

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

6,900

Open access books available

186,000

International authors and editors

200M

Downloads

Our authors are among the

154

Countries delivered to

TOP 1%

most cited scientists

12.2%

Contributors from top 500 universities



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](mailto:book.department@intechopen.com)

Numbers displayed above are based on latest data collected.  
For more information visit [www.intechopen.com](http://www.intechopen.com)



## Vascularization in the Bone Repair

Jian Zhou and Jian Dong\*

*Department of Orthopedic Surgery, Zhongshan Hospital, Fudan University, Shanghai, China*

### 1. Introduction

The repair of critical-size bone defects resulted from severe traumatic injury, infection, surgery for bone cancer or congenital malformation remains a continuous challenge for orthopedists. Currently, autogenous bone grafting is a clinical gold standard for bone repair, and provides excellent osteoconduction, osteoinduction, high-healing rate and absence of immunogenic reaction after surgery. However, autogenous bone grafts are associated with the morbidity of donors, additional surgical procedures for harvest, and limitations in the quantity and available bone size. Bone tissue engineering has become a new and promising alternative approach for the repair of bone defects. Moreover, the clinical application of these advanced technologies in the field of tissue engineering seldom leads to satisfactory results. Increasing evidences have demonstrated that the key factor for the poor repair with tissue-engineered bone is poor vascularization (Nakasa et al. 2005; Kawamura et al. 2006). Bone is a highly vascularized tissue that relies on the supply of essential nutrients and oxygen from blood vessels for maintaining skeletal integrity (Kanczler and Oreffo 2008). Under the circumstance of a well-developed vascular network, the osteoblasts can produce osteoid tissues, differentiate to osteocytes, and form healthy bone. In order to provide sufficient oxygen for survival, osteoblasts must reside within 150-200  $\mu$ m of a capillary lumen and no cells are greater than 0.2 mm from a blood vessel (Kannan et al. 2005). Without the perfusion of blood supply, the osteoblasts in the middle of tissue-engineered constructs will be necrosis due to ineffective transportation of oxygen, nutrients and metabolites (Smith et al. 2004; Rouwkema et al. 2008). Insufficient vascularization can often restrain the formation of new bones and delay the healing of bones. Therefore, vascularization plays a key role in bone regeneration. The rate and range of vascular growth are the determinants of the efficiency and consequence of new bone formation.

### 2. The role of angiogenesis in bone development

Besides providing nutrients and removing waste products, intraosseous vasculature also can accomplish other important functions including bone development and remodeling. Bone formation and development occurs through two distinct processes: intramembraneous and endochondral ossification. The vascularization is the prerequisite of two different processes of ossification (Clarkin et al. 2008). In intramembraneous bone formation, mesenchymal stem cells (MSCs) can be transported through capillaries and differentiate

---

\* Corresponding Author

directly into mature osteoblasts. These osteoblasts then deposit bone matrix and lead to bone formation. On the other hand, during the endochondral ossification, the chondrocytes secrete angiogenic growth factors promoting the invasion of blood vessels, which then bring along a number of highly specialized cells and replace the cartilage mold with bone and bone marrow (Chung et al. 2004). Vasculature also plays a key role in bone formation by the production of growth factors that control the recruitment, proliferation, differentiation and function of various cells including osteoblasts and osteoclasts. These growth factors are secreted by endothelial cells (ECs) (Red-Horse et al. 2007).

During the bone remodeling, the osteoblasts play an important role in the balance of resorption and bone deposition by secreting osteoprotegerin that is an inhibitor of osteoclast activity. However, the mature osteocytes lose the capability to produce this molecule (Marx et al. 2007). Blood vessels transport osteoprogenitor cells for the deposition of new bones (Barou et al. 2002). The invading vasculature, thus, serves as both a reservoir and a conduit for the recruitment of essential cells involved in bone remodeling, and provides critical signals necessary for bone morphogenesis (Brandi et al. 2006).

### 3. Interaction between osteoblasts and endothelial cells

The intercellular signaling between vessel-forming cells and bone-forming cells plays a critical role in bone integrity. The cell-to-cell communication is crucial to coordinate cell behavior, which is necessary for the development and remodeling of bones (Rivron et al. 2008). Several models have been established for studying cellular interactions between osteoblasts (OBs) and ECs in two-dimensional culture dishes (Guillotin et al. 2008 and Grellier et al. 2009), three-dimensional (3D) scaffolds (Choong et al. 2006 and Unger et al. 2007), or 3D spheroids (Wenger et al. 2004 and Stahl et al. 2005). The OBs and ECs can communicate through a couple of mechanisms such as indirect cell contact (Guillotin et al. 2004) through the secretion of diffusible factors with paracrine and autocrine action, and gap junction communication mediating direct cytoplasmic connections between adjacent cells (Villars et al. 2002).

Many diffusible factors released from ECs and OBs that affect the growth and differentiation of both cell types has been identified. Some diffusible factors secreted by ECs include platelet-derived growth factor AB (PDGF-AB), transforming growth factor  $\beta_1$  (TGF- $\beta_1$ ), transforming growth factor  $\beta_2$  (TGF- $\beta_2$ ), fibroblast growth factors-2 (FGF-2), epidermal growth factor (EGF), osteoprotegerin (OPG), and bone morphogenetic protein 2 (BMP-2) (Bouletreau et al. 2002), which can affect the migration and proliferation of OBs and the differentiation of osteoprogenitor cells. In contrast, vascular endothelial growth factor (VEGF) secreted by OBs can promote the proliferation of ECs, and stimulate the differentiation and angiogenesis through the activation of specific receptors (Clarkin et al. 2008 and Clarkin et al. 2008).

Gap junction is another mechanism for direct cell-to-cell communications. Some special membrane domains composed of aqueous intracellular channels provide direct cytoplasmic connections between cells, and allow for the passage of ions or small molecules between adjacent cells, thus ECs and OBs can communicate and exchange information (Dbouk et al. 2009). Several predominant gap junction proteins including Cx43, Cx37 and Cx40 have been identified to express in ECs and OBs (Yeh et al. 2006). Similarly, the communication via Cx43 gap junctions can promote the expression of osteoblastic differentiation markers such as alkaline phosphatase (ALP), osteocalcin (OC) and bone sialoprotein in OBs (Guillotin et al. 2008).

## 4. Strategies for improving vascularization

Several strategies for improving vascularization have been proposed. These strategies include the modification of scaffold design, the delivery of angiogenic factors, cell-based techniques, and microsurgery strategies (Rouwkema et al. 2008; Phelps et al. 2010).

### 4.1 Modification of scaffold design

Biomaterial scaffold, a key component in the bone tissue engineering, serves as a template for cell interactions and the formation of extracellular matrix in bones. The scaffolds should match certain criteria including biocompatibility, biodegradability and mechanical properties similar to the bone repair site. However, the scaffold itself should also be engineered to promote rapid and effective vascularization, and the architecture and design of a scaffold is the key factor for controlling the rate of vascularization after implantation. Currently, the effect of pore size and interpore distance on the scaffolds during the growth of endothelial cells has been evaluated (Narayan and Venkatraman 2008). The growth of endothelial cells can be improved by a smaller pore size (5-20  $\mu\text{m}$ ) and lower interpore distance. However, the growth of blood vessels is more extensive in scaffolds with larger pore size ( $> 250 \mu\text{m}$ ) than those with smaller pore size (Druecke et al. 2004). Other *in vivo* studies have also confirmed that a higher porosity and pore size can result in extensive osteogenesis and sufficient vascularization (Bonfield, 2006), which can be explained by the fact that large pores facilitate vascular ingrowth and osteoblastic cell migration into the scaffold and promote the vascularization and osteogenesis. Porosity also plays an important role in the vascularization of scaffolds. The high porosity allows for the maximum space of vascularization, osteoblast migration and bone deposition (Karageorgiou and Kaplan 2005). In addition, high porosity has a beneficial effect on the diffusion of nutrients and oxygen, transportation and vascularization (Park et al. 2009). The scaffold for bone tissue engineering must possess interconnecting open pores for the maximum potential of vascularization; otherwise, it will be inhibited (Karageorgiou and Kaplan 2005). The interconnected pores facilitate cell migration and vascularization (Jovanovic et al. 2010). This strategy for promoting vascularization still relies on the vessel ingrowth from the host. Limited benefits can be achieved due to the single use. Therefore, it is strongly recommended to combine the scaffold design with other strategies.

### 4.2 Delivery of angiogenic factors

It is well understood that the local and controlled release of growth factors from a tissue-engineered scaffold can effectively enhance the vascularization of engineered tissues (De Laporte et al. 2010; Zhu et al. 2008). Many angiogenic factors such as vascular endothelial growth factor (VEGF) (des Rieux et al. 2011; Anderson et al. 2011), fibroblast growth factor (FGF) (Kim et al. 2010; Zhu et al. 2008), TGF- $\beta$  (Lee et al. 2006) and angiopoietin 1 (Ang1) (Chiu and Radisic 2010) have been used for promoting the vascularization of scaffolds. VEGF has gained considerable attention due to its central role in physiology and neovascularization of endothelial cells. The VEGF diffused from the scaffolds or released as the scaffold degrades can stimulate local vessels to sprout towards the implanted tissue-engineered constructs. Current reports have demonstrated that the controlled release of FGF-1 from alginate microbeads can result in an increase of initial vessel invasion into the collagen scaffolds and a longer persistence of vascular network formation (Moya et al. 2010; Uriel et al. 2006). However, the dosage must be tightly controlled because excessive amounts

of VEGF and FGF can cause high permeability and poor long-term stability (Ozawa et al. 2004; Zisch et al. 2003). Growth factors including TGF- $\beta$  and Ang1 for the stabilization of new vessels are also important because subsequent stabilization of newly-formed vessels is critical for the generation of functional vascular networks within tissue-engineered constructs. TGF- $\beta$  can stimulate the mobilization and recruitment of endothelial cells, and thus accelerating vascularization. Ang 1 plays a key role in regulating vessel homeostasis and stabilization of newly-generated capillaries (Fiedler et al. 2006; Zisch et al. 2003). The neovascularization requires the temporal and spatial expression of multiple angiogenic growth factors, which stimulates different stages of blood-vessel formation to enhance the vascularization of tissue-engineered bones. More and more researchers are investigating the delivery of two sets of factors to mimics under *in vivo* conditions (Tengood et al. 2010; Sun et al. 2011). The combinatorial application of angiogenic factors for stimulating new blood-vessel formation and maturation is highly necessary for the optimal vascularization of tissue-engineered constructs.

#### 4.3 Cell-based techniques

Regardless of the approach adopted to improve vascularization, all of these strategies include endothelial cells. Previous studies have shown that the addition of endothelial cells to tissue cultures can result in the formation of vascular structures *in vitro* and can anastomose to the vessels of the host after implantation (Tremblay et al. 2005; Levenberg et al. 2005). Another approach to accelerate the vascularization of tissue-engineered graft is the co-culture with endothelial cells based on the principle that the transplanted ECs will interact with host ECs and vasculature to establish faster blood supply. The sources of ECs used in the promotion of vascularization in bone tissue engineering included mature ECs, endothelial progenitor cells (EPCs) and MSCs-derived ECs.

Mature ECs can be isolated from a wide variety of sources such as umbilical cords, kidney vasculars, fat tissues and saphenous veins. Previous studies have revealed the 3D pre-vascular network formation when the human umbilical vein endothelial cells are co-cultured with human mesenchymal stem cells in a spheroid co-culture model. After implantation, the pre-vascular network can be developed further and the structures containing lumen can be observed regularly (Rouwkema et al. 2006). The co-culture of rat bone marrow MSCs with kidney vascular ECs on 3D scaffolds exhibits a pre-vascular network-like structure after *in vivo* implantation and results in the increased amount and size of new bone formation when compared with the control group (Sun et al. 2007). These results suggest that mature ECs can efficiently enhance the vascularization of the tissue-engineered grafts. However, the low availability and proliferation capability will severely restrict its large-scale applications (Kim and Von Recum, 2008).

An alternative source of ECs to promote vascularization in tissue engineering is endothelial progenitor cells. The EPCs are enriched in bone marrow, peripheral blood and umbilical cord blood. EPCs have greater proliferation capability than mature ECs (Lin et al. 2000) and can differentiate into ECs *in vitro*, thus contributing to the formation of vascular networks (Rafii and Lyden 2003). Physical and biochemical interactions between EPCs derived from bone marrow and MSCs in a co-culture system *in vitro*. These studies suggest the co-culture of EPCs derived from bone marrow and MSCs can induce endothelial phenotype and angiogenesis without the addition of exogenous growth factors (Aguirre et al. 2010). The co-culture of MSCs and peripheral blood EPCs in Matrigel with 2-3 mm of biphasic calcium



phosphate particles for the analysis of bone formation at 6 weeks after implantation in nude mice has demonstrated that co-implantation of EPCs isolated from peripheral blood can significantly enhance osteogenic differentiation *in vitro* and support bone formation *in vivo* (Fedorovich et al. 2010). The influence of EPCs combined with mesenchymal stem cells on early vascularization and bone healing in critical-size defect *in vivo* has also been evaluated to reveal an improvement of early vascularization in the combinatorial group of EPCs and MSCs. Meanwhile, more bony bridges also can be observed in the combinatorial group between EPCs and MSCs at 8 weeks after implantation. These studies suggest that the combinatorial delivery of MSCs and EPCs can support early vascularization and accelerate bone healing.

Similarly, previous studies have been proved that MSCs can be induced to differentiate into ECs and these ECs have more proliferation potential than the terminally-differentiated ECs (Oswald et al. 2004). The MSCs-derived ECS should be ideal for pre-vascularized bone tissue engineering and the pre-vascularized bone tissue engineering construct can be prepared by a single, easily accessible, bone marrow biopsy. ECs and osteogenic cells derived from bone marrow have been seeded in an apatite-coated poly(lactide-co-glycolide)/hydroxyapatite composite scaffolds and then transplanted into critical-size calvarial defects in immunodeficient mice (Kim et al. 2010). The bone regeneration reveals a significant enhancement due to the addition of ECs derived from bone marrow. Critical-size ulnar defects in the rabbits have also been repaired through vascularized tissue-engineered bone (Zhou et al. 2010). The vascularized tissue-engineered bone is constructed with MSCs and MSC-derived ECs and then co-cultured in porous  $\beta$ -tricalcium phosphate ceramic. The rabbits treated with vascularized tissue-engineered bone exhibit more extensive osteogenesis and better vascularization. Therefore, the ECs derived from bone marrow can be used as a source for pre-vascularized bone tissue engineering with multiple advantages. First, bone marrow aspiration is less invasive. Second, the use of autologous bone marrow cell grafts can avoid immune rejection.

#### 4.4 Microsurgery strategies

Another promising approach for enhancing vascularization in tissue engineering is the hybrid strategy coupled with microsurgery approaches with bone tissue-engineered constructs such as flap fabrication and arteriovenous (AV) loop (Kneser et al. 2006). The vascularization of tissue-engineered grafts basically consists of a two-stage surgical procedure. In the first stage, the scaffolds loaded with cells and/or growth factors are implanted into a site of rich vascularization, usually a muscle or the forearm. Then the capillaries are grown into the scaffold to form a microvascular network in the engineered graft at the initial implantation site after several weeks (Kneser et al. 2006). In the second stage, the tissue-engineered construct with microvascular network is harvested and then re-implanted at the defect site. The microvascular network in the tissue-engineered grafts will anastomose with the host vessels and result in instantaneous perfusion of the entire construct (Kneser et al. 2006). For example, the studies have been conducted the *in situ* implantation of prefabricated tissue-engineered bone flaps and recombinant human bone morphogenetic protein-2 (rhBMP-2) to accomplish the mandible reconstruction (Zhou et al. 2010). The AV-loop model provides a new approach for the fabrication of axially vascularized tissue so that the vascularization of tissue-engineered grafts can be emanated from internal vascular pedicle independent of local conditions. This AV-loop approach has

applied to induce axial vascularization in a bovine cancellous bone matrix (Beier et al. 2011). The micro-CT scans and histomorphometry have showed a significant increase of axial vascularization in bovine cancellous bone matrix constructs, and immunohistochemistry has confirmed the endothelial linking of newly-formed vessels. Similarly, a vascularized tissue-engineered bone graft composed of implanted MSCs and a vascular bundle into the xenogeneic deproteinized cancellous bone (XDCB) scaffold has also constructed (Zhao et al. 2011). The histological and biomechanical examinations have showed that the combination of MSCs and a vascular bundle implantation can result in the promotion of vascularization and osteogenesis in the XDCB graft, and the improvement of new bone formation and mechanical properties during the repair of radius defects. These studies suggest that the vascular bundle implantation is a promising strategy for promoting vascularization in the tissue-engineered grafts.

## 5. Conclusion and future directions

Insufficient vascularization remains one of the major problems in bone tissue engineering. The critical factor for the limitation of clinical application of tissue-engineered bone is poor vascularization. Multiple approaches such as scaffold design, angiogenic factor delivery, cell-based technique and microsurgery strategy have been explored to promote the vascularization in the field of tissue engineering. These approaches may generate capillary-like structures within the tissue-engineered graft, however, the best method for successful application *in vivo* is still uncertain because there is no convincing evidence. Therefore, the integration of several strategies for enhancing the repair of bone defects is highly desired in the future.

## 6. References

- Anderson, S.M.; Siegman, S.N.; Segura, T. (2011) The effect of vascular endothelial growth factor (VEGF) presentation within fibrin matrices on endothelial cell branching. *Biomaterials*, 32 (30): 7432-7443.
- Aguirre, A.; Planell, J.A.; Engel, E. (2010) Dynamics of bone marrow-derived endothelial progenitor cell/mesenchymal stem cell interaction in co-culture and its implications in angiogenesis. *Biochem Bioph Res Co*, 400(2): 284-291.
- Barou, O.; Mekraldi, S.; Vico, L.; Boivin, G.; Alexandre, C.; Lafage-Proust, M.H. (2002) Relationships between trabecular bone remodeling and bone vascularization: A quantitative study. *Bone*, 30(4): 604-612.
- Beier, J. P.; Hess, A.; Loew, J.; Heinrich, J.; Boos, A. M.; Arkudas, A.; Polykandriotis, E.; Bleiziffer, O.; Horch, R. E.; Kneser, U. (2011) De novo Generation of an Axially Vascularized Processed Bovine Cancellous-Bone Substitute in the Sheep Arteriovenous-Loop Model. *Eur Surg Res*, 46 (3): 148-155.
- Bonfield, W. (2006) Designing porous scaffolds for tissue engineering. *Philos Trans A: Math Phys Eng Sci*, 364: 227-232.
- Brandi, M.L.; Collin-Osdoby, P. (2006) Vascular biology and the skeleton. *J Bone Miner Res*, 21(2): 183-192.
- Bouletreau, P.J.; Warren, S.M.; Spector, J.A.; Peled, Z.M; Gerrets, R.P.; Greenwald, J.A.; Longaker, M.T. (2002) Hypoxia and VEGF up-regulate BMP-2 mRNA and protein

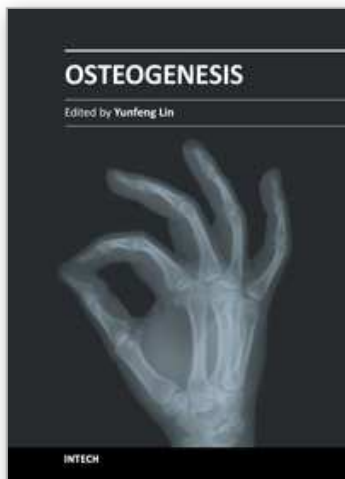
- expression in microvascular endothelial cells: implications for fracture healing. *Plast Reconstr Surg*, 109(7): 2384–2397.
- Chiu, L.L.Y.; Radisic, M. (2010) Scaffolds with covalently immobilized VEGF and Angiopoietin-1 for vascularization of engineered tissues. *Biomaterials*, 31 (2): 226–241.
- Choong, C.S.; Hutmacher, D.W.; Triffitt J.T. (2006) Co-culture of bone marrow fibroblasts and endothelial cells on modified polycaprolactone substrates for enhanced potentials in bone tissue engineering. *Tissue Eng*. 12(9): 2521–2531.
- Chung, U.I.; Kawaguchi, H.; Takato, T.; Nakamura, K. (2004) Distinct osteogenic mechanisms of bones of distinct origins. *J Orthop Sci*, 9 (4): 410–414.
- Clarkin, C.E.; Emery, R.J.; Pitsillides, A.A.; Wheeler-Jones, C.P.D. (2008) Evaluation of VEGF-Mediated signaling in primary human cells reveals a paracrine action for VEGF in osteoblast-mediated crosstalk to endothelial cells. *J Cell Physiol*, 214 (2): 537–544.
- Clarkin, C.E.; Garonna, E.; Pitsillides, A.A.; Wheeler-Jones, C.P.D. (2008) Heterotypic contact reveals a COX-2-mediated suppression of osteoblast differentiation by endothelial cells: A negative modulatory role for prostanooids in VEGF-mediated cell: cell communication? *Exp Cell Res*, 314(17): 3152–3161.
- Dbouk, H.A.; Mroue, R.M.; El-Sabban, M.E.; Talhouk, R.S. (2009) Connexins: a myriad of functions extending beyond assembly of gap junction channels. *Cell Commun Signal*, 7, 4.
- De Laporte, L.; des Rieux, A.; Tuinstra, H.M.; Zelivyanskaya, M.L.; De Clerck, N.M.; Postnov, A.A.; Preat, V.; Shea, L.D. (2011) Vascular endothelial growth factor and fibroblast growth factor 2 delivery from spinal cord bridges to enhance angiogenesis following injury. *J Biomed Mater Res A*, 98A (3): 372–382.
- Des Rieux, A.; Ucar, B.; Mupendwa, B.P.K.; Colau, D.; Feron, O.; Carmeliet, P.; Preat, V. (2011) 3D systems delivering VEGF to promote angiogenesis for tissue engineering. *J Control Release*, 150(3): 272–278.
- Druecke, D.; Langer, S.; Lamme, E.; Pieper, J.; Ugarkovic, M.; Steinau, H.U.; Homann, H.H. (2004) Neovascularization of poly(ether ester) blockcopolymer scaffolds in vivo: long-term investigations using intravital fluorescent microscopy. *J Biomed Mater Res A*, 68A (1): 10–18.
- Fedorovich, N.E.; Haverslag, R.T.; Dhert, W.J.A.; Alblas, J. (2010) The Role of Endothelial Progenitor Cells in Prevascularized Bone Tissue Engineering: Development of Heterogeneous Constructs. *Tissue Eng A*, 16 (7): 2355–2367.
- Fiedler, U.; Augustin, H.G. (2006) Angiopoietins: a link between angiogenesis and inflammation. *Trends Immunol*, 27 (12): 552–558.
- Grellier, M.; Ferreira-Tojais, N. Bourget, C.; Bareille, R.; Guillemot, F.; Amedee, J. (2009) Role of vascular endothelial growth factor in the communication between human osteoprogenitors and endothelial cells. *J Cell Biochem*, 106(3): 390–398.
- Guillotin, B.; Bareille, R.; Bourget, C.; Bordenave, L.; Amedee, J. (2008) Interaction between human umbilical vein endothelial cells and human osteoprogenitors triggers pleiotropic effect that may support osteoblastic function. *Bone* 42(6): 1080–1091.
- Guillotin, B.; Bourget, C.; Remy-Zolghadri, M.; Bareille, R.; Fernandez, P.; Conrad, V.; Amedee-Vilamitjana, J. (2004) Human primary endothelial cells stimulate human osteoprogenitor cell differentiation *Cell Physiol Biochem*, 14(4–6): 325–332.



- Jovanovic, D.; Engels, G.E.; Plantinga, J.A.; Bruinsma, M.; van Oeveren, W.; Schouten, A.J.; van Luyn, M.J.A.; Harmsen, M.C. (2010) Novel polyurethanes with interconnected porous structure induce in vivo tissue remodeling and accompanied vascularization. *J Biomed Mater Res A*, 95A (1): 198-208.
- Kanczler, J.M. & Oreffo, R.O. (2008). Osteogenesis and angiogenesis: the potential for engineering bone. *Eur Cell Mater*, 15: 100-114.
- Kannan, R.Y.; Salacinski, H.J.; Sales, K.; Butler, P. & Seifalian, A.M. (2005). The roles of tissue engineering and vascularisation in the development of micro-vascular networks: a review. *Biomaterials*, 26 (14): 1857-1875.
- Karageorgiou, V.; Kaplan, D. (2005) Porosity of 3D biomaterial scaffolds and osteogenesis. *Biomaterials*, 26(27): 5474-5491.
- Kawamura, K.; Yajima, H.; Ohgushi, H.; Tomita, Y.; Kobata, Y., Shigematsu, K. & Takakura, Y. (2006). Experimental study of vascularized tissue-engineered bone grafts. *Plastic and Reconstructive Surgery*, 117 (5): 1471-1479.
- Kneser, U.; Polykandriotis, E.; Ohnolz, J.; Heidner, K.; Grabinger, L.; Euler, S.; Amann, K.U.; Hess, A.; Brune, K.; Greil, P.; Sturzl, M.; Horch, R.E. (2006) Engineering of vascularized transplantable bone tissues: induction of axial vascularization in an osteoconductive matrix using an arteriovenous loop. *Tissue Eng*, 12 (7), 1721-1731.
- Kneser, U.; Schaefer, D.J.; Polykandriotis, E.; Horch, R.E. (2006) Tissue engineering of bone: the reconstructive surgeon's point of view. *Cell. Mol. Med*, 10 (1): 7-11.
- Kim, M.S.; Bhang, S.H.; Yang, H.S.; Rim, N.G.; Jun, I.; Kim, S.I.; Kim, B.S.; Shin, H. (2010) Development of Functional Fibrous Matrices for the Controlled Release of Basic Fibroblast Growth Factor to Improve Therapeutic Angiogenesis. *Tissue Eng A*, 16 (10): 2999-3010.
- Kim, S.S.; Park, M.S.; Cho, S.W.; Kang, S.W.; Ahn, K.M.; Lee, J.H.; Kim, B.S. (2010) Enhanced bone formation by marrow-derived endothelial and osteogenic cell transplantation. *J Biomed Mater Res A*, 92A (1): 246-253.
- Kim, S.; Von Recum, H. (2008) Endothelial stem cells and precursors for tissue engineering: Cell source, differentiation, selection, and application. *Tissue Eng B*, 14 (1): 133-147.
- Lee, J.Y.; Kim, K.H.; Shin, S.Y.; Rhyu, I.C.; Lee, Y.M.; Park, Y.J.; Chung, C.P.; Lee, S.J. (2006) Enhanced bone formation by transforming growth factor-beta 1-releasing collagen/chitosan microgranules. *J Biomed Mater Res A*, 76A (3): 530-539.
- Levenberg, S.; Rouwkema, J.; Macdonald, M.; Garfein, E.S.; Kohane, D.S.; Darland, D.C.; Marini, R.; van Blitterswijk, C.A.; Mulligan, R.C.; D'Amore, P.A.; Langer, R. (2005) Engineering vascularized skeletal muscle tissue. *Nat Biotechnol* 23 (7): 879-884.
- Lin, Y.; Weisdorf, D.J.; Solovey, A.; Hebbel, R.P. (2000) Origins of circulating endothelial cells and endothelial outgrowth from blood. *J Clin Invest*, 105 (1): 71-77.
- Marx, R.E. (2007) Bone and bone graft healing. *Oral Maxillofac Surg Clin. North Am.* 19(4): 455-466.
- Moya, M.L.; Garfinkel, M.R.; Liu, X.; Lucas, S.; Opara, E.C.; Greisler, H.P.; Brey, E.M. (2010) Fibroblast growth factor-1 (FGF-1) loaded microbeads enhance local capillary neovascularization. *J Surg Res*, 160(2): 208-212.
- Nakasa, T.; Ishida, O.; Sunagawa, T.; Nakamae, A.; Yasunaga, Y.; Agung, M. & Ochi, M. (2005). Prefabrication of vascularized bone graft using a combination of fibroblast growth factor-2 and vascular bundle implantation into a novel interconnected

- porous calcium hydroxyapatite ceramic. *Journal of Biomedical Materials Research Part A*, 75A (2): 350-355.
- Narayan, D.; Venkatraman, S. S. (2008) Effect of pore size and interpore distance on endothelial cell growth on polymers. *J Biomed Mater Res A*, 87A (3): 710-718.
- Oswald, J.; Boxberger, S.; Jorgensen, B.; Feldmann, S.; Ehninger, G.; Bornhauser, M.; Werner, C. (2004) Mesenchymal stem cells can be differentiated into endothelial cells in vitro. *Stem Cells*, 22 (3): 377-384.
- Ozawa, C.R.; Banfi, A.; Glazer, N.L.; Thurston, G.; Springer, M.L.; Kraft, P.E.; McDonald, D.M.; Blau, H.M. (2004) Microenvironmental VEGF concentration, not total dose, determines a threshold between normal and aberrant angiogenesis. *J Clin Invest*, 113 (4): 516-527.
- Park, S.; Kim, G.; Jeon, Y.C.; Koh, Y.; Kim, W. (2009) 3D polycaprolactone scaffolds with controlled pore structure using a rapid prototyping system. *J Mater Sci-Mater M*, 20 (1): 229-234.
- Phelps EA, García AJ. (2010) Engineering more than a cell: vascularization strategies in tissue engineering. *Current Opinion in Biotechnology*, 21: 704-709.
- Rafii, S.; Lyden, D. (2003) Therapeutic stem and progenitor cell transplantation for organ vascularization and regeneration. *Nat Med*, 9 (6): 702-712.
- Red-Horse, K.; Crawford, Y.; Shojaei, F.; Ferrara, N. (2007) Endothelium-microenvironment interactions in the developing embryo and in the adult. *Dev Cell*, 12 (2): 181-194.
- Rivron, N.C.; Liu, J.; Rouwkema, J.; de Boer, J.; van Blitterswijk, C.A. (2008) Engineering vascularised tissues in vitro. *Eur Cell Mater*, 15: 27-40.
- Rouwkema, J.; De Boer, J.; Van Blitterswijk, C.A. (2006) Endothelial cells assemble into a 3-dimensional prevascular network in a bone tissue engineering construct. *Tissue Eng*, 12 (9): 2685-2693.
- Rouwkema J, Rivron NC, van Blitterswijk CA. (2008) Vascularization in tissue engineering. *Trends in Biotechnology*, 26(8): 434-441.
- Seebach, C.; Henrich, D.; Kahling, C.; Wilhelm, K.; Tami, A.E.; Alini, M.; Marzi, I. (2010) Endothelial Progenitor Cells and Mesenchymal Stem Cells Seeded onto b-TCP Granules Enhance Early Vascularization and Bone Healing in a Critical-Sized Bone Defect in Rats. *Tissue Eng A*, 16 (6): 1961-1970.
- Smith, M.K.; Peters, M.C.; Richardson, T.P.; Garbern, J.C. & Mooney, D.J. (2004) Locally enhanced angiogenesis promotes transplanted cell survival. *Tissue Engineering*, 10(1-2): 63-71.
- Stahl, A.; Wu, X.; Wenger, A.; Klagsburn, M.; Kurschat, P. (2005) Endothