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Influence of Angiogenesis on Osteogenesis

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1. Introduction

No bone without vasculature. Current research reveals the molecular biology behind this simple statement. Bone formation and regeneration and the associated physiological and pathologically altered angiogenesis is not only of vital importance in the omnipresent fracture repair but also plays a constantly growing role in the diagnosis and treatment of chronically infected bone or osteonecrosis. The jaw and especially the mandible with its exceptional vascularisation in the human body are prone to these diseases.
The understanding of the molecular mechanisms of osteogenic-angiogenic coupling is the fundament of a sufficient diagnosis and therapy.
From the description of the physiological vascularisation of the jaw bones to pure autologous tissue engineering in the context of regenerative medicine we follow the process of bone formation under the influence of a sufficient angiogenesis with special regard to the key protagonist VEGF, the osteogenic-angiogenic interface and their interaction.

2. Vascular anatomy of the jaw bones

Head and neck are supplied with blood by branches of the external carotid arteries (ECA). The anterior and posterior superior alveolar arteries originate from the maxillary artery and provide the nutrient supply for the upper jaw and teeth; the inferior alveolar artery supplies the mandible and lower teeth. The jaw bones are drained of blood by the correspondent veins and eventually by the subclavian and the jugular vein.
The outline of the bony vasculature is closely related to the mechanism of osteogenesis. The comparison between upper and lower jaw is the most striking example for this correlation in the human body: the ramified vessel system of the maxilla is a direct consequence of its intramembraneous ossification. In contrast, the mandible is supplied by a central artery and vein in the wake of a partly enchondral ossification as observed in long bones. This difference is of vital importance for physiological or iatrogenic bone regeneration.
Coupling interface of bone and vasculature is the periosteum. It provides osteoprogenitor cells and keeps the capillary vessel system.
Age dependent changes in the vascular and bony anatomy are the vertical and sagittal bone loss in the edentulous jaw, circulatory disorders, osteoporosis or bisphosphonate associated osteonecrosis. These alterations pose a challenge for successful regenerative intervention.

2.1 Development

The base of any form of growth and development is the continuous and sufficient supply with (progenitor) cells, oxygen and nutrients.

The first appearance of a primitive vessel formation happens shortly after gastrulation, between the 19. and 21. day of embryonal growth. At this moment the first cells that express a VEGF receptor can be visualized. These cells proliferate in the yolk sac, form so called blood islands with endothelial and hemapoetic cells and expand to the first vessel network, the capillary plexus (Ferkowicz et al., 2003).

In a parallel development, first angioblasts form the pairs of dorsal aortas following the midline of the embryo (Lawson et al., 2002).

With the onset of heartbeat and the permanent presence of erthyroblasts in the newly formed vessel structures, the first vascular network is established.

From now on a permanent remodelling and adaptation process set in, that lasts during the whole lifetime of the individual.

Mechanical signals from the blood flow are pivotal factors in the differentiation of the vessel system, of the development of veins and arteries, their walls and the diameters of the growing vasculature.

The head and neck vasculature derives from the first three aortal arches.

2.2 Anatomy

The carotid artery develops as a self-contained vessel from the ventral aorta.

The external carotid artery (ECA) provides the blood supply for the face and the jaws. The maxillary artery as a terminal branch of the ECA supplies the profound regions of the face. It rises behind the mandibular neck to the pterygopalatine fossa; its course is divided into three portions: the mandibular, the pterygoid and the pterygopalatine portion.

The facial bones are supplied by the superior and inferior alveolar artery, branches of the maxillary artery.

The posterior superior alveolar artery divides into numerous branches to supply the upper teeth and the jawbone. The anterior superior artery originates from the infraorbital artery and supplies the upper incisor and canine teeth. Between these branches ramify many anastomoses.

The inferior alveolar artery runs with the inferior alveolar nerve through the mandibular canal, divides into incisor and mental branch and provides the nutrient supply for the bone, the pulp of the lower teeth and the lower lip.

In striking contrast to the ramified vessel system of the upper jaw, the mandible is perfused by the inferior alveolar artery alone.

The venous drainage occurs via concomitant homonymous veins and anastomoses in the jugular and subclavian veins finally (Bouthillier et al., 1996).

Figure 1 illustrates the arterial vascularisation of the upper and lower jaw originating from the internal carotid artery.

