We are IntechOpen,
the world's leading publisher of
Open Access books
Built by scientists, for scientists

5,400
Open access books available

133,000
International authors and editors

165M
Downloads

Our authors are among the

154
Countries delivered to

TOP 1%
most cited scientists

12.2%
Contributors from top 500 universities

CLARIVATE ANALYTICS
BOOK CITATION INDEX
INDEXED

WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Angiogenesis — The Key to Regeneration

Susanne Jung and Johannes Kleinheinz

Additional information is available at the end of the chapter

1. Introduction

Regenerative concepts are one of the basic ideas of modern biomedical research. Regeneration means origination in stark contrast to substitution. This process aims not to replace or to reconstruct but to restore the physical integrity of cells, tissues and organs by means of the organisms' own repair mechanisms.

Especially the regeneration of neuronal tissues has been in the focus of interest, but these restorative concepts will also become applicable in the treatment of metabolic or degenerative diseases. Gentherapy and tissue engineering procedures are two established areas of research in which regeneration plays a major role and has already proven its significance.

The fundament of regeneration is the tissue's potential to grow, to differentiate and therefore to continually bridge permanently emerging damages. It is a stepless coexistence of build-up and degradation processes in which a plethora of enzymes, signal proteins, ligands and their corresponding receptors on different regulatory levels are involved. These processes concern every part of the body; the least common denominator of all these physiological events that include a transition from single cells to a complex tissue structure is their demand for energy and substrates. It becomes obvious that there can never arise a regenerative course without a functioning vasculature to provide the essential cells and proteins, to ensure the oxygen and nutrient supply and to evacuate accumulating metabolic products. Any regeneration is only able to develop with a simultaneously developing vessel system. The realisation seems to be trivial, but the vital importance of a functional vasculature is not generally considered in regenerative concepts.

On the following pages the role of vasculogenesis and angiogenesis in regeneration is to be described; after a depiction of the angiogenic cascade, the regulation by hypoxia is tracked. From the description of the physiological molecular course of the angiogenic cascade and the interrelation of its key protagonists we trace the impact of macro- and microscopic vascularisation in various regenerative processes and clarify its central position in tissue engineering models.

Finally the role of pluripotent cells in modern tissue engineering concepts is summarized.

Fundamental research with special respect to cell culture, immunohistochemistry, in vitro and in vivo trials, circulation modelling and gene expression profiling provides the scientific basis for this survey.

2. The angiogenic cascade

Angiogenesis is the complex physiological sequence of vasodilatation, degradation of base‐ment membrane, endothelial cell migration, chemotaxis, increasing vascular permeability and eventually endothelial cell proliferation and vessel formation. The fine-tuned balance of vasculo- and angiogenesis is controlled by many growth and transcription factors (Pandya, Dhalla et al., 2006).

2.1. Angiogenesis versus vasculogenesis

Lack of oxygen and nutrients threatens the tissue integrity and viability fundamentally. In these situations of undersupply, the organisms´ reaction is to improve the local perfusion by inducing the growth of the vasculature.

Angiogenesis is one crucial mechanism; it describes the sprouting of a vascular system on the base of pre-existing capillaries via endothelial migration and proliferation. This kind of vascular regeneration is the more common one.

When endothelial progenitor cells are mobilized from the bone marrow to differentiate and proliferate to form new vessel architecture, vasculogenesis takes place. An alternative way to vasculogenesis bears on the potential of local endothelial to differentiate and proliferate without a pre-existing vessel structure (Tepper, Cappla et al., 2005, Hankenson et al., 2005).

Both processes have in common that the smooth frictionless procedure depends on a continu‐ing dynamic crosstalk between endothelial cells and surrounding connective tissue. Other important cell types are monocytes, macrophages, fibroblasts, pericytes or smooth muscle cells (Nyberg, Salo et al., 2008).

The biochemical and morphological roll out of vessel formation is standardized: angiogenesis starts with the vasodilatation of the original vessels followed by the degradation of the basement membrane, migration and proliferation of endothelial cells, their arrangement in luminal structures, loop formation and establishment of new basement membranes (Moulton, Folkman et al., 1998).

2.2. VEGF pathway

The healthy vasculature is one prerequisite of every regenerative process. It its governed by many interacting signalling pathways, the VEGF pathway is considered as one of the most crucial ones; it is certainly the most investigated and understood one (Dyer, Portbury et al., 2010).

The first observed function of VEGF was its ability to enhance the permeability of tumour vasculature. Later its power as endothelial mitogen was described: VEGF attracts endothelial cells and promotes their differentiation, proliferation and survival. Today the role of VEGF as one of the angiogenic factors to keep up and promote vascular homoeostasis in the organism has become clear.

The striking significance of VEGF becomes obvious considering the fact that the first definitive marker protein on ripening endothelial cells in the yolk sac is the VEGF receptor 2 (VEGFR 2 or Flk 1). Under the influence of VEGF A these endothelial progenitors marked by VEGFR 2 form areas of blood islands; these formations are characterized by clusters of initial erythroblasts lined by the endothelial precursors (Park, Afrikanova et al., 2004, Pearson, Sroczynska et al., 2008).

VEGF-A exists in several splice variants, with different characteristics; VEGF120 for example is thought to be an especially diffusible isoform due to the lack of a heparin-binding domain.

Three relevant receptors transmit the signal of specific VEGF binding: VEGFR-1 (flt-1), VEGFR-2 (KDR/flk-1) and VEGFR-3 (flt-4). The ligand-receptor interaction leads to cellular response on the base of receptor phosphorylation (Autiero, Waltenberger et al., 2003).

One regulative factor is the appearance of a soluble VEGF receptor, VEGFR-1 (sFlt-1) that acts as a so-called VEGF trap, catching VEGF-A and so inhibiting the initiation of angiogenesis (Maynard, Min et al., 2003).

The VEGF pathway is summarized in the KEGG signalling pathway: in this survey it becomes obvious that the most important receptor on endothelial cells is VEGFR-2 to transmit the angiogenic information. Starting from there several cascades are initiated. Their common outcome is the up regulation of genes that accomplishes endothelial cell proliferation and migration, focal adhesion and cell survival.

Relevant pathways are the calcium-signalling pathway, the MAPK signalling pathway and the arachidonic acid metabolism.

2.3. Role of endothelial cells

Endothelial cells are the cellular key element of angiogenesis and play a significant role in all crucial steps:

Endothelial cells are able to produce and release growth factors.

Endothelial cells express different growth factor receptors on their surface and are regulated by their impact.

Endothelial cells are actively involved in the dissolution of the surrounding matrix.

Simultaneously the migration adhesion and proliferation of endothelial cells continues.

Endothelial cells start to express characteristic integrins to anchor and pull forward the sprouting vessels.

Special endothelial cells, the tip cells secrete matrix metalloproteinases to pave the way and loosen the connective tissue in front of the sprouting vessels´ tip to facilitate the further out growth.
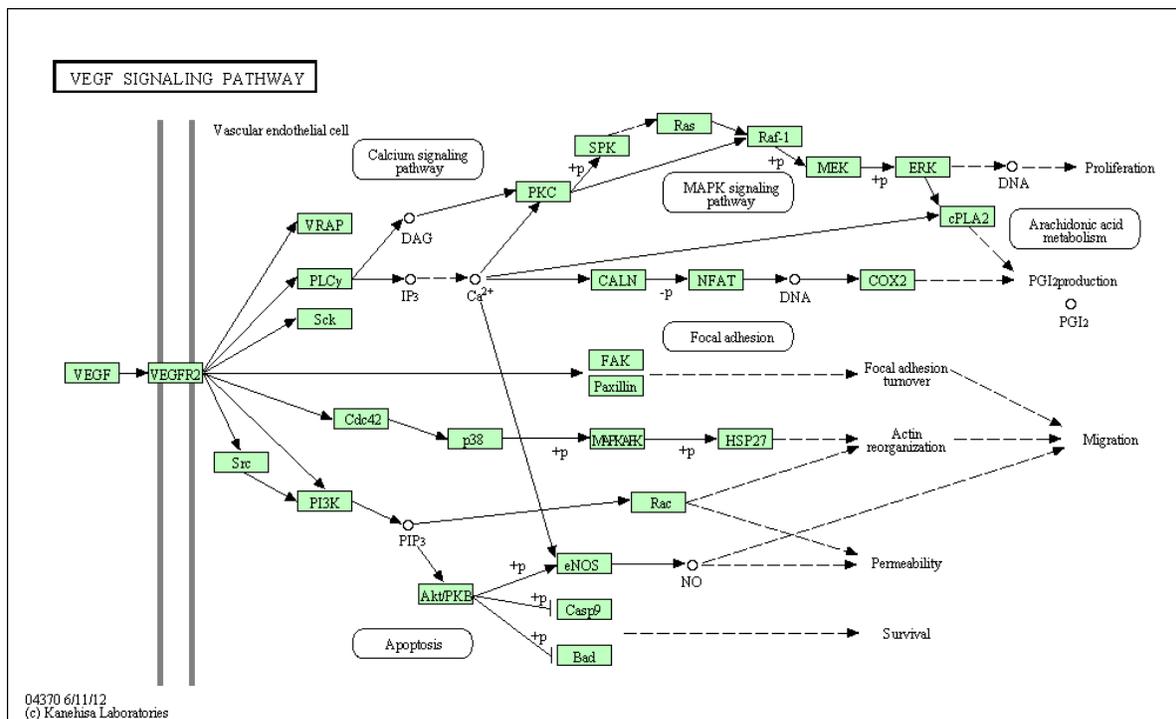
Figure 1. KEGG VEGF signalling pathway (Homo sapiens)

Proliferating endothelial cells are capable of forming three-dimensional structures as tubes and loops, the structural fundament of a functioning circulation.

Considering all these key functions it becomes clearly obvious that only an intact endothelial property can effectively lead to angiogenesis and provides the prerequisite for any regenerative process (Pandya, Dhalla et al., 2006).

These special demands during vascular regeneration are reflected in a significantly increased turn over time. Normally the endothelial turnover is up to hundreds of days. Under angiogenic conditions the turnover time speeds up rapidly to a turnover of under five days, which corresponds with the proliferation of bone marrow cells. This adaptation is of vital importance for the cells to live up to the regenerative demands (Kalluri, 2003).

Other, non-endothelial cells are regulated by VEGF via autocrine control and contribute directly or indirectly to the stimulated processes: monocytes, macrophages, mast cells, dendritic cells, lymphocytes, hematopoietic cells, epithelia, hepatocytes and many others (Breen, 2007).

3. Hypoxia: Master and commander of vascular regeneration

The lack of oxygen immediately threatens the organism's integrity. Few minutes without oxygen supply lead to irreparable damages in the affected organs. The oxygen sensing and the

quick and efficient induction of regulative measures are among the most sensitive and fine-tuned processes in physiology.

3.1. Physiology

Hypoxia is one of the most potent inductors of angiogenesis. Hypoxia is defined by a deficiency of oxygen that can concern the whole organism or parts of it.

The standard is age dependent and varies from 80-100 mmHg, the formula is paO2=102-(age in years x 0.33).

A disturbance of the oxygen haemostasis can be caused on different levels: the partial pressure of the tidal air, the gaseous exchange in the lung or the peripheral tissues or the binding capacity of the erythrocytes.

One has to differentiate different forms of hypoxia: the hypoxic hypoxia refers to a lack of oxygen caused among others reasons by a low partial pressure in heights or by the inability of the lung tissue to perform the necessary gaseous exchange.

The anaemic hypoxia reposes on a reduced capacity of oxygen transport in the blood, e.g. caused by a reduced content of haemoglobin or a carbon monoxide poisoning.

The ischemic hypoxia is caused by a disturbed perfusion of single organs, e.g. due to an embolic insult.

In histotoxic hypoxia the concerned cells are not able to exploit the present oxygen. It is observed in cyanide or alcohol intoxication.

The pathologies resulting from hypoxia are various: on cellular level an alteration of oxygen tension can lead to endothelial changes, among others.

Systemically persisting hypoxia results in pulmonary hypertension. The aim of the increased perfusion of the pulmonary vessels is an optimal oxygen profit during the gaseous exchange.

On bio molecular level hypoxia interferes with gene expression; via oxygen sensing molecules and their downstream signalling cascade the transcription of genes is promoted that induce an enhanced haematopoiesis and angio- and vasculogenesis.

3.2. Oxygen sensing

Cellular mechanisms of oxygen regulation concern the aerobe glycolysis, the arrest of the cell cycle end the initiation of apoptosis.

Systemic regulation includes the release of erythropoietin from the kidneys, hyperventilation and finally angiogenesis.

The interesting question is: which molecule represents the sensor of a low intracellular oxygen tension?

3.3. HIF-1 alpha

Hypoxia inducible factor 1 alpha (HIF-1 alpha) is the key regulator of cellular and systemic oxygen haemostasis. It has been described in 1995 for the first time by Wang et al. (Wang, Jiang, et al., 1995)

HIF-1 alpha consists of two subunits: the alpha subunit is the virtual oxygen sensor. It is O2-sensitive and very unstable. In presence of oxygen, the alpha subunit is not detectable. Under normoxic conditions a quick ubiquination and immediate proteosomic degradation is observed. Oxygen-dependent enzymes, the prolyl-hydroxylases (PHDs) bind oxygen and couple it to HIF1-alpha. Von Hippel Lindau (VHL) protein attacks this complex and initiates its degradation.

The necessary co-factors for the degradation are oxygen and iron. Under hypoxic conditions HIF-1 alpha cannot be degraded for the lack of the co-factor oxygen and accumulates. The molecules reach the nucleus where the come in contact with the HIF-1 beta subunit.

The beta subunit, the so-called aryl hydrocarbon receptor nuclear translocator, ARNT, is expressed in the nucleus constantly.

Triggered by hypoxia there is a dimerisation of alpha and beta subunit that finally leads to the activation of target genes via binding to so-called hypoxia responsive elements (HREs) (Semenza, 2001).
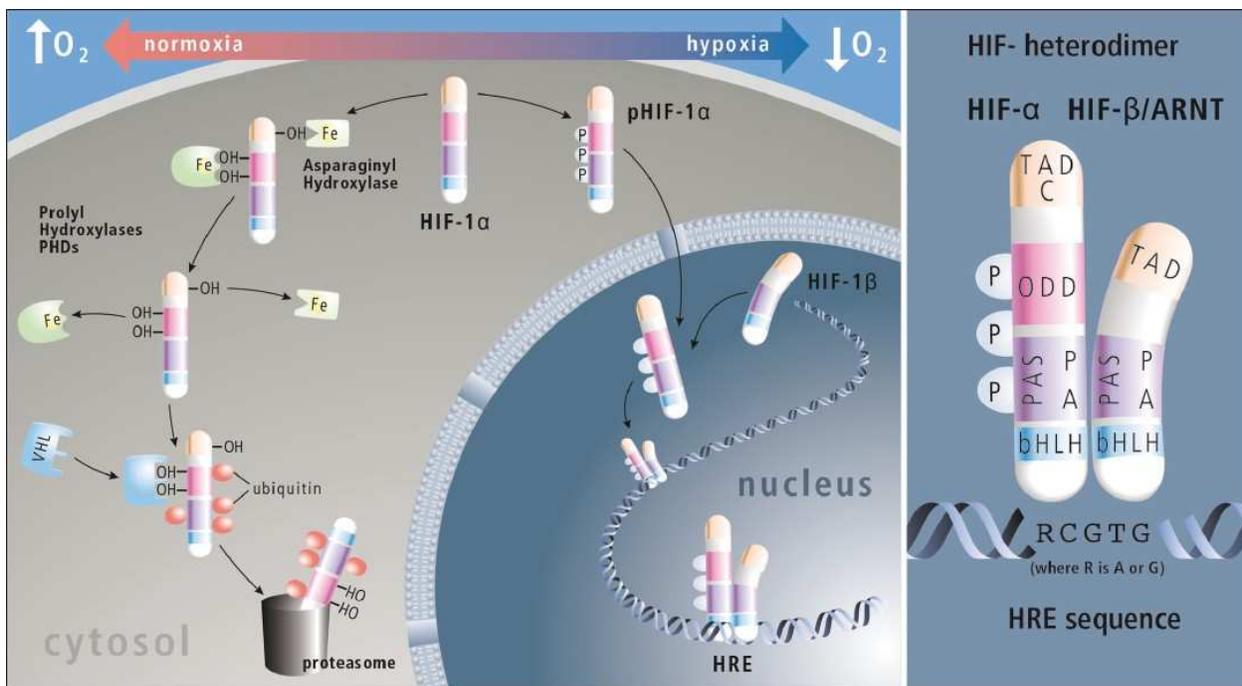

Figure 2. Oxygen sensing (Zagórska, Dulak et al., 2004)

Thus HIF-1 transmits the gene activation that is initiated by the existing hypoxia; many genes – directly or indirectly regulated by hypoxia - are involved in the unleashed cascade that

basically leads to cell differentiation, migration and glycolysis. Next to the famous players VEGF, Flk-1 and Flt-1, EPO, LDH-A, platelet-derived growth factor-ß (PDGF-ß) or basic fibroblast growth factor (bFGF) are involved.

3.4. Target genes

A plethora of target genes are governed by hypoxia via HIF-1. The following tables summarize the most relevant genes according to their function in the context of angio- and vasculogenesis

Extracellular matrix proteins and enzymes	Angiogenic growth factors and cytokines	Cell surface receptors
MMP2	ANGPT1 and 2	CXCR4
FN1	EPO	VEGFR 2
COL5A1	IGF2	TFRC
PLAUR	VEGF	
	TGFA and B	

Table 1. Extracelullar targets

Cytoskeletal proteins	Proapoptotic proteins	Transcription factors	Glucose tranporters and glycolytic enzymes
KRT14	RTP801	DEC1	GLUT1 and 3
KRT18	NIP3	DEC2	ENO1
KRT19	NIX	ETS1	HK1 and 2
VIM		CITED2	LDHA
			TPI
			PGK1

(Metzen, Ratcliffe et al, 2004, Marxsen, Stengel et al., 2004)

Table 2. Intracellular targets

3.5. Clinical relevance

The functionality of HIF-1 alpha, its capacity to transmit the need for oxygen and therefore for more vasculature has been the basic idea of innovative therapeutic concepts.

Recently several drugs have been developed which act as selective HIF prolyl-hydroxylase inhibitors; the inhibited degradation of HIF-1α persuades the system of a severe lack of oxygen and leads to an initiation of counter-measures.

By inhibiting HIF prolyl-hydroxylase, the activity of HIF-1 alpha in the bloodstream is prolonged, which results in an increase in endogenous production of erythropoietin (Bruegge, Jelkmann et al., 2007).

HIF activity is involved in angiogenesis required for cancer tumour growth, so HIF inhibitors are under investigation for anti-cancer effects (Semenza, 2006).

In addition, there have been observations that suggest that HIF pathway is not only a pivotal inductor of neo-angiogenesis but also is relevant in questions of bone regeneration for example in fracture repair.

The mechanism behind this hypothesis postulates the ability of osteoblasts to instrumentalize HIF-1 alpha as oxygen sensor and the corresponding signalling cascade to improve angio- and osteogenesis concurrently; the molecular interconnection is not finally elucidated. A dynamic crosstalk between osteoblasts and endothelial progenitors is assumed.

Therefore the application of HIF activators might improve bone healing by optimizing the angiogenic properties of the wounded bone but more important by inducing bone regeneration itself. Encouraging observations have been made in mouse fracture models where an overexpression of HIF and VEGF in long bones of mice results in pronounced vascularisation. Even a separate cultivation of the special osteoblasts without the corresponding endothelial cells does not affect their proliferation and differentiation (Wan, Gilbert et al., 2008, Wang, Wan et al, 2007).

4. Influence of angiogenesis during bone regeneration

The statements and examples of the preceding sections underlined clearly the impact of angiogenic processes on any tissue regeneration.

One well investigated area of research in this context in the regeneration of bone, e.g. in terms of skeletal development or fracture repair. The vasculatures´ job is to bring oxygen and nutrients to the metabolically active areas, but also to provide the bone with precursors or inflammatory cells. As far as the cytokines are concerned, there are many factors that act as key protagonists in angiogenesis as well as in bone regeneration and remodelling: VEGF, especially its isoforms VEGF120, 164 and 188 play a significant role. But there are other relevant players: bFGF, TGFβ, HIF are among the most potential ones.

VEGF in its isoforms with the corresponding receptors have emerged as the decisive coupling factors between epi- and metaphyseal vascularisation and cartilage development and enchondral ossification. A block of VEGFR-1 and -2 with selective antibodies leads to a reduced VEGF signalling and consecutively to a reduced intramembraneous bone formation in distraction osteogenesis; VEGF in this setting is produced by local inflammatory cells (Jacobsen, Al-Aql et al. 2005).

The angiopoietins Ang-1 and Ang-2, hepatocyte growth factor HGF, platelet-derived growth factor PDGF, the IGF family and the neurotrophins NGFs also have angiogenic properties.

The effect of HIF 1-alpha as stimulator of bone regeneration also has been observed: in a mouse model with increased HIF activity the animals showed significantly higher bone mass. The stimulated HIF activity led to enhanced intramembraneous bone regeneration

in a mouse distraction model (Wang et al., 2007, Portal-Núñez, Lozano et al., 2012, Hankenson, Dishowitz et al., 2011)

In fracture vascularisation and repair VEGF function is required: here the matrix-bound forms of VEGF are activated by matrix metalloproteinases, enzymes that fulfil many functions during bone and matrix degradation and remodelling.

MMP9, expressed in osteo- and chondroclasts during fracture repair, initiates cartilage resorption. This degradation process releases matrix-bound VEGF from the cartilage matrix and thus stimulates the vascularisation. This callus degradation in addition provides the base for bony fracture repair in contrast to persisting cartilage non-union (Colnot, Thompson 2003).

MMP 13 activates VEGF relase independently: whereas MMP9 depends on osteo- can chondroclast functionality, MMP13 is expressed by hypertrophic osteo- and chondroblasts. Lack of MMP13 interferes with the proteoglycan degradation leading to a reduced permeability of the cartilage matrix for recruited inflammatory cells and sprouting blood vessels. The result is delayed callus resorption and altered vascular invasion (Behonick, Xing et al., 2007).

5. Autologous bone tissue engineering

Established concepts in the management of bony segmental defects or non-union after fracture rest upon the surgical implantation of either autologous bone as free grafts or micro-surgically anastomosed or artificial substitutes.

The concept of autologous bone tissue engineering wants to make available an amount of bone chips or bars that the organism itself is not able to supply without severe consequences. The base of the ideal three dimensional vascularised bone scaffold comprises the presence of a mechanically stable scaffold, seeded with different autologous cell populations and precursors, loaded with growth factors, embedded from the moment of implantation in a functioning vasculature to provide oxygen and nutrients and to remove metabolic by-products. Whereas many demands of this regenerative model can be met during in vitro culture in bioreactors, the vascular continuity remains the big problem to be solved.

As the supply of nutrition and oxygen via diffusion in three-dimensional tissue formations is restricted to an area of 100 μm around the nutritive capillary, resorption and devitalisation in the centre of the implant lead to a loss of mechanical stability.

The improvement of vascularisation therefore is an important demand on bone tissue engineering concepts. As far as the scaffold itself is concerned, there are different aspects to be considered; one decisive factor is the porosity of the material. In in-vitro studies the scaffolds with smaller pores (5-20 μm) come with increased endothelial cell growth and enhanced osteogenesis (Narayan, Venkatraman et al., 2008). In vivo the opposed effect is observed: higher porosity leads to more efficient osteo- and angiogenesis (Santos, Reis et al., 2010).

The modern materials provide the base for successful vascularisation simply by their design. In the structure of biodegradable polymers the negative of a vascular network can be imprinted

and thus provide the architectural structure of an efficient vasculature; the endothelial (progenitor) cells have to populate the form, the structure is pre-fabricated. The predefined geometry has to fulfil special demand to grant for optimal results, so the network should be designed in branches with defined numbers and localization of vertical nodes.

This concept of microfabrication has been upgraded: with CAD/CAM techniques three-dimensional scaffolds can be designed (Ciocca, De Crescenzio et al., 2009). So far these techniques are mainly applied in soft tissue engineering.

The loading of the scaffolds with growth factors is an established concept. The systems of drug delivery and release have become more refined, due to a combination of advanced scaffold materials and bio molecular perception concerning the anigogenic and osteogenic character-istics of the applied factors, their interconnection and vice-versa regulation. Combined application of several interacting growth factors is regarded as one of the pivotal steps towards successful factor application: from a polymeric scaffold a combination of VEGF and PDGF is delivered with defined dose and release kinetics. The advantage is the interaction of VEGF as endothelial mitogen and initiator of angiogenesis whereas PDGF impact on muscle cells and pericytes leads to vessel maturation and stabilization (Richardson, Peters et al., 2001).

In vitro pre-vascularisation of the scaffold often requires the colonisation with a co-culture of osteoblasts and endothelial (progenitor) cells, the duration of the in vitro phase ranges from hours to weeks. Investigations with poly-lactides implanted with a co-culture of endothelial progenitors and osteoblasts resulted in improved osteogenesis and vascularisation. The ischemic necrosis that was observed in the center of a graft that has only been implanted with osteoblast was not shown in the co-cultured scaffold (Yu, Vandevord et al., 2008).

Finally the success of any implant relies on a quick and efficient perfusion. In this context microsurgical techniques are combined with tissue engineering concepts in hybrid approaches that combine the respective advantages (Santos, Reis et al, 2010).

Modern approaches aim to design a custom made scaffold, loaded with autologous cells and growth factors including autologous vessel loops to grant for a spontaneous microvascular supply to support the expanding tissue. The details of this technique will have to be refined, but the first results are promising (Locmic, Stillaert et al., 2007). In autologous bone tissue engineering the combination of different regenerative strategies including tissue support and angiogenesis on various levels of the implant design and prefabrication finally will lead to successful therapeutic concepts.

6. Regenerative concepts

The support of developing vasculature happens on different levels from the (systemic) application of growth factors to the local application of loaded implants.

Next to the selection of growth factors, the colonisation with prefabricated cell populations or the nano-structural design of the implants are decisive factors considering the implant integration and the development of a functional vessel network.

6.1. Therapeutic angiogenesis

In different clinical applications the angiogenic effect of different growth and transcription factors could be observed. In the context of angio- and osteogenesis, their coupling and the chance of therapeutic intervention, the administration of VEGF is the best investigated one. In fracture healing and bone regeneration therapeutic angiogenesis finds many points of attack. Beside the acute trauma the especially interesting indications considering bony regeneration are non-unions and distraction osteogenesis.

There are several approaches to stimulate angiogenesis and consecutively bone regeneration. The administration of angiogenic factors, VEGF or FGF, is supposed to effect a direct angiogenic up regulation. Another initiator of angiogenesis is HIF; its application or the inhibition of its degradation results in angiogenic effects. Generally these therapies aim to promote angiogenesis, to block anti-angiogenic processes and to bring endothelial progenitor cells to the wounded bone (Hankenson, Dishowitz et al., 2011).

The effects of VEGF as a promoter not only of angiogenesis but also of bone regeneration have been reported in a femur fracture model in mice and in a rabbit radial segmental defect; improved ossification and callus maturation where observed (Street et al, 2002).

Another growth factor with angiogenic and osteogenic characteristics is platelet derived growth factor (PDGF) that acts as mitogen for osteoblasts and up-regulates VEGF expression. In animal models the administration of PDGF came with increased mechanical stability and callus density (Hollinger, Onikepe et al., 2008). In human pilot projects these positive results of PDGF application in combination with fracture stabilization could be verified.

A modern area of research dealing with VEGF as a means of vascular protection and regeneration aims to neuroprotection; VEGF has been reported to protect motor neurons in vitro from hypoxia induced toxicity, reactive oxygen and other degrading factors (Svensson, Peters et al., 2002). In addition, VEGF seems to be able to stimulate growth and development of neuronal stem cells as well as to recruit neuronal progenitor cells (Schaenzer, Wachs et al., 2004). In ALS rat models the protective effect of VEGF in the cerebrospinal fluid has recently been reported, showing a protracted course of disease with delayed paralysis and increased survival time (Storkebaum, Lambrechts et al., 2005).

The potential of angiogenesis as a pivotal factor in many areas of tissue maintenance and regeneration is obvious; in future regenerative medical concepts the manipulation of angiogenesis in parallel with tissue regeneration will be integral part and lead to successful strategies.

The most important growth and transcription factors enhancing vascularisation are summarized in table 3. The variety of different angiogenic factors with similar functions implies the idea of redundancy: the quick and undisturbed succession of events of angiogenesis is too important for the function of the whole organism to take the risk of relying on unique regulators or promoters.

Growth factor	Molecular target	Effects on progenitor cells
VEGF	VEGF receptors expressed on endothelial cells, monocytes, hematopoietic stem cells; stimulates proliferation, migration, and tube formation	Mobilization of EPC Improves survival and differentiation of EPC
Placenta-derived growth factor (PIGF)	VEGF receptor 1 (cross talk with VEGF receptor 2)	Mobilization of hematopoietic stem cells and EPC
Fibroblast growth factor (FGF)	FGF receptors expressed on endothelial cells, smooth muscle cells, and myoblasts; stimulates proliferation	Included in EPC culturing media
Angiopoietin-1	Tie-2 receptor expressed on endothelial cells; enhances vessel maturation and stability	Mobilizes EPC and hematopoietic progenitor cells
Insulin-like growth factor (IGF)	IGF receptor expressed on vascular cells and satellite cells; enhances skeletal muscle regeneration	Included in EPC culturing media
Erythropietin (EPO)	Activates the Epo receptor, which is expressed on hematopoietic stem cells, EPC, endothelial cells, and cardiac myocytes; improves survival	Mobilization of EPC
Hypoxia inducible factor 1 (HIF-1)	Activation of gene expression (eg, VEGF, VEGF receptor 2, erythropoietin, IGF-2, and NO synthase)	

Table 3. Growth and transcription factors stimulating angiogenesis (Losordo, Dimmeler et al., 2004)

6.2. Nanotechnology in regenerative medicine

Modern biomaterials have to meet many requirements: not only do they have to provide mechanic support and stability; they have also to enhance regenerative processes, to be anti-infective and non-inflammatory. The problems of bone implants so far were seen in the poor osseointegration and bone regeneration on the one hand and implant loosening and fracture on the other (Dhillon, Schwarz et al., 2011).

The relevance of nanotechnology in the area of tissue engineering research is reflected by the fact that it has become an independent field of interest in regenerative medicine: the nanomedicine. Nanomedicine deals with implant structures that have (surface) dimensions of fewer than 100 nm. The used materials include fibres and particles, imitating natural bone structure to improve the mechanic and biological properties of the implants.

Figure 3 illustrates the dimension of nanostructures in the context of bone anatomy (Sato, Webster et al., 2004, Khang, Lu et al., 2008).
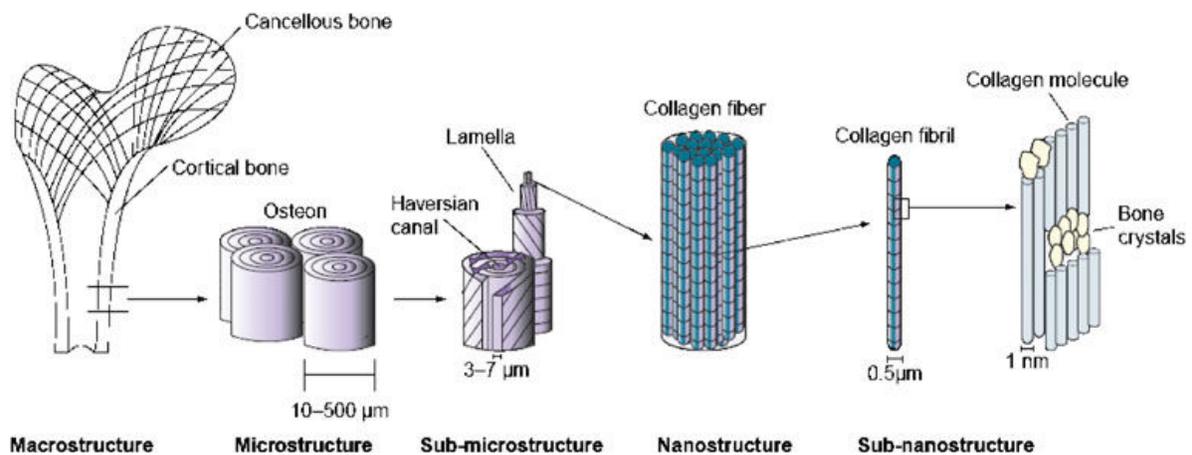
Figure 3. Nanostructure in bone structure (Sato, Webster et al., 2004)

One important task of nanomedicine is to come up with improved, probably intelligent biomaterial. In fact there are two different strategies to modify established materials. One deals with the materials chemistry, the other cares for the surface properties. By individually adapting chemical and physical characteristics the idea scaffold can be designed (Khang, Carpenter et al., 2010).

The established nano-materials in bone regeneration are nano-hydroyapatite, silk and nano structured titanium surfaces. To reach optimal cell adhesion and function there have been efforts to imitate the physiological anisotropy of natural bone. These modifications led to enhanced adhesion and mineralisation (Khang, Lu et al., 2008).

These nano-materials will find their way into clinical practice as far as orthopaedic indications or dental implants are concerned.

These developments are also realized in vascular tissue regeneration: the material surfaces are supposed to promote endothelial cell migration, adhesion and proliferation of vascular graft or stents. In an investigation of polylactide-co-clycolic acid (PLGA) surfaces with spherical surfaces features with ascending diameters a positive correlation of vertical surface feature dimension to cell adhesion and protein adsorption was measured; the optimal dimension was 20 nm (Carpenter, Khang et al., 2008). In special etching techniques, titanium inductively coupled plasma deep etching (TIDE), a linear nano-structured surface pattern is created that allows for increased endothelial cell proliferation compared to smooth titanium surfaces and even to randomly nano-structures titanium surfaces after five days of cultivation (Lu, Rao et al., 2008). Recent concepts deal with mechanical strain applying pulsed or sustained pressure to the implanted scaffolds to meet the demands of physiological vascular tension.

Nanotechnology in current tissue engineering concepts investigates the cell- biomaterial interaction and perfects the surface properties to achieve maximum regenerative support in combination with a prolonged implant lifetime. In the context of angiogenesis the development and integration of nano-materials will be of vital importance in regenerative strategies.

6.3. Critical aspects

Many promising investigations featuring growth factor therapy are performed in cell culture or healthy young animals. The therapeutic use of these developments especially addresses the ageing population suffering from ischemic diseases and vascular degeneration. Special attention in coming investigations has to be shifted to the functionality and regenerative demands of diseased of damages cells and tissues. Therapeutic angiogenic strategies have to be scrutinized under the focus of safety and effectiveness in systems with impaires endogenous endothelial function (Sun, Bai et al., 2009).

Beside the form of application, the definition of the ideal dose of angiogenic growth factor is one the most difficult questions to answer. When dealing with loaded scaffolds one has to define release kinetics. Additionally, in histological investigations, the vasculature that develops under the influence of high doses of VEGF repeatedly showed malformations and an insufficiency in the cell-cell junctions (Zisch, Lutolf 2003).

In most therapeutic concepts the desired effect of VEGF is a local one and aims to improve the formation of nutritive vasculature supporting tissue regeneration in a limited area of tissue as well as a in a limited period of time. VEGF application has to take place locally and only during defined period; considering the fact, the VEGF coming with increased angiogenesis is part of many pathologic processes, e.g. tumour vascularisation or proliferative retinopathy, the control of VEGF effect has to be granted. These demands require a lot of conceptional research considering the therapeutic application of VEGF. The angiogenic and osteogenic effects of VEGF delivered from poly-lactide scaffolds in irradiated osseous defects was illustrated impressively in increased vascularisation and bone formation, the application of a potent growth factor in tumour patients however bears many risks (Kaigler, Wang et al., 2006)

In every therapeutic concept the medical gain and the patients´ profit has to be weighed against the impending costs. Nowadays the production of recombinant VEGF in the desired doses is enormous. For routine clinical application the methods of generation, application and delivery have to be refined (Barralet, Gbureck et al., 2009).

7. Role of stem cells in regenerative medicine

The potency of multilineage differentiation and self-renewal makes stem cells an attractive target for scientific approaches concerning tissue repair and regeneration. The interesting cell populations in this context are embryonic and mesenchymal stem cells.

7.1. Embryonic stem cells

Human embryonic stem cells are capable of self-renewal and differentiation in cells from all germ layers. These regenerative characteristics are very attractive in basic science as well as in potential clinical applications. The pros and cons of embryonic stem cell therapy are discussed controversially.

Current investigations describe the cells potential to differentiate into vascular cell lines and therefore to support tissue repair via angiogenesis. The difficulty is to control and regulate the cell proliferation and differentiation to avoid spontaneous development of teratoma from undifferentiated embryonic stem cells.

In figure 4 an overview of different culture protocols to induce vascular differentiation from embryonic stem cells is given (Descamps, Emanueli et al., 2012).
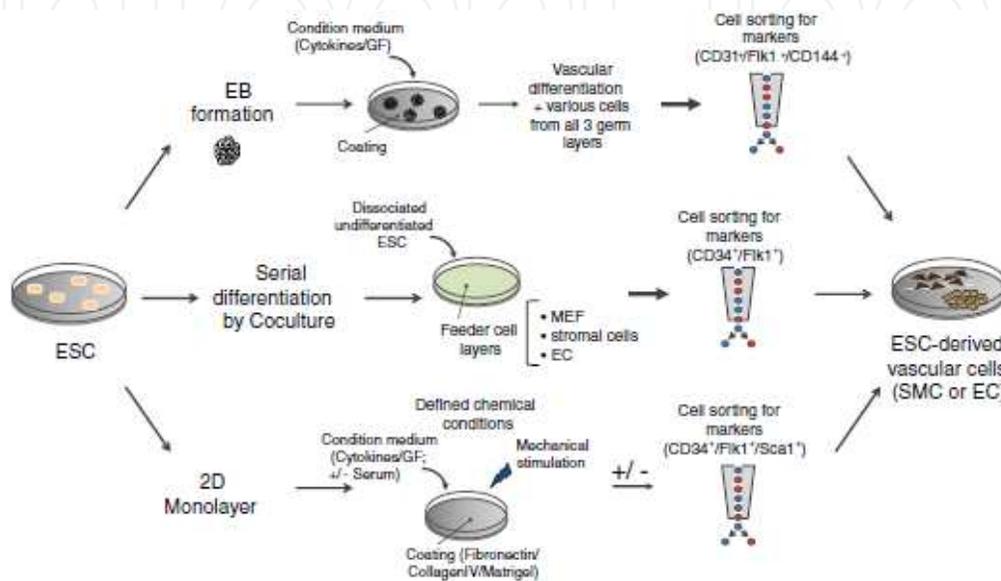

Figure 4. Vascular differentiations

Therapeutic neo-vascularisation is of extraordinary interest in the therapy of cerebral or heart ischemia and regenerative strategies. In mouse models with limb ischemia the implantation of embryonic stem cells – alone or in combination with muscle cells – proved a significantly better perfusion via neo-vascularisation in stark contrast to an implantation of adult endothelial cells (Kane, Xiao et al., 2010).

The positive results in animal models are promising. Yet the pitfalls of stem cell transfer represented by tumorigenicity and immunocompatibility have to be overcome (Descamps, Emanueli et al., 2012).

7.2. Mesenchymal stem cells

In contrast to embryonic stem cells, mesenchymal stem cells are characterized by pluripotency. These adult stem or progenitor cells, harvested from fat, skin or dental pulp are less prone to ethical concerns and directly available for autologous approaches.

Especially in cardiovascular regeneration mesenchymal stem cells hold great promise for further advanced in therapeutic strategies.

In different investigations these cell lines have shown their potential: not only are they able to differentiate into vascular cells, endothelial cells or pericytes, in addition they stimulate the

local cells via paracrine secretion of growth and transcription factors, among others VEGF or IGF. In this context, a secretion of microparticles by the pluripotent cells has been observed; these particles seem to support the regenerative process, the mechanism behind this phenomenon is not understood (Vono, Spinetti et al., 2012).

8. Conclusion

Angiogenesis in vivo and vitro, in physiologic and pathologic processes is a multifactorial process. It includes a plethora of signalling molecules and pathways, dynamically cross-talking cells and innumerable cytokines and growth factors to generate a functional and stable vessel system. This vasculature, however, is one prerequisite of tissue regeneration for it grants a continuous supply of nutrients and oxygen. These considerations found their entry to modern tissue engineering when the vital importance of a vascular network was more and more focussed.

The future of regenerative approaches to bone healing and regeneration will inevitably combine the field-tested strategies of tissue engineering with modern bio molecular techniques in the scientific environment of stem cells and gene therapy.

To define the fragile equilibrium between mechanical properties, tissue support and angiogenic stimulation will be the interest of research in regenerative medicine for the coming years.

Author details

Susanne Jung and Johannes Kleinheinz

Department of Cranio-Maxillofacial Surgery, University Hospital Muenster, Germany, Research Unit Vascular Biology of Oral Structures (VABOS), Germany

References

[1] Autiero, M, Waltenberger, J, Communi, D, Kranz, A, Moons, L, Lambrechts, D, Kroll, J, Plaisance, S, De Mol, M, Bono, F, Kliche, S, Fellbrich, G, Ballmer-hofer, K, Maglione, D, Mayr-beyrle, U, Dewerchin, M, Dombrowski, S, Stanimirovic, D, Van Hummelen, P, Dehio, C, Hicklin, D. J, Persico, G, Herbert, J. M, Communi, D, Shibuya, M, Collen, D, Conway, E. M, & Carmeliet, P. (2003). *Role of PlGF in the intra- and intermolecular cross talk between the VEGF receptors Flt1 and Flk1,* Nat Med. , 9(7), 936-43.

[2] Barralet, J, Gbureck, U, Habibovic, P, Vorndran, E, Gerard, C, & Doillon, C. J. (2009). *Angiogenesis in calcium phosphate scaffolds by inorganic copper ion release,* Tissue Eng Part A. , 15(7), 1601-9.

[3] Behonick, D. J, Xing, Z, Lieu, S, Buckley, J. M, Lotz, J. C, Marcucio, R. S, Werb, Z, Miclau, T, & Colnot, C. (2007). *Role of matrix metalloproteinase 13 in both endochondral and intramembranous ossification during skeletal regeneration,* PLoS One. 2(11):e1150.

[4] Breen, E. C. (2007). *VEGF in biological control,* J Cell Biochem. , 102(6), 1358-67.

[5] Bruegge, K, Jelkmann, W, & Metzen, E. (2007). *Hydroxylation of hypoxia-inducible transcription factors and chemical compounds targeting the HIF-alpha hydroxylases,* Curr. Med. Chem. , 14(17), 1853-62.

[6] Carpenter, J, Khang, D, & Webster, T. J. (2008). *Nanometer polymer surface features: the influence on surface energy, protein adsorption and endothelial cell adhesion,* Nanotechnology. 19(50):505103.

[7] Cima, L. G, Vacanti, J. P, Vacanti, C, Ingber, D, Mooney, D, & Langer, R. (1991). *Tissue engineering by cell transplantation using degradable polymer substrates,* J Biomech Eng , 113, 143-51.

[8] Ciocca, L, De Crescenzio, F, Fantini, M, & Scotti, R. (2009). *CAD/CAM and rapid prototyped scaffold construction for bone regenerative medicine and surgical transfer of virtual planning: a pilot study,* Comput Med Imaging Graph. , 33(1), 58-62.

[9] Colnot, C, Thompson, Z, Miclau, T, Werb, Z, & Helms, J. A. (2003). *Altered fracture repair in the absence of MMP9,* Development. , 130(17), 4123-33.

[10] Descamps, B, & Emanueli, C. (2012). *Vascular differentiation from embryonic stem cells: novel technologies and therapeutic promises,* Vascul Pharmacol. 56(5-6):267-79. Epub 2012 Apr 2.

[11] Dhillon, R. S, & Schwarz, E. M. (2011). *Teriparatide Therapy as an Adjuvant for Tissue Engineering and Integration of Biomaterials,* J Mater Res. , 4(6), 1117-1131.

[12] Dyer, L. A, Portbury, A. L, & Patterson, C. (2010). *Gyrate: CCM3 dances with a different angiogenic partner,* Sci Signal. 3(122):pe17.

[13] Frerich, B, Kurtz-hoffmann, J, Lindemann, N, & Müller, S. (2000). *Untersuchungen zum Tissue engineering vaskularisierter knöcherner und weichgewebiger Transplantate,* 4 (Suppl 2):490-95.

[14] Glowacki, J, Shustermann, M, Troulis, M, Holmes, R, Perrott, D, & Kaban, L. B. (2004). *Distraction Osteogenesis of the Porcine Mandible: Histomorphometric Evaluation of Bone,* Plast Reconstr Surg 113: 566.

[15] Hankenson, K. D, Dishowitz, M, Gray, C, & Schenker, M. (2011). *Angiogenesis in bone regeneration,* Injury. 2011 Jun;, 42(6), 556-61.

[16] Hollinger, J. O, & Onikepe, A. O. MacKrell J, Einhorn T, Bradica G, Lynch S, Hart CE. ((2008). *Accelerated fracture healing in the geriatric, osteoporotic rat with recombinant human platelet-derived growth factor-BB and an injectable beta-tricalcium phosphate/collagen matrix,* J Orthop Res. , 26(1), 83-90.

[17] Jacobsen, K. A, Al-aql, Z. S, Wan, C, Fitch, J. L, Stapleton, S. N, Mason, Z. D, Cole, R. M, Gilbert, S. R, Clemens, T. L, Morgan, E. F, Einhorn, T. A, & Gerstenfeld, L. C. (2008). *Bone formation during distraction osteogenesis is dependent on both VEGFR1 and VEGFR2 signaling,* J Bone Miner Res. , 23(5), 596-609.

[18] Kaigler, D, Wang, Z, Horger, K, Mooney, D. J, & Krebsbach, P. H. (2006). *VEGF scaffolds enhance angiogenesis and bone regeneration in irradiated osseous defects,* J Bone Miner Res. , 21(5), 735-44.

[19] Kalluri, R. (2003). *Basement membranes: structure, assembly and role in tumour angiogenesis,* Nat Rev Cancer. , 3(6), 422-33.

[20] Kane, N. M, Xiao, Q, Baker, A. H, Luo, Z, Xu, Q, & Emanueli, C. (2011). *Pluripotent stem cell differentiation into vascular cells: a novel technology with promises for vascular re(generation).* Pharmacol Ther. , 129(1), 29-49.

[21] Khang, D, Carpenter, J, Chun, Y. W, Pareta, R, & Webster, T. J. (2010). *Nanotechnology for regenerative medicine,* Biomed Microdevices. , 12(4), 575-87.

[22] Khang, D, Lu, J, Yao, C, Haberstroh, K. M, & Webster, T. J. (2008). *The role of nanometer and sub-micron surface features on vascular and bone cell adhesion on titanium,* Biomaterials. , 29(8), 970-83.

[23] Lokmic, Z, Stillaert, F, Morrison, W. A, Thompson, E. W, & Mitchell, G. M. (2007). *An arteriovenous loop in a protected space generates a permanent, highly vascular, tissue-engineered construct,* FASEB J. , 21(2), 511-22.

[24] Losordo, D. W, & Dimmeler, S. (2004). *Therapeutic angiogenesis and vasculogenesis for ischemic disease. Part I: angiogenic cytokines,* Circulation. , 109(21), 2487-91.

[25] Lu, J, & Rao, M. P. MacDonald NC, Khang D, Webster TJ. ((2008). *Improved endothelial cell adhesion and proliferation on patterned titanium surfaces with rationally designed, micrometer to nanometer features,* Acta Biomater. , 4(1), 192-201.

[26] Maynard, S. E, Min, J. Y, Merchan, J, Lim, K. H, Li, J, Mondal, S, Libermann, T. A, Morgan, J. P, Sellke, F. W, Stillman, I. E, Epstein, F. H, Sukhatme, V. P, & Karumanchi, S. A. (2003). *Excess placental soluble fms-like tyrosine kinase 1 (sFlt1) may contribute to endothelial dysfunction, hypertension, and proteinuria in preeclampsia,* J Clin Invest. , 111(5), 649-58.

[27] Metzen, E, & Ratcliffe, P. J. (2004). *HIF hydroxylation and cellular oxygen sensing,* Biol Chem. 385(3-4):223-30.

[28] Marxsen, J. H, Stengel, P, Doege, K, Heikkinen, P, Jokilehto, T, Wagner, T, Jelkmann, W, Jaakkola, P, & Metzen, E. (2004). *Hypoxia-inducible factor-1 (HIF-1) promotes its degradation by induction of HIF-alpha-prolyl-4hydroxylases,* Biochem J. 381(Pt 3):761-7.

[29] Narayan, D, & Venkatraman, S. S. (2008). *Effect of pore size and interpore distance on endothelial cell growth on polymers,* J Biomed Mater Res A. , 87(3), 710-8.

[30] Nomi, M, Atala, A, Coppi, P. D, & Soker, S. (2002). *Principals of neovascularization for tissue engineering,* Mol Aspects Med. , 23(6), 463-83.

[31] Nyberg, P, Salo, T, & Kalluri, R. (2008). *Tumor microenvironment and angiogenesis,* Front Biosci. , 13, 6537-53.

[32] Pandya, N. M, Dhalla, N. S, & Santani, D. D. (2006). *Angiogenesis--a new target for future therapy,* Vascul Pharmacol. , 44(5), 265-74.

[33] Park, C, Afrikanova, I, Chung, Y. S, Zhang, W. J, & Arentson, E. Fong Gh G, Rosendahl A, Choi K. ((2004). *A hierarchical order of factors in the generation of FLK1- and SCL-expressing hematopoietic and endothelial progenitors from embryonic stem cells,* Development. , 131, 2749-2762.

[34] Pearson, S, Sroczynska, P, Lacaud, G, & Kouskoff, V. (2008). *The stepwise specification of embryonic stem cells to hematopoietic fate is driven by sequential exposure to Bmp4, activin A, bFGF and VEGF,* Development. , 135, 1525-1535.

[35] Pelissier, P, Villars, F, Mathouli-pelissier, S, Bareille, R, Lafage-proust, M. H, & Vilamitjana-amedee, J. (2003). *Influence of vascularisation and osteogenic cells on ectopic bone formation within a madreporic ceramic in rats,* Plast Reconstr Surg 111(6); , 1932-41.

[36] Portal-núñez, S, Lozano, D, & Esbrit, P. (2012). *Role of angiogenesis on bone formation,* Histol Histopathol. , 27(5), 559-66.

[37] Putnam, A. J, & Mooney, D. J. (1996). *Tissue engineering using synthetic extracellular matrizes,* Nat Med , 2, 824-26.

[38] Richardson, T. P, Peters, M. C, Ennett, A. B, & Mooney, D. J. (2001). *Polymeric system for dual growth factor delivery,* Nat Biotechnol. , 19(11), 1029-34.

[39] Saadeh, P. B, Khoslah, R. K, Mehrara, B. J, Steinbrech, D. S, Mccormick, S. A, Devore, D. P, & Longaker, M. T. (2001). *Repair of a Critical Size Defect in the Rat Mandible Using allogenic Type I Collagen,* J Craniof Surg , 12

[40] Santos, M. I, & Reis, R. L. (2010). *Vascularization in bone tissue engineering: physiology, current strategies, major hurdles and future challenges,* Macromol Biosci. , 10(1), 12-27.

[41] Sato, M, & Webster, T. J. (2004). *Nanobiotechnology: implications for the future of nanotechnology in orthopedic applications,* Expert Rev Med Devices. , 1(1), 105-14.

[42] Schänzer, A, Wachs, F. P, Wilhelm, D, Acker, T, Cooper-kuhn, C, Beck, H, Winkler, J, Aigner, L, Plate, K. H, & Kuhn, H. G. (2004). *Direct stimulation of adult neural stem cells*

in vitro and neurogenesis in vivo by vascular endothelial growth factor, Brain Pathol. , 14(3), 237-48.

[43] Semenza, G. L. (2001). HIF-1 and mechanisms of hypoxia sensing. Curr Opin Cell Biol. , 13(2), 167-71.

[44] Storkebaum, E, Lambrechts, D, Dewerchin, M, Moreno-murciano, M. P, Appelmans, S, Oh, H, Van Damme, P, Rutten, B, Man, W. Y, De Mol, M, Wyns, S, Manka, D, & Vermeulen, K. Van Den Bosch L, Mertens N, Schmitz C, Robberecht W, Conway EM, Collen D, Moons L, Carmeliet *Treatment of motoneuron degeneration by intracerebroventricular delivery of VEGF in a rat model of ALS,* Nat Neurosci. 8(1):85-92., 2005.

[45] Svensson, B, Peters, M, König, H. G, Poppe, M, Levkau, B, Rothermundt, M, Arolt, V, Kögel, D, & Prehn, J. H. (2002). *Vascular endothelial growth factor protects cultured rat hippocampal neurons against hypoxic injury via an antiexcitotoxic, caspase-independent mechanism,* J Cereb Blood Flow Metab. , 22(10), 1170-5.

[46] Sun, L, Bai, Y, & Du, G. (2009). *Endothelial dysfunction--an obstacle of therapeutic angiogenesis,* Ageing Res Rev. , 8(4), 306-13.

[47] Tepper, O. M, Capla, J. M, Galiano, R. D, Ceradini, D. J, Callaghan, M. J, Kleinman, M. E, & Gurtner, G. C. (2005). *Adult vasculogenesis occurs through in situ recruitment, proliferation, and tubulization of circulating bone marrow-derived cells,* Blood. , 105(3), 1068-77.

[48] Vono, R, Spinetti, G, Gubernator, M, & Madeddu, P. (2012). *What's New in Regenerative Medicine: Split up of the Mesenchymal Stem Cell Family Promises New Hope for Cardiovascular Repair,* J Cardiovasc Transl Res.

[49] Wan, C, Gilbert, S. R, Wang, Y, Cao, X. M, Shen, X, Ramaswamy, G, Jacobsen, K. A, Alaql, Z. S, Gerstenfeld, L. C, Einhorn, T. A, Eberhardt, A. W, Deng, L, Guldberg, R. E, & Clemens, T. L. (2008). *Role of hypoxia inducible factor-1 alpha pathway in bone regeneration,* J Musculoskelet Neuronal Interact. , 8(4), 323-4.

[50] Wang, G. L, Jiang, B. H, Rue, E. A, & Semenza, G. L. (1995). *Hypoxia-inducible factor 1 is a basic-helix-loop-helix-PAS heterodimer regulated by cellular O2 tension,* Proc. Natl.Acad. Sci. USA , 92, 5510-5514.

[51] Wang, Y, Wan, C, Deng, L, Liu, X, Cao, X, Gilbert, S. R, Bouxsein, M. L, Faugere, M. C, Guldberg, R. E, Gerstenfeld, L. C, Haase, V. H, Johnson, R. S, Schipani, E, & Clemens, T. L. (2007). *The hypoxia-inducible factor alpha pathway couples angiogenesis to osteogenesis during skeletal development,* J Clin Invest. , 117(6), 1616-26.

[52] Wang, Y, Wan, C, Gilbert, S. R, & Clemens, T. L. (2007). *Oxygen sensing and osteogenesis,* Ann N Y Acad Sci. , 1117, 1-11.

[53] Yu, H, Vandevord, P. J, Gong, W, Wu, B, Song, Z, Matthew, H. W, Wooley, P. H, & Yang, S. Y. (2008). *Promotion of osteogenesis in tissue-engineered bone by pre-seeding endothelial progenitor cells-derived endothelial cells,* J Orthop Res. , 26(8), 1147-52.

[54] Zagórska, A, & Dulak, J. (2004). *HIF-1: the knowns and unknowns of hypoxia sensing*, Acta Biochim Pol. , 51(3), 563-85.

[55] Zisch, A. H, Lutolf, M. P, & Hubbell, J. A. (2003). *Biopolymeric delivery matrices for angiogenic growth factors*, Cardiovasc Pathol. , 12(6), 295-310.