] Buck BE, Malinin TI, Brown MD. Bone transplantation and human immunodeficiency virus. An estimate of risk of acquired

immunodeficiency syndrome (AIDS). Clin Orthop Relat Res 1989; 240: 129-36.
[11] Lewandrowski KU, Rebmann V, Passler M, *et al*. Immune response to perforated and partially demineralized bone allografts. J Orthop Sci. 2001; 6(6): 545-55.
[12] Moreau MF, Gallois Y, Basle MF, Chappard D. Gamma irradiation of human bone allografts alters medullary lipids and releases toxic compounds for osteoblast-like cells. Biomaterials 2000; 21(4): 369-76.
[13] Mankin HJ, Hornicek FJ, Raskin KA. Infection in massive bone allografts. Clin Orthop Relat Res 2005; 432: 210-6.
[14] Langer R, Tirrell DA. Designing materials for biology and medicine. Nature 2004; 428(6982): 487-92.
[15] Jadlowiec JA, Celil AB, Hollinger JO. Bone tissue engineering: recent advances and promising therapeutic agents. Expert Opin Biol Ther 2003; 3(3): 409-23.
[16] Murphy WL, Mooney DJ. Molecular-scale biomimicry. Nat Biotechnol 2002; 20(1): 30-1.
[17] Hollister SJ, Maddox RD, Taboas JM. Optimal design and fabrication of scaffolds to mimic tissue properties and satisfy biological constraints. Biomaterials 2002; 23(20): 4095-103.
[18] Hollister SJ, Murphy WL. Scaffold Translation: Barriers Between Concept and Clinic. Tissue Eng Part B Rev 2011; 17(6): 459-74
[19] Khan Y, Yaszemski MJ, Mikos AG, Laurencin CT. Tissue engineering of bone: material and matrix considerations. J bone joint surg Am vol 2008; 90 (Suppl 1): 36-42.
[20] Mikos AG, Herring SW, Ochareon P, *et al*. Engineering complex tissues. Tissue Eng 2006; 12(12): 3307-39.
[21] Balasundaram G, Webster TJ. Nanotechnology and biomaterials for orthopedic medical applications. Nanomedicine (Lond) 2006; 1(2): 169-76.
[22] Gloria A, De Santis R, Ambrosio L. Polymer-based composite scaffolds for tissue engineering. J Appl Biomater Biomech; 8(2): 57-67.
[23] Rezwan K, Chen QZ, Blaker JJ, Boccaccini AR. Biodegradable and bioactive porous polymer/inorganic composite scaffolds for bone tissue engineering. Biomaterials 2006; 27(18): 3413-31.
[24] Liu X, Ma PX. Polymeric scaffolds for bone tissue engineering. Ann Biomed Eng 2004; 32(3): 477-86.
[25] Schliephake H, Weich HA, Dullin C, Gruber R, Frahse S. Mandibular bone repair by implantation of rhBMP-2 in a slow release carrier of polylactic acid-an experimental study in rats. Biomaterials 2008; 29(1): 103-10.
[26] Larsson S, Bauer TW. Use of injectable calcium phosphate cement for fracture fixation: a review. Clin Orthop Relat Res 2002; 395: 23-32.
[27] Balasundaram G, Sato M, Webster TJ. Using hydroxyapatite nanoparticles and decreased crystallinity to promote osteoblast adhesion similar to functionalizing with RGD. Biomaterials 2006; 27(14): 2798-805.
[28] Habraken WJ, Wolke JG, Mikos AG, Jansen JA. Injectable PLGA microsphere/calcium phosphate cements: physical properties and degradation characteristics. J Biomater Sci Polym Ed 2006; 17(9): 1057-74.
[29] Khan YM, Katti DS, Laurencin CT. Novel polymer-synthesized ceramic composite-based system for bone repair: an *in vitro* evaluation. J Biomed Mater Res A 2004; 69(4): 728-37.
[30] Saito E, Kang H, Taboas JM, Diggs A, Flanagan CL, Hollister SJ. Experimental and computational characterization of designed and fabricated 50: 50 PLGA porous scaffolds for human trabecular bone applications. J Mater Sci Mater Med; 21(8): 2371-83.
[31] Spalazzi JP, Dagher E, Doty SB, Guo XE, Rodeo SA, Lu HH. *In vivo* evaluation of a multiphased scaffold designed for orthopaedic interface tissue engineering and soft tissue-to-bone integration. J Biomed Mater Res A 2008; 86(1): 1-12.
[32] Athanasiou KA, Agrawal CM, Barber FA, Burkhart SS. Orthopaedic applications for PLA-PGA biodegradable polymers. Arthroscopy 1998; 14(7): 726-37.
[33] Laurencin CT, Attawia MA, Lu LQ, *et al.* Poly(lactide-co-glycolide)/hydroxyapatite delivery of BMP-2-producing cells: a regional gene therapy approach to bone regeneration. Biomaterials 2001; 22(11): 1271-7.
[34] Crane GM, Ishaug SL, Mikos AG. Bone tissue engineering. Nat med 1995; 1(12): 1322-4.
[35] Lieberman JR, Daluiski A, Einhorn TA. The role of growth factors in the repair of bone. Biology and clinical applications. J Bone Joint Surg Am 2002; 84-A(6): 1032-44.
[36] Kempen DH, Creemers LB, Alblas J, *et al*. Growth factor interactions in bone regeneration. Tissue Eng Part B Rev; 16(6): 551-66.
[37] Solheim E. Growth factors in bone. Int Orthop 1998; (6): 410-6.
[38] Kofron MD, Laurencin CT. Bone tissue engineering by gene delivery. Adv Drug Deliv Rev 2006 ; 58(4): 555-76.
[39] Wang EA, Rosen V, Dalessandro JS, *et al*. Recombinant Human Bone Morphogenetic Protein Induces Bone-Formation. Proc NatAcad Sci USA 1990; 87(6): 2220-4.
[40] Rosen V, Thies RS. The Bmp Proteins in Bone-Formation and Repair. Trends Genet 1992; 8(3): 97-102.
[41] Riley EH, Lane JM, Urist MR, Lyons KM, Lieberman JR. Bone morphogenetic protein-2: Biology and applications. Clin Orthop Related Res 1996; 324: 39-46.
[42] Kempen DH, Lu L, Hefferan TE, *et al*. Enhanced bone morphogenetic protein-2-induced ectopic and orthotopic bone formation by intermittent parathyroid hormone (1-34) administration. Tissue Eng Part A 2010; 16(12): 3769-77.
[43] Ripamonti U, Heliotis M, Ferretti C. Bone morphogenetic proteins and the induction of bone formation: from laboratory to patients. Oral Maxillofac Surg Clin North Am 2007; 19(4): 575-89, vii.
[44] El-Amin SF, Hogan MV, Allen AA, Hinds J, Laurencin CT. The indications and use of bone morphogenetic proteins in foot, ankle, and tibia surgery. Foot Ankle Clin 2010; 15(4): 543-51.
[45] Sun H, Feng K, Hu J, Soker S, Atala A, Ma PX. Osteogenic differentiation of human amniotic fluid-derived stem cells induced by bone morphogenetic protein-7 and enhanced by nanofibrous scaffolds. Biomaterials 2010; 31(6): 1133-9.
[46] Lu HH, Vo JM, Chin HS, *et al.* Controlled delivery of platelet-rich plasma-derived growth factors for bone formation. J Biomed Mater Res A 2008 ; 86(4): 1128-36.
[47] Botchwey EA, Pollack SR, El-Amin S, Levine EM, Tuan RS, Laurencin CT. Human osteoblast-like cells in three-dimensional culture with fluid flow. Biorheology 2003; 40(1-3): 299-306.
[48] Shea LD, Wang D, Franceschi RT, Mooney DJ. Engineered bone development from a pre-osteoblast cell line on three-dimensional scaffolds. Tissue Eng 2000 ; 6(6): 605-17.
[49] Peter SJ, Lu LC, Kim DJ, Mikos AG. Marrow stromal osteoblast function on a poly(propylene fumarate)/beta-tricalcium phosphate biodegradable orthopaedic composite. Biomaterials 2000; 21(12): 1207-13.
[50] Malekzadeh R, Hollinger JO, Buck D, Adams DF, McAllister BS. Isolation of human osteoblast-like cells and *in vitro* amplification for tissue engineering. JPeriodontol 1998; 69(11): 1256-62.
[51] Mauney JR, Volloch V, Kaplan DL. Role of adult mesenchymal stem cells in bone tissue engineering applications: current status and future prospects. Tissue Eng 2005; 11(5-6): 787-802.
[52] Kroeze RJ, Knippenberg M, Helder MN. Osteogenic differentiation strategies for adipose-derived mesenchymal stem cells. Methods Mol Biol 2011; 702: 233-48.
[53] Petrie C, Tholpady S, Ogle R, Botchwey E. Proliferative capacity and osteogenic potential of novel dura mater stem cells on poly-lactic-co-glycolic acid. J Biomed Mater Res A 2008; 85(1): 61-71.
[54] Seong JM, Kim BC, Park JH, Kwon IK, Mantalaris A, Hwang YS. Stem cells in bone tissue engineering. Biomed Mater 2010; 5(6): 062001.
[55] Levi B, Longaker MT. Concise review: adipose-derived stromal cells for skeletal regenerative medicine. Stem Cells; 29(4): 576-82.
[56] Rada T, Santos TC, Marques AP, *et al*. Osteogenic differentiation of two distinct subpopulations of human adipose-derived stem cells: an *in vitro* and *in vivo* study. J Tissue Eng Regen Med 2012; 6(1): 1-11
[57] Tamama K, Fan VH, Griffith LG, Blair HC, Wells A. Epidermal growth factor as a candidate for *ex vivo* expansion of bone marrow-derived mesenchymal stem cells. Stem Cells 2006; 24(3): 686-95.
[58] Du YA, Ghodousi M, Qi H, Haas N, Xiao WQ, Khademhosseini A. Sequential Assembly of Cell-Laden Hydrogel Constructs to Engineer Vascular-Like Microchannels. Biotech Bioeng 2011; 108(7): 1693-703.
[59] Du Y, Cropek D, Mofrad MRK, Weinberg EJ, Khademhosseini A, Borenstein J. Microfluidic Systems for Engineering Vascularized Tissue Constructs. In: Tian W-C, Finehout E, editors. Microfluidics for Biological Applications. New York: Springer; 2008.

[60] Goshima J, Goldberg VM, Caplan AI. The Origin of Bone Formed in Composite Grafts of Porous Calcium-Phosphate Ceramic Loaded with Marrow-Cells. Clin Orthop Related Res 1991; 269: 274-83.

[61] Hausman MR, Schaffler MB, Majeska RJ. Fracture healing impairment in rats by an angiogenesis inhibitor indicates a requirement for vasculature in early fracture callus development. J Bone Miner Res 2001; 16: S181-S.

[62] Akita S, Tamai N, Myoui A, *et al*. Capillary vessel network integration by inserting a vascular pedicle enhances bone formation in tissue-engineered bone using interconnected porous hydroxyapatite ceramics. Tissue Eng 2004; 10(5-6): 789-95.

[63] Carano RAD, Filvaroff EH. Angiogenesis and bone repair. Drug Discov Today 2003; 8(21): 980-9.

[64] Hsiong SX, Mooney DJ. Regeneration of vascularized bone. Periodontology 2000 2006; 41: 109-22.

[65] Fang TD, Salim A, Xia W, *et al*. Angiogenesis is required for successful bone induction during distraction osteogenesis. J Bone Miner Res 2005; 20(7): 1114-24.

[66] Santos MI, Reis RL. Vascularization in bone tissue engineering: physiology, current strategies, major hurdles and future challenges. Macromol Biosci 2010; 10(1): 12-27.

[67] Johnson PC, Mikos AG, Fisher JP, Jansen JA. Strategic directions in tissue engineering. Tissue Eng 2007; 13(12): 2827-37.

[68] Guillotin B, Guillemot F. Cell patterning technologies for organo-typic tissue fabrication. Trends Biotechnol 2011; 29(4): 183-90.

[69] Haller AV. Opera Minora. Francisci Grasset1763.

[70] Colnot CI, Helms JA. A molecular analysis of matrix remodeling and angiogenesis during long bone development. Mech Dev 2001; 100(2): 245-50.

[71] Gerber HP, Ferrara N. Angiogenesis and bone growth. Trends Cardiovasc Med 2000; 10(5): 223-8.

[72] Street J, Winter D, Wang JH, Wakai A, McGuinness A, Redmond HP. Is human fracture hematoma inherently angiogenic? Clin Orthop Related Res 2000; 378): 224-37.

[73] Street J, Bao M, deGuzman L, *et al*. Vascular endothelial growth factor stimulates bone repair by promoting angiogenesis and bone turnover. Proc Natl Acad Sci USA 2002 ; 99(15): 9656-61.

[74] Duvall CL, Taylor WR, Weiss D, Wojtowicz AM, Guldberg RE. Impaired angiogenesis, early callus formation, and late stage remodeling in fracture healing of osteopontin-deficient mice. J Bone Miner Res 2007; 22(2): 286-97.

[75] Ratner BD. Reducing capsular thickness and enhancing angiogenesis around implant drug release systems. J Control Release 2002 ; 78(1-3): 211-8.

[76] Dziubla TD, Torjman MC, Joseph JI, Murphy-Tatum M, Lowman AM. Evaluation of porous networks of poly(2-hydroxyethyl methacrylate) as interfacial drug delivery devices. Biomaterials 2001; 22(21): 2893-9.

[77] Mooney DJ, Organ G, Vacanti JP, Langer R. Design and fabrication of biodegradable polymer devices to engineer tubular tissues. Cell Transplant 1994; 3(2): 203-10.

[78] Jabbarzadeh E, Abrams CF. Strategies to enhance capillary formation inside biomaterials: a computational study. Tissue Eng 2007 ; 13(8): 2073-86.

[79] Deryugina EI, Soroceanu L, Strongin AY. Up-regulation of vascular endothelial growth factor by membrane-type 1 matrix metalloproteinase stimulates human glioma xenograft growth and angiogenesis. Cancer Res 2002 ; 62(2): 580-8.

[80] Diaz VM, Planaguma J, Thomson TM, Reventos J, Paciucci R. Tissue plasminogen activator is required for the growth, invasion, and angiogenesis of pancreatic tumor cells. Gastroenterology 2002; 122(3): 806-19.

[81] Grossfeld GD, Carroll PR, Lindeman N, *et al*. Thrombospondin-1 expression in patients with pathologic stage T3 prostate cancer undergoing radical prostatectomy: association with p53 alterations, tumor angiogenesis, and tumor progression. Urology 2002 ; 59(1): 97-102.

[82] Jones A, Harris AL. New developments in angiogenesis: a major mechanism for tumor growth and target for therapy. Cancer J Sci Am 1998 ; 4(4): 209-17.

[83] Hobson B, Denekamp J. Endothelial proliferation in tumours and normal tissues: continuous labelling studies. Br J Cancer 1984; 49(4): 405-13.

[84] Fox SB, Gatter KC, Bicknell R, *et al*. Relationship of endothelial cell proliferation to tumor vascularity in human breast cancer. Cancer Res 1993; 53(18): 4161-3.

[85] Ingber DE. Fibronectin controls capillary endothelial cell growth by modulating cell shape. Proc Natl Acad Sci USA 1990; 87(9): 3579-83.

[86] Sepp N, Fritsch P, Luger TA. Endothelial cells and angiogenesis. Exp Dermatol 1997; 6(5): 272-3.

[87] Shepherd BR, Enis DR, Wang FY, Suarez Y, Pober JS, Schechner JS. Vascularization and engraftment of a human skin substitute using circulating progenitor cell-derived endothelial cells. FASEB J 2006; 20(10): 1739.

[88] Vacanti CA, Upton J. Tissue-Engineered Morphogenesis of Cartilage and Bone by Means of Cell Transplantation Using Synthetic Biodegradable Polymer Matrices. Clin Plastic Surg1994 ; 21(3): 445-62.

[89] Mooney DJ, Organ G, Vacanti JP, Langer R. Design and Fabrication of Biodegradable Polymer Devices to Engineer Tubular Tissues. Cell Transplant 1994; 3(2): 203-10.

[90] Carmeliet P, Jain RK. Angiogenesis in cancer and other diseases. Nature 2000; 407(6801): 249-57.

[91] Muschler GF, Nakamoto C, Griffith LG. Engineering principles of clinical cell-based tissue engineering. J Bone Joint Surg Am 2004; 86-A(7): 1541-58.

[92] Colton CK. Implantable Biohybrid Artificial Organs. Cell Transplantation 1995; 4(4): 415-36.

[93] Freshney RI. Culture of animal cells: A manual of basic technique 1994.

[94] Schuch G, Machluf M, Bartsch G, Jr., *et al*. *In vivo* administration of vascular endothelial growth factor (VEGF) and its antagonist, soluble neuropilin-1, predicts a role of VEGF in the progression of acute myeloid leukemia *in vivo*. Blood 2002; 100(13): 4622-8.

[95] Mikos AG, Sarakinos G, Lyman MD, Ingber DE, Vacanti JP, Langer R. Prevascularization of porous biodegradable polymers. Biotechnol Bioeng 1993; 42(6): 716-23.

[96] Arnold F, West DC. Angiogenesis in wound healing. Pharmacol Ther 1991; 52(3): 407-22.

[97] Loughman MS, Chatzistefanou K, Gonzalez EM, *et al*. Experi-mental corneal neovascularisation using sucralfate and basic fibroblast growth factor. Aust N Z J Ophthalmol 1996; 24(3): 289-95.

[98] Nguyen M, Shing Y, Folkman J. Quantitation of angiogenesis and antiangiogenesis in the chick embryo chorioallantoic membrane. Microvasc Res 1994; 47(1): 31-40.

[99] Shing Y, Folkman J, Haudenschild C, Lund D, Crum R, Klagsbrun M. Angiogenesis is stimulated by a tumor-derived endothelial cell growth factor. J Cell Biochem 1985; 29(4): 275-87.

[100] Sato Y, Rifkin DB. Autocrine activities of basic fibroblast growth factor: regulation of endothelial cell movement, plasminogen activator synthesis, and DNA synthesis. J Cell Biol 1988; 107(3): 1199-205.

[101] Benjamin LE, Hemo I, Keshet E. A plasticity window for blood vessel remodelling is defined by pericyte coverage of the preformed endothelial network and is regulated by PDGF-B and VEGF. Development 1998; 125(9): 1591-8.

[102] Benjamin LE, Keshet E. Conditional switching of vascular endothelial growth factor (VEGF) expression in tumors: induction of endothelial cell shedding and regression of hemangioblastoma-like vessels by VEGF withdrawal. Proc Natl Acad Sci USA 1997; 94(16): 8761-6.

[103] Yang Z, Birkenhauer P, Julmy F, *et al*. Sustained release of heparin from polymeric particles for inhibition of human vascular smooth muscle cell proliferation. J Control Release 1999; 60(2-3): 269-77.

[104] Perets A, Baruch Y, Weisbuch F, Shoshany G, Neufeld G, Cohen S. Enhancing the vascularization of three-dimensional porous alginate scaffolds by incorporating controlled release basic fibroblast growth factor microspheres. J Biomed Mater Res Part A 2003; 65A(4): 489-97.

[105] Leach JK, Kaigler D, Wang Z, Krebsbach PH, Mooney DJ. Coating of VEGF-releasing scaffolds with bioactive glass for angiogenesis and bone regeneration. Biomaterials 2006; 27(17): 3249-55.

[106] Richardson TP, Peters MC, Ennett AB, Mooney DJ. Polymeric system for dual growth factor delivery. Nat Biotechnol 2001; 19(11): 1029-34.

[107] Kneser U, Polykandriotis E, Ohnolz J, *et al*. Engineering of vascularized transplantable bone tissues: induction of axial vascularization in an osteoconductive matrix using an arteriovenous loop. Tissue Eng 2006; 12(7): 1721-31.

[108] Wieghaus KA, Capitosti SM, Anderson CR, *et al*. Small molecule inducers of angiogenesis for tissue engineering. Tissue Eng 2006; 12(7): 1903-13.

[109] Patel ZS, Mikos AG. Angiogenesis with biomaterial-based drug- and cell-delivery systems. J Biomater Sci Polym Ed 2004; 15(6): 701-26.

[110] Chen RR, Silva EA, Yuen WW, Mooney DJ. Spatio-temporal VEGF and PDGF delivery patterns blood vessel formation and maturation. Pharm Res 2007; 24(2): 258-64.

[111] Zisch AH, Lutolf MP, Ehrbar M, *et al*. Cell-demanded release of VEGF from synthetic, biointeractive cell ingrowth matrices for vascularized tissue growth. FASEB J 2003; 17(15): 2260-2.

[112] Silva GA, Coutinho OP, Ducheyne P, Shapiro IM, Reis RL. Starch-based microparticles as vehicles for the delivery of active platelet-derived growth factor. Tissue Eng 2007; 13(6): 1259-68.

[113] Golub JS, Kim YT, Duvall CL, *et al*. Sustained VEGF delivery via PLGA nanoparticles promotes vascular growth. Am J Physiol Heart Circ Physiol 2010; 298(6): H1959-65.

[114] Tabata Y, Miyao M, Yamamoto M, Ikada Y. Vascularization into a porous sponge by sustained release of basic fibroblast growth factor. J Biomater Sci Polymer Ed 1999; 10(9): 957-68.

[115] Sheridan MH, Shea LD, Peters MC, Mooney DJ. Bioabsorbable polymer scaffolds for tissue engineering capable of sustained growth factor delivery. J Control Release 2000; 64(1-3): 91-102.

[116] Perets A, Baruch Y, Weisbuch F, Shoshany G, Neufeld G, Cohen S. Enhancing the vascularization of three-dimensional porous alginate scaffolds by incorporating controlled release basic fibroblast growth factor microspheres. J Biomed Mater Res Part A 2003; 65(4): 489-97.

[117] Jabbarzadeh E, Nair LS, Khan YM, Deng M, Laurencin CT. Apatite nano-crystalline surface modification of poly(lactide-co-glycolide) sintered microsphere scaffolds for bone tissue engineering: implications for protein adsorption. J Biomater Sci Polym Ed 2007; 18(9): 1141-52.

[118] Jabbarzadeh E, Jiang T, Deng M, Nair LS, Khan YM, Laurencin CT. Human endothelial cell growth and phenotypic expression on three dimensional poly(lactide-co-glycolide) sintered microsphere scaffolds for bone tissue engineering. Biotechnol Bioeng 2007; 98(5): 1094-102.

[119] Epstein SE, Fuchs S, Zhou YF, Baffour R, Kornowski R. Therapeutic interventions for enhancing collateral development by administration of growth factors: basic principles, early results and potential hazards. Cardiovasc Res 2001 ; 49(3): 532-42.

[120] Emanueli C, Madeddu P. Angiogenesis gene therapy to rescue ischaemic tissues: achievements and future directions. Br J Pharmacol 2001 ; 133(7): 951-8.

[121] Iwaguro H, Yamaguchi J, Kalka C, *et al*. Endothelial progenitor cell vascular endothelial growth factor gene transfer for vascular regeneration. Circulation 2002 ; 105(6): 732-8.

[122] Fields RC, Solan A, McDonagh KT, Niklason LE, Lawson JH. Gene therapy in tissue-engineered blood vessels. Tissue Eng 2003; 9(6): 1281-7.

[123] Peterson B, Zhang J, Iglesias R, *et al*. Healing of critically sized femoral defects, using genetically modified mesenchymal stem cells from human adipose tissue. Tissue Eng 2005; 11(1-2): 120-9.

[124] Schreiber RE, Blease K, Ambrosio A, Amburn E, Sosnowski B, Sampath TK. Bone induction by AdBMP-2/collagen implants. Journal Bone Joint Surg Am Vol 2005; 87(5): 1059-68.

[125] Ohno N, Itoh H, Ikeda T, *et al*. Accelerated reendothelialization with suppressed thrombogenic property and neointimal hyperplasia of rabbit jugular vein grafts by adenovirus-mediated gene transfer of C-type natriuretic peptide. Circulation 2002; 105(14): 1623-6.

[126] Verma IM, Somia N. Gene therapy - promises, problems and prospects. Nature 1997; 389(6648): 239-42.

[127] Kafri T, Morgan D, Krahl T, Sarvetnick N, Sherman L, Verma I. Cellular immune response to adenoviral vector infected cells does not require de novo viral gene expression: implications for gene therapy. Proc Natl Acad Sci USA 1998; 95(19): 11377-82.

[128] Benihoud K, Esselin S, Descamps D, *et al*. Respective roles of TNF-alpha and IL-6 in the immune response-elicited by adenovirus-mediated gene transfer in mice. Gene Therapy 2007; 14(6): 533-44.

[129] Jabbarzadeh E, Starnes T, Khan YM, *et al*. Induction of angiogenesis in tissue-engineered scaffolds designed for bone repair: a combined gene therapy-cell transplantation approach. Proc Natl Acad Sci USA 2008; 105(32): 11099-104.

[130] Nor JE, Polverini PJ. Role of endothelial cell survival and death signals in angiogenesis. Angiogenesis 1999; 3(2): 101-16.

[131] Folkman J. What is the role of endothelial cells in angiogenesis? Lab Invest 1984; 51(6): 601-4.

[132] Folkman J, Browder T, Palmblad J. Angiogenesis research: guidelines for translation to clinical application. Thromb Haemost 2001; 86(1): 23-33.

[133] Madri JA, Pratt BM, Tucker AM. Phenotypic Modulation of Endothelial-Cells by Transforming Growth Factor-Beta Depends Upon the Composition and Organization of the Extracellular-Matrix. J Cell Biol 1988; 106(4): 1375-84.

[134] Auerbach R, Lewis R, Shinners B, Kubai L, Akhtar N. Angiogenesis assays: A critical overview. Clin Chem 2003; 49(1): 32-40.

[135] Koike N, Fukumura D, Gralla O, Au P, Schechner JS, Jain RK. Creation of long-lasting blood vessels. Nature 2004; 428(6979): 138-9.

[136] Nor JE, Peters MC, Christensen JB, *et al*. Engineering and characterization of functional human microvessels in immunodeficient mice. Lab Invest 2001; 81(4): 453-63.

[137] Schechner JS, Nath AK, Zheng L, *et al*. *In vivo* formation of complex microvessels lined by human endothelial cells in an immunodeficient mouse. Proc Natl Acad Sci USA 2000; 97(16): 9191-6.

[138] Melero-Martin JM, Khan ZA, Picard A, Wu X, Paruchuri S, Bischoff J. *In vivo* vasculogenic potential of human blood-derived endothelial progenitor cells. Blood 2007; 109(11): 4761-8.

[139] Au P, Daheron LM, Duda DG, *et al*. Differential *in vivo* potential of endothelial progenitor cells from human umbilical cord blood and adult peripheral blood to form functional long-lasting vessels. Blood 2008; 111(3): 1302-5.

[140] Scherberich A, Galli R, Jaquiery C, Farhadi J, Martin I. Three-dimensional perfusion culture of human adipose tissue-derived endothelial and osteoblastic progenitors generates osteogenic constructs with intrinsic vascularization capacity. Stem Cells 2007; 25(7): 1823-9.

[141] Soker S, Machado M, Atala A. Systems for therapeutic angiogenesis in tissue engineering. World J Urol 2000; 18(1): 10-8.

[142] Tremblay PL, Hudon V, Berthod F, Germain L, Auger FA. Inosculation of tissue-engineered capillaries with the host's vasculature in a reconstructed skin transplanted on mice. Am J Transplant 2005; 5(5): 1002-10.

[143] Visconti RP, Kasyanov V, Gentile C, Zhang J, Markwald RR, Mironov V. Towards organ printing: engineering an intra-organ branched vascular tree. Expert Opin Biol Ther 2010; 10(3): 409-20.

[144] Visconti RP, Richardson CD, Sato TN. Orchestration of angiogenesis and arteriovenous contribution by angiopoietins and vascular endothelial growth factor (VEGF). Proc Natl Acad Sci USA 2002; 99(12): 8219-24.

[145] Visconti RP, Markwald RR. Recruitment of new cells into the postnatal heart: potential modification of phenotype by periostin. Ann NY Acad Sci 2006; 1080: 19-33.

[146] Pruett ND, Visconti RP, Jacobs DF, *et al*. Evidence for Hox-specified positional identities in adult vasculature. BMC Dev Biol 2008; 8: 93.

[147] Abderrahim-Ferkoune A, Bezy O, Astri-Roques S, Elabd C, Ailhaud G, Amri EZ. Transdifferentiation of preadipose cells into smooth muscle-like cells: role of aortic carboxypeptidase-like protein. Exp Cell Res 2004; 293(2): 219-28.

[148] Rodriguez LV, Alfonso Z, Zhang R, Leung J, Wu B, Ignarro LJ. Clonogenic multipotent stem cells in human adipose tissue differentiate into functional smooth muscle cells. Proc Natl Acad Sci USA 2006; 103(32): 12167-72.

[149] Yun DH, Song HY, Lee MJ, *et al*. Thromboxane A(2) modulates migration, proliferation, and differentiation of adipose tissue-derived mesenchymal stem cells. Exp Mol Med 2009; 41(1): 17-24.

[150] Kim MR, Jeon ES, Kim YM, Lee JS, Kim JH. Thromboxane a(2) induces differentiation of human mesenchymal stem cells to smooth muscle-like cells. Stem Cells 2009; 27(1): 191-9.

[151] Gerritsen ME, Bloor CM. Endothelial cell gene expression in response to injury. FASEB J 1993; 7(6): 523-32.

[152] Cassavaugh J, Lounsbury KM. Hypoxia-mediated biological control. J Cell Biochem 2011; 112(3): 735-44.
[153] Takenaga K. Angiogenic signaling aberrantly induced by tumor hypoxia. Front Biosci 2011; 16: 31-48.
[154] Lu X, Kang Y. Hypoxia and hypoxia-inducible factors: master regulators of metastasis. Clin Cancer Res 2010; 16(24): 5928-35.
[155] Semenza GL. HIF-1 and human disease: one highly involved factor. Genes Dev 2000; 14(16): 1983-91.
[156] Semenza GL. Hypoxia-inducible factor 1: oxygen homeostasis and disease pathophysiology. Trends Mol Med 2001; 7(8): 345-50.
[157] Wang Y, Wan C, Deng L, *et al*. The hypoxia-inducible factor alpha pathway couples angiogenesis to osteogenesis during skeletal development. J Clin Invest 2007; 117(6): 1616-26.
[158] Heppenstall RB, Grislis G, Hunt TK. Tissue gas tensions and oxygen consumption in healing bone defects. Clin Orthop Relat Res 1975; 106: 357-65.
[159] Malda J, Klein TJ, Upton Z. The roles of hypoxia in the *in vitro* engineering of tissues. Tissue Eng 2007; 13(9): 2153-62.
[160] Zachar V, Duroux M, Emmersen J, *et al*. Hypoxia and adipose-derived stem cell-based tissue regeneration and engineering. Expert Opin Biol Ther 2011; 11(6): 775-86.
[161] Rubinstein I, Abassi Z, Milman F, *et al*. Hyperbaric oxygen treatment improves GFR in rats with ischaemia/reperfusion renal injury: a possible role for the antioxidant/oxidant balance in the ischaemic kidney. Nephrol Dial Transplant 2009; 24(2): 428-36.
[162] Forsythe JA, Jiang BH, Iyer NV, *et al*. Activation of vascular endothelial growth factor gene transcription by hypoxia-inducible factor 1. Mol Cell Biol 1996; 16(9): 4604-13.
[163] Manalo DJ, Rowan A, Lavoie T, *et al*. Transcriptional regulation of vascular endothelial cell responses to hypoxia by HIF-1. Blood 2005; 105(2): 659-69.
[164] Ceradini DJ, Kulkarni AR, Callaghan MJ, *et al*. Progenitor cell trafficking is regulated by hypoxic gradients through HIF-1 induction of SDF-1. Nat Med 2004; 10(8): 858-64.
[165] Asahara T, Takahashi T, Masuda H, *et al*. VEGF contributes to postnatal neovascularization by mobilizing bone marrow-derived endothelial progenitor cells. EMBO J 1999; 18(14): 3964-72.
[166] Kinnaird T, Stabile E, Burnett MS, Epstein SE. Bone-marrow-derived cells for enhancing collateral development: mechanisms, animal data, and initial clinical experiences. Circ Res 2004; 95(4): 354-63.
[167] Kinnaird T, Stabile E, Burnett MS, *et al*. Local delivery of marrow-derived stromal cells augments collateral perfusion through paracrine mechanisms. Circulation 2004; 109(12): 1543-9.
[168] des Rieux A, Ucakar B, Mupendwa BP, *et al*. 3D systems delivering VEGF to promote angiogenesis for tissue engineering. J Control Release 2010; 150(3): 272-8.
[169] Odedra D, Chiu LL, Shoichet M, Radisic M. Endothelial cells guided by immobilized gradients of vascular endothelial growth factor on porous collagen scaffolds. Acta Biomater 2011; 7(8): 3027-35.
[170] Korff WL, McHenry MJ. Environmental differences in substrate mechanics do not affect sprinting performance in sand lizards (Uma scoparia and Callisaurus draconoides). J Exp Biol 2011; 214(Pt 1): 122-30.
[171] Skiles ML, Fancy R, Topiwala P, Sahai S, Blanchette JO. Correlating hypoxia with insulin secretion using a fluorescent hypoxia detection system. J Biomed Mater Res B Appl Biomater 2011; 97(1): 148-55.
[172] Borenstein JT, Terai H, King KR, Weinberg EJ, Kaazempur-Mofrad MR, Vacanti JP. Microfabrication technology for vascularized tissue engineering. Biomed Microdevice 2002; 4(3): 167-75.
[173] Tan W, Desai TA. Layer-by-layer microfluidics for biomimetic three-dimensional structures. Biomater Engl 2004; 25(7-8): 1356-64.
[174] Raghavan S, Nelson CM, Baranski JD, Lim E, Chen CS. Geometrically controlled endothelial tubulogenesis in micropatterned gels. Tissue Eng Part A 2010; 16(7): 2255-63.
[175] Mironov V, Markwald R, Forgacs G. Organ printing: self-assembling cell aggregates as "Bioink". Sci Med 2003; 9: 69-71.
[176] Choi NW, Cabodi M, Held B, Gleghorn JP, Bonassar LJ, Stroock AD. Microfluidic scaffolds for tissue engineering. Nat Mater 2007; 6(11): 908-15.
[177] Ling Y, Rubin J, Deng Y, Huang C, Demirci U, Karp JM, *et al*. A cell-laden microfluidic hydrogel. Lab Chip 2007; 7(6): 756-62.
[178] Golden AP, Tien J. Fabrication of microfluidic hydrogels using molded gelatin as a sacrificial element. Lab Chip 2007; 7(6): 720-5.
[179] Park JH, Chung BG, Lee WG, *et al*. Microporous Cell-Laden Hydrogels for Engineered Tissue Constructs. Biotechnol Bioeng 2010; 106(1): 138-48.
[180] Fidkowski C, Kaazempur-Mofrad MR, Borenstein J, Vacanti JP, Langer R, Wang Y. Endothelialized microvasculature based on a biodegradable elastomer. Tissue Eng 2005; 11(1-2): 302-9.
[181] Aubin H, Nichol JW, Hutson CB, *et al*. Directed 3D cell alignment and elongation in microengineered hydrogels. Biomaterials 2010; 31(27): 6941-51.
[182] Fedorovich NE, Alblas J, de Wijn JR, Hennink WE, Verbout AJ, Dhert WJ. Hydrogels as extracellular matrices for skeletal tissue engineering: state-of-the-art and novel application in organ printing. Tissue Eng 2007; 13(8): 1905-25.
[183] Varghese D, Deshpande M, Xu T, Kesari P, Ohri S, Boland T. Advances in tissue engineering: Cell printing. J Thor Cardiovasc Surg 2005; 129(2): 470-2.
[184] Sadr N, Zhu MJ, Osaki T, *et al*. SAM-based cell transfer to photopatterned hydrogels for microengineering vascular-like structures. Biomaterials 2011; 32(30): 7479-90.
[185] Ingber DE. Mechanical signaling and the cellular response to extracellular matrix in angiogenesis and cardiovascular physiology. Circ Res. 2002; 91(10): 877-87.
[186] Ingber DE. Cellular mechanotransduction: putting all the pieces together again. FASEB J 2006; 20(7): 811-27.
[187] Mammoto A, Mammoto T, Ingber DE. Rho signaling and mechanical control of vascular development. Curr Opin Hematol 2008; 15(3): 228-34.
[188] Shiu YT, Weiss JA, Hoying JB, Iwamoto MN, Joung IS, Quam CT. The role of mechanical stresses in angiogenesis. Crit Rev Biomed Eng 2005; 33(5): 431-510.
[189] Krishnan L, Underwood CJ, Maas S, *et al*. Effect of mechanical boundary conditions on orientation of angiogenic microvessels. Cardiovasc Res 2008; 78(2): 324-32.
[190] Mammoto A, Connor KM, Mammoto T, *et al*. A mechanosensitive transcriptional mechanism that controls angiogenesis. Nature 2009; 457(7233): 1103-8.
[191] Wang N, Naruse K, Stamenovic D, Fredberg JJ, Mijailovich SM, Tolic-Norrelykke IM, *et al*. Mechanical behavior in living cells consistent with the tensegrity model. Proc Natl Acad Sci USA 2001; 98(14): 7765-70.
[192] Ingber DE. Tensegrity II: How structural networks influence cellular information processing networks. J Cell Sci 2003; 116(Pt 8): 1397-408.
[193] Ingber DE. Tensegrity I: Cell structure and hierarchical systems biology. J Cell Sci 2003; 116(Pt 7): 1157-73.
[194] Ingber DE. Opposing views on tensegrity as a structural framework for understanding cell mechanics. J Appl Physiol 2000; 89(4): 1663-70.
[195] Myers KA, Applegate KT, Danuser G, Fischer RS, Waterman CM. Distinct ECM mechanosensing pathways regulate microtubule dynamics to control endothelial cell branching morphogenesis. J Cell Biol 2011; 192(2): 321-34.
[196] Volokh KY. On tensegrity in cell mechanics. Mol Cell Biomech 2011; 8(3): 195-214.
[197] Mustata T, Rusu V. [Mechanotransduction and tensegrity (I)]. Rev Med Chir Soc Med Nat Iasi 1998; 102(3-4): 25-35.
[198] Ingber DE. Tensegrity and mechanotransduction. J Bodyweight Mov Ther 2008; 12(3): 198-200.
[199] Humphrey JD. Mechanisms of arterial remodeling in hypertension: coupled roles of wall shear and intramural stress. Hypertension 2008; 52(2): 195-200.
[200] Humphrey JD. Vascular adaptation and mechanical homeostasis at tissue, cellular, and sub-cellular levels. Cell Biochem Biophys 2008; 50(2): 53-78.
[201] Ausprunk DH, Folkman J. Migration and proliferation of endothelial cells in preformed and newly formed blood vessels during tumor angiogenesis. Microvasc Res 1977; 14(1): 53-65.

Received: March 14, 2012 **Revised: May 10, 2012** **Accepted: May 15, 2012**