2.3 Remodelling

During foetal development the circulation is not only essential to provide the vital oxygen; the mechanical caused by the blood stream exert fundamental impact on the maturating vessel network.

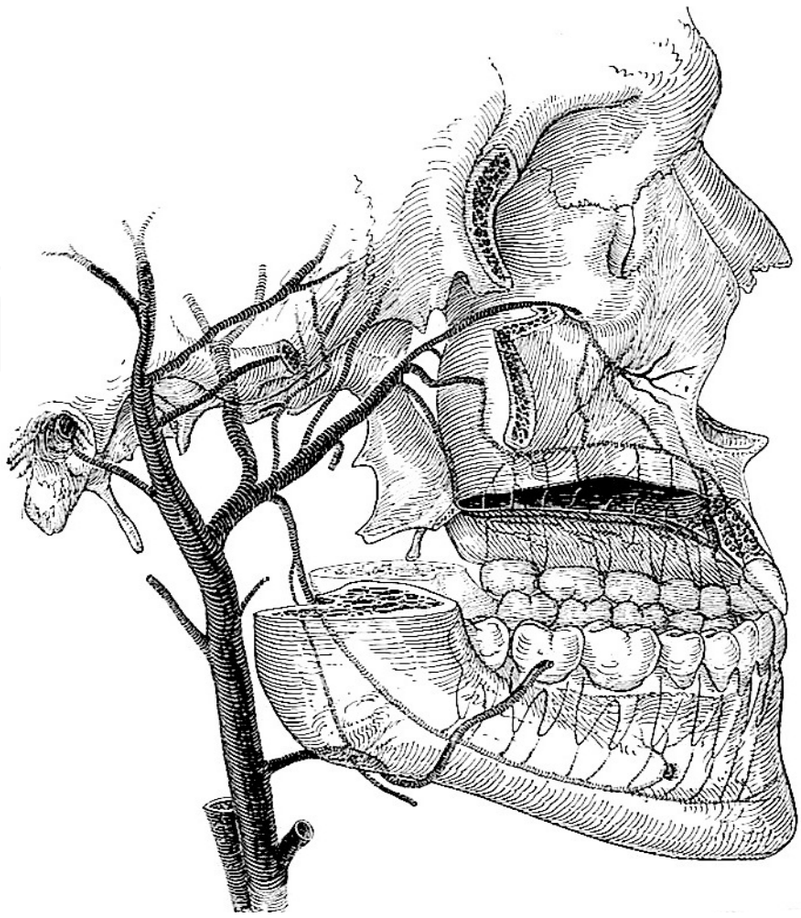

Fig. 1. Vascularisation of the jaw

The development and maturation of the vessel system varies significantly form embryos to adults. One important and often neglected factor is the mechanical influence of an intact blood circulation on vessel anatomy and remodelling.
Vascular remodelling in adults in a physiological surrounding is basically influenced by shear stress and circumferential stretch. These mechanical forces are relevant factors in vessel development and maturation. The molecular mechanisms of transforming a mechanical requirement to a histological response of the involved cells are so far not fully understood (Jones et al., 2011).

3. Basic course of osteogenesis

Bone tissue formation rests upon the balanced interaction between the secretory function of osteoblasts and the degrading activity of osteoclasts. There is intramembraneous and enchondral ossification: both processes are dependent on vascular ingrowth. Most of the facial bones, flat cranial bones, parts of the mandible and the clavicle are formed by enchondral ossification. It combines the growth potential of cartilage and bone to promote skeletal formation and development.
Intramembraneous ossification is the direct ossification of the mesenchym, the primitive connective tissue. It starts with the invasion of capillaries into the mesenchymal zone, where

the resident cells transform into mature osteoblasts and begin to deposit bone matrix extracellularly. The growing bone formations build woven bone, which is eventually organized in stable lamellar bone.
Osteogenesis in general takes place in the vicinity of neo-vessels that mediate the delivery of osteoprogenitors, secrete mitogen for osteoblasts, and transport nutrient and oxygen.
Blood vessels provide a conduit for the recruitment of cells involved in resorption and bone deposition and are therefore a crucial condition for any bone formation or regeneration. Decisive factor of the rate of bone increase is the level of vascularisation of the growth plate.
Bone is the rigid form of connective tissue. It is a dynamic construct, composed of secretory cells, the osteocytes, degrading cells, the osteoclasts and the surrounding matrix, the osteoid. The interaction of its major constituents define its mechanical characteristics: collagen type I guarantees its elasticity; calcium/ phosphate as structural components stand for stability and resistance; dermatan- and chondroitinsulfate represent together with collagen the main part of the extracellular matrix and determine the structural integrity of the tissue.
Skeletal bone is the reservoir of calcium and phosphate that can be activated and released under endocrinological control. Its dynamic (re)modelling is a result of a permanent adaptation to changing physiological and pathological requirements. A fine-tuned balance of (physiological) stress and phases of regeneration leads to a continuous coexistence of osteogenesis and bone degradation and simultaneously to a corresponding vasculature (Martin et al., 2008).
Osteogenesis takes place not only during foetal development but also in the course of facture healing or during infection. One differentiates chondral and intramembranous ossification.
Essential osteoblastic markers are collagen type I, alpha 1 (Col1a1) and alkaline phosphatase (ALP); osteoclasts are characterized by the presence of tartrate-resistant acid phosphatase (TRAP), cathepsin K and dendritic cell-specific transmembrane protein (DC-STAMP) (Suzuki et al., 2007).

3.1 Chondral ossification

The bone formation on the basis of a cartilaginous scaffold is the basic principle of chondral ossification.
One has to distinguish between an enchondral and a perichondral process.
The course of enchondral ossification starts with the ingrowth of nutritive blood vessels in the growth centre of the epiphyseal growth plate. The blood supplies the tissue not only with oxygen and nutrients but also provides a new cell population: Chondroclasts to disintegrate the chondral gantry and osteoblasts and –clasts to construct the bone. Histologically, the growth area of enchondral ossification is characterized by a pillar-like alignment of the chondrocytes in an ascending order from reposing chondral cells to secreting ripe chondrocytes. These active cells produce the initial osteoid that is subsequently mineralized by the locally proliferating osteoblasts. The apoptosis of hypertrophic chondrocytes induces their replacement by osteoblasts in combination with endothelial progenitor cell invasion.
The proliferative chondrocytes produce among other growth and transcription factors VEGF that regulates the vascular ingrowth.

Parts of the os petrosum maturate via chondral ossification.
Perichondral ossification concerns the shafts of the long bones in the extremities: osteoblasts deriving from the well-perfused perichondrium build a three-dimensional cell collar around the cartilage and start to form bone appositionally (Berkowitz et al., 2002).

3.2 Intramembranous ossification

Intramembranous ossification describes the direct bony transformation of the primitive connective tissue and affects mainly the flat bones.
Starting point is the compaction of mesenchymal cells into ossification centres. The cells differentiate to osteoblasts and start to produce the bony matrix, the osteoid. With the emplacement of calcium and phosphate the developing bone maturates; the osteoblasts become resting osteocytes.
Intramembranous ossification takes place in the flat bones of the skull, for example the os frontale (Berkowitz et al., 2002).

3.3 Ossification of the mandible

The skull structures develop from the first and second branchial arch. These mesenchymal tissue areas appear during the fourth week of embryonic growth and determine the base of the individual facial features.
The central vessel/ nerve bundle characterizes the anatomy of the mandible. This special anatomical feature determines the course of the bony development of the lower jaw.
First ossification centres in the mandible can be detected in the area of the branching mandibular nerve in close relation to the developing mental foramen. From here the Meckel´s cartilage indicates course and direction. The Meckel´s cartilage is a transient chondral check rail derived from the first brachial arch to guide the developing bony mandible. It degrades until the 24. week of embryonal development. From its posterior remnants the auditory ossicles malleus and incus are formed.
From the mental foramen to the base of the condylar processus, the course of the mandibular nerve with its corresponding vessels defines the guardrail for the maturating bone. The growing nerval structure with its concomitant vessel system and the tide of growth factors seems to influence and guide the bone maturation.
In an investigation of 25 fetuses the prenatal development and remodelling af the mandible was studied with regard to the morphometry, the histological evaluation of the remodelling processes and a 3D analysis.
The results suggest a strong influence of Meckel´s cartilage configuration and dental primordial on the later anatomy, the signal cascading and specific role of the involved growth and transcription factors in the 3D moulding of the human mandible are not clear (Radlanski et al., 2003).
Another region of interest as far as the ossification of the mandible is concerned, is the mental foramen, the area where nerve, vessels and surrounding extracellular matrix pass the bone. The mental foramen is a centre of bone development and remodelling of the mandible. The pattern of bone configuration and dynamics around the mental foramen differs from the ossification in the bony gutter.
The presence and proximity of the nerve and concomitant vessels does have significant impact on mandibular ossification (Radlanski et al., 2004).

3.4 Role of VEGF in the growth plate

The vital importance of a sufficient VEGF induced vessel formation in the physiological morphological progression of the growth plate was underlined impressively by the work of Gerber et el. 1999: in an mouse model the inactivation of VEGF via soluble antibodies resulted in a significant reduction of vascularisation and consequently ossification. Their data suggests that VEGF-dependent angiogenesis is essential for the processing of he growth plate, cell differentiation and the coupling of chondral degradation and bone formation.

The lack of trabecular bone and the hypertrophy of the chondrocytes were reversible after cessation of VEGF suppression (Gerber et al. 1999).

4. Angiogenic cascade

VEGF and its receptors are the key regulators of angiogenesis.

Angiogenesis is the growth of new blood vessels from pre-existing vasculature that occurs in physiological and pathological contexts. This process is often triggered by a signalling cascade that occurs upon ligand-receptor binding between vascular endothelial growth factor (VEGF) and its receptors (VEGFR1/Flt-1, VEGFR2/KDR). These receptors are expressed by endothelial cells that line the blood vessels. Most potent trigger for angiogenesis is hypoxia.

Angiogenesis, the complex physiological sequence of vasodilatation, degradation of basement membrane, endothelial cell migration, chemotaxis, increasing vascular permeability and eventually endothelial cell proliferation and vessel formation is regulated by VEGF(R). Osteogenic and angiogenic cascade are inseparably interconnected by the functional alliance of endothelial cells, osteoblasts and growth factors.

Angiogenesis is defined as the growth of new blood vessels on the base of existing vascular structures in contrast to vasculogenesis, the development of a new vessel system by sprouting, differentiating endothelial cells. These pivotal processes mark the starting point of any form of vascular development or regeneration processes. In malignant growth, the appearance of new vessels can be a determinant of progression and poor prognosis, whereas a lack of vasculature inevitably leads to loss of functional efficiency and tissue necrosis from the brain to the limbs.

The fine-tuned balance of vasculo- and angiogenesis is controlled by many growth and transcription factors (Pandya, Dhalla et al., 2006).

4.1 Angiogenic cascade – in vitro

The angiogenic cascade in vitro follows a characteristic course of events: Initiation, proliferation or invasion and maturation.

The starting point – the initiation - is the release of angiogenic factors, VEGF, basic fibroblast growth factor (bFGF) or tumour necrosis factor alpha (TGFα). These mitogens and stimulators are released by or inflammatory cells.

Proliferation and invasion includes the degradation and remodelling of the extracellular matrix, the chemotaxis of endothelial (progenitor) cells and the altered expression of adhesion molecules. During the maturation phase, the newly formed vessel ripens, a lumen develops and finally the basal lamina envelops the structure.

These processes can be retraced in vitro:

After the sterile preparation of endothelial cells from an umbilical cord vein according to standard procedure follows the adhesion and proliferation of endothelial cells in their culture well under defined conditions.
After 5 to 7 days a (sub-) confluence of the proliferating cells and formation of a monolayer can be observed.
The formation of circular ring-structures with characteristic cell-cell contacts marks the angiogenic potency of the cultured cells. Special marker of the cell-cell contact is VE-Cadherin, CD 144 antigen.
The adjustment of the culture conditions influences the tissue architecture: in a three dimensional scaffold the formation of a network of capillaries can be observed.
Immunohistochemical markers to characterize the proliferating cell population are CD 31 or von Willebrand factor, for example.
Growth and proliferation of endothelial cells and their surrounding matrix in the course of angiogenesis are governed by a multitude of regulating factors (Fraisl et al., 2009).

4.2 Angiogenic growth factors

Stimulators and inhibitors govern Angiogenesis. The most important pro-angiogenic factors are VEGF, Angiopoietin-1, β-Estradiol, bFGF, IL-8, Leptin, MMPs, NOS, PDGF-BB, TNF-α, Angiogenin and TGFα. They exert their influence on different stages during the angiogenic cascade.
Table 1 subsumes the respective function of the most prominent angiogenic factors.

Factor	Function
VEGF	Cell mitogen, vascular permeability
Angiopoietin-1	Maturation, stability
Angiotropin	Cell migration
bFGF	Angiogenesis in wound healing
IL-8	Chemotaxis, inflammation
NOS	Vasodilatation
PDGF-BB	Chemotaxis, proliferation
Angiogenin	Indirect stimulation, release of angiogenic factors
TGFα	Mitogen, differentiation of endothelial cells

Table 1. Pro-angiogenic factor

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.

The interconnection of angio- and osteogenesis is reflected in the expression of angiopoietin by osteoblasts. The role of angiopoietin in bone formation is not yet clear. The results of in vivo studies suggest that one essential osteogenic effect is the induction of angiogenesis. An over expression of angiopoietin led to an augmentation of bone mass (Suzuki et al., 2007).
The anti-angiogenic antagonists are among others angiostatin, anti-angiogenic anti-thrombin III, canstatin, endostatin (collagen XIII fragment), fibronectin fragment, heparinases, IFN-α,β,χ IL4, IL12, IL18, plasminogen activator inhibitor or pigment epithelium derived factor, PEDF.
Their function is to balance angiogenesis and to interfere with excessive vessel formation. The most important inhibiting factors with their functions are summarized in table 2.
Angiogenesis in vivo is a complex, multilayer process involving the degradation of the vessel basement lamina, the migration and proliferation of endothelial cells and the formation of luminal structures. It includes the cell interaction – activation and silencing by a variety of different growth and transcription factors – and depends on the information and provided by the extracellular matrix. Excessive growth has the same devastating pathological impact as a deficient vasculature (Klagsbrun et al., 1991).

Factor	Function
TGFß	Inhibition of endothelial cell motility
Canstatin	Collagen IV, inhibitor
Endostatin	Collagen XVIII, inhibitor
Platelet factor IV	Inhibitor of growth factor-dependent endothelial stimulation
TNFα	Inhibitor of endothelial cell proliferation
Protamin	Block of heparin-binding growth factors
PEDF	Inductor of endothelial cell apoptosis

Table 2. Anti-angiogenic factors

Another pivotal factor in the regulation of angiogenesis is the microRNA (miRNA) expression. MiRNAs are noncoding small RNAs the modulate postransscriptional gene expression. Their up- and down-regulation is partly regulated by hypoxia and is involved in the stimulation or inhibition of VEGF or other (anti-)angiogenic factors. The most prominent angiogenic miRNAs are miR-126 and miR-27b, anti-angiogenic miRNAs are miR-221 or miR-20a (Fraisl et al., 2009).

4.3 Oxygen sensing

How is a lack of oxygen and therefore an urgent need for further vascularisation detected?
VEGF is release is controlled by hypoxia, oxidative stress, pH or glucose concentration. VEGF binds to membrane tyrosine kinase receptors and initiates chemotaxis, cell migration and vascular permeability (Bikfalvi et al., 2002).

The key of oxygen sensing lies in the HIF transcription factor complex. Oxygen-dependent enzymes, the prolyl-hydroxylases (PHDs) bind oxygen and couple it to HIF1-α. Von Hippel Lindau (VHL) protein attacks this complex and initiates its degradation. Decreasing levels of oxygen lead to a rising concentration of HIF1α (Riddle et al., 2009).
HIF initiates pronounced tissue oxygenation, among many other factors by the stimulation of VEGF expression.
In addition, a tight osteogenic-angiogenic coupling was demonstrated by an increase of bone mass and volume up to 70% after HIF1α activation.
The pharmacological activation of the HIF pathway resulted in an accelerated bone healing (Wang et al., 2008, Wan et al., 2007, Towler, 2008).
Under hypoxic conditions the expression of a variety of genes is altered, adapted to the reduced oxygen supply. Among the hypoxia-induced genes there are not genes involved in metabolism, angiogenesis, erythropoiesis, chondrocyte differentiation and the development and maturation of the collagen matrix (Araldi et al., 2010).

5. Interaction between endothelial cells and osteoblasts on different matrix structures

The potency of the matrix has an enormous impact on the development of the new tissue. It influences cell adhesion, spreading and signalling, cell growth and migration as well as the differentiation of the extracellular matrix and the tissue morphogenesis.
One decisive prerequisite of a matrix structure – especially in the context of regenerative medicine - is the ability to support the growth of blood vessels.
As biological surfaces, collagen membranes are accepted as reliable surfaces to support the growth and adherence of endothelial cells.
Titanium emerged as an artificial surface, which clearly supports the growth and adhesion of endothelial cells and therefore complies with the basic requirements for osseointegration of titanium implants.
The differentiation, growth and function of cellular systems not only relies on their interaction and the interference by synthesized soluble factors but also to a considerable extent on the alloplastic or autologous surfaces of the scaffold on which they are supposed to proliferate. This undisturbed or even conducive environment is the precondition for any healing process where the application of any form of implant is involved.
The biological properties of autologous and alloplastic surfaces have been under scrutiny.

5.1 Collagen

The successful clinical application of materials should involve detailed investigations on interaction between them and tissue with which they will contact.
One relevant approach to gain information about the influence of the scaffold on the proliferating cells is to observe the behaviour of endothelial cells on a collagen material, using histological and immunohistochemical methods.
In an in-vitro essay, isolated human umbilical cord vein cells (HUVECs) identified by means of endothelial-specific antibodies were cultivated to perform the trials.
Cells were seeded in a standard density on a collagen membrane (Lycoll, Resorba, Nuernberg, Germany) and on gelatin-coated control plastic surfaces, after two passages. These were maintained for periods of 1, 7, or 14 days. The cells adhered, spread and

proliferated. Within 24 hours the HUVECs started forming a subconfluent monolayer. It could be observed that the cultured cells expressed integrins and synthesized fibronectin.
The results suggested that the collagen material supported growth and attachment of endothelial cells. In addition, the attachment seemed to be related to the fibronectin synthesized by the cells and to its highly expressed receptor, the α5β1 integrin. (Breithaupt-Faloppa et al., 2006).
The undisturbed growth and proliferation on a collagen scaffold is the basis of standard tissue engineering protocols.

5.2 Titanium

Osteogenesis on titanium surfaces is the prerogative of any form of osseointegration of alloplastic titanium implants from the knee to the teeth.
The mechanisms of cell-cell interaction and dependence meet the prerequisites and requirements of an alloplastic interface with its special mechanical and biochemical material properties of the surface, such as roughness or hydrophilicity.
The coculture of osteoblast-like and endothelial cells on titanium surfaces represents a reliable experimental model to evaluate their biological behaviour. It is a simple and reduced model of osteo- and angiogenesis without the complexity of an in vivo investigation.
The cellular integrity and their function ability are measured by the synthesis of characteristic cell markers via immunohistochemistry.
Gene expression of osteo- and angiogenic factors is used to consolidate and quantify the histological results.
In an in vitro trial the endothelial cell behaviour on a titanium surface was investigated by the immunhistochemical detection of characteristic endothelial markers CD 31 and von Willebrand factor, fibronectin and its receptor, vitronectin and its receptor and VE cadherin.
The results indicated that the attachment of ECs on titanium could be related to cellular-derived fibronectin and the binding to its specific receptor, the α5β1 integrin. It was observed that titanium effectively serves as a suitable substrate for endothelial cell attachment, growth and proliferation in the initial phase. It is suggested to describe this feature of titanium if not angiogenic then at least angio-conductive.
Nevertheless the studied cells did not show the physiological cytological development after titanium contact of 7 days. These findings do not correlate with good clinical results.
The high degree of biocompatibility of titanium and its alloys is intimately related to the passively formed oxide film on the metallic surface (Breithaupt-Falloppa et al., 2008).

6. Influence of angiogenesis during osteogenesis

The level of angiogenesis is a pivotal element of osteogenesis. The vasculature provides the transport of oxygen, nutrients, growth regulating factors and metabolites to all tissues in the body.
Many factors act as key protagonists of bone angiogenesis: VEGF, especially its isoforms VEGF120, 164 and 188, bFGF, TGFβ, HIF are 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 therefore enchondral ossification.

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 course of osteogenesis paves the way for the vascular anatomy: anastomosing vessel net or central artery and vein.

6.1 VEGF in angiogenesis

The development of a new vessel network provides multipotent mesenchymal cells, endothelial progenitor cells and the substrates for any form of regeneration. The sprouting of vessels from surrounding local bone tissue into a defect or regeneration area is considered to be a decisive factor for undisturbed osteogenesis. Quality of bone in traumatology is assessed mostly by its structure and mechanical stability. In the treatment of many other diseases of the skeleton the clinical evaluation of the bony vascularisation is the benchmark for its quality.
The latter is especially disturbed in most patients needing solid and reliable bone regeneration: in clinical routine it is the problem of patients with osteomyelitis, tumour patients after radiation, or patients after trauma in whom local vascularisation is considerably disturbed and not predictable.
For this reason, extensive evaluation of this part of the regeneration process is of great significance as a means of gaining basic information for specific therapy.
Not only the physiological environment of an intact sprouting vessel system, but also its special stimulation by decisive growth and differentiation factors has an enormous impact on the development and maturation of the surrounding bone. The outstanding and field-tested in this context is VEGF. Many publications underline its efficacy in angiogenesis.
The stimulation of bone regeneration by rhVEGF165 was investigated, among many other trials, in a rabbit animal model.
Mandibular critical size defects in 56 animals were treated with VEGF-laden collagen carriers. The significant effects of the stimulation became most obvious during the phase of physiological regression during the regeneration process:
This physiologic process, activated by hyperoxygenation caused by hyperperfusion led to delamination of endothelial cells from the basal membrane and endothelial apoptosis.
The effect of rhVEGF165 as an endothelial cell survival factor led to persisting angiogenesis in the study group, extending beyond day 14. Morphologic and structural effects of angiogenesis on osteogenesis could not be observed until after at least 28 days, because maximum bone regeneration occurs at that time.
In addition to the indirect influence of the increased supply of osteogenic progenitor cells by the developing vascular system, the increased expression of osteogenic growth factors expressed by endothelial cells seemed to play a crucial role in this experimental setting (Kleinheinz et al., 2005).

6.2 Impact of osteoid

In addition to established angiogenic growth stimulators the microenvironment of secretory osteoblasts shows a significant pro-angiogenic effect. The produced extracellular matrix influences demonstrably migration, proliferation and differentiation of endothelial progenitor cells.

Cell culture investigation proved the significant stimulation of endothelial cells to form vessels-like structures.
Post-translationally glycosylated collagen I was unmasked as decisive factor concerning the effective activation of endothelial stimulation via p38/MAPK signalling pathway.
It was demonstrated that the adhesion of endothelial cells to collagen I, one of the major constituents of osteoid, not only supports their growth but represents an essential condition to perform the angiogenic switch.
In cell culture observations the role glycosylated collagen type I became clear:
It induces the expression of molecules like MMP-2 and IL-12. These factors were connected with an increased vessel networking, at the same time an increasing amount of mature bone tissue. The close connection of these processes seems to be linked by the many involved growth factors as well as the variety of progenitors and regenerative cells (Palmieri et al., 2010).

7. Autologous bone tissue engineering

Today approaches to restore the osseous integrity of such primarily non-regenerating lesions rely either on the integration of autologous bone or on the implantation of substitute materials. Autologous bone chips or bars reducing the defect size below the critical threshold enable primary healing processes, but their supply is limited.
Bone tissue engineering subsumes an abundance of approaches to the artificial engineering of osseous structures based on the principle of an extracorporeal linking of cells, matrix and bioactive factors.
As the supply of nutrition and oxygen via diffusion in three-dimensional tissue formations is restricted to an area of 150 µm around the nutritive capillary, resorption and devitalisation in the center of the implant lead to a loss of mechanical stability. A successful approach to bone tissue engineering therefore has to incorporate both osteogenic and vascular development as a combination of osteogenic and angiogenic processes will lead to an improved and accelerated regeneration with simultaneous proliferation of the new complex tissue of these cell types.
A pure autologous combination of all components with an autologous scaffold seems to be of elementary importance for further improved cell behaviour.
Promising approaches concentrate mainly on fibrin as the best possible basis for tissue engineering as fibrin plays an important role in haemostasis and also represents a physiological temporary scaffold for the cell invasion in the following healing process.
Large bone defects or osseous deficiency caused by congenital malformation, trauma, tumour surgery or osseous infection exceeding a critical threshold and showing no signs of spontaneous healing are still a great challenge to reconstructive surgery. These critical size defects (CSDs) are individually determined for every species and vary by age, size and sex (Cima et al., 1991, Glowacki et al., 2004).
Today approaches to restore the osseous integrity of such primarily non-regenerating lesions rely either on the integration of autologous bone or on the implantation of substitute materials. Autologous bone chips or bars reducing the defect size below the critical threshold enable primary healing processes, but their supply is limited. Xenogenous or alloplastic materials seem to be a simple and gentle method with bone regeneration from the

surrounding bone, but malresorption and decreased osteogenic potency of substitute material affect the stability of the defect area.
Besides the repair and reconstruction the in vivo – regeneration of lost tissue using the activation of local regeneration cascades therefore seems to be the most promising method.
Bone tissue engineering subsumes an abundance of approaches to the artificial engineering of osseous structures based on the principle of an extracorporal linking of cells, matrix and bioactive factors. Many approaches to promote the adherence and proliferation of transplanted, osteopotent cells expressing of extracellular matrix proteins (ECM) used xenogenous or alloplastic scaffolds such as corals (Putnam et al., 1996), collagen (Saadeh et al., 2001), PLA/PGA or fibrin glue, but there is no record for the use of autologous scaffolds. Despite the success in some parts of the substitute materials such as the avoidance of donor site morbidity, optimization of the three-dimensional configuration of the transplant and healing processes, infection and non-integration of the implanted materials occur (Frerich et al., 2000).
The potency of the matrix has an enormous impact on the development of the new tissue. This potency should include: a) an optimal scaffold for specific tissue engineering, b) matrix biodegradation with contemporaneous new tissue formation, c) a guaranteed interaction of newly formed tissue with new specific cells forming coherent structures with mechanical function and stability.
As the supply of nutrition and oxygen via diffusion in three-dimensional tissue formations is restricted to an area of 150 µm around the nutritive capillar, resorption and devitalisation in the center of the implant lead to a loss of mechanical stability. A successful approach to bone tissue engineering therefore has to incorporate both osteogenic and vascular development as a combination of osteogenic and angiogenic processes will lead to an improved and accelerated regeneration with simultaneous proliferation of the new complex tissue of these cell types (Pellisier et al., 2003).
A pure autologous combination of all components with an autologous scaffold seems to be of elementary importance for further improved cell behaviour.
Promising approaches concentrate mainly on fibrin as the best possible basis for tissue engineering as fibrin plays an important role in haemostasis and also represents a physiological temporary scaffold for the cell invasion in the following healing process (Nolting et al., not published yet).

8. Conclusion

Chronic infections of the bone, necrosis and drug associated osteomyelitis are diseases that will become of growing dominance in the coming decades. These pathologies result not only from a deficient bone formation or regeneration but are closely related to an impaired vasculature.
To understand and to better evaluate the co-dependence of developing or regenerating bone and its accompanying vasculature it is of vital importance to retrace their serial interface from fetal development to adulthood, from macroscopic anatomy to biomolecular processes.
The future of regenerative approaches to bone healing and regeneration will inevitably combine the field-tested strategies of tissue engineering with modern biomolecular techniques in the scientific environment of stem cells and gene therapy.

Our work showed conclusively the enormous reciprocal impact of angio- and osteogenesis. To stimulate the osteogenesis one clearly has to optimize – or at least: incorporate – the vascular regeneration in the context of regenerative medicine.
Promising approaches for the near future will investigate the potential of stem and regenerative cells: mesenchymal stem cells or cells from the dental pulp have been examined particularly with regard to their ability to differentiate in an angio- or osteogenic direction. The experimental results encourage further experimental work in this field of regenerative medicine to develop an applicable therapeutic strategy of personalized regenerative medicine.

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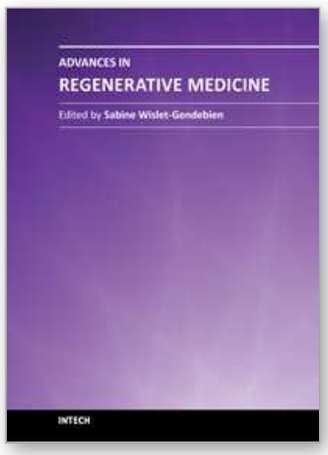

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Even if the origins of regenerative medicine can be found in Greek mythology, as attested by the story of Prometheus, the Greek god whose immortal liver was feasted on day after day by Zeus' eagle; many challenges persist in order to successfully regenerate lost cells, tissues or organs and rebuild all connections and functions. In this book, we will cover a few aspects of regenerative medicine highlighting major advances and remaining challenges in cellular therapy and tissue/organ engineering.

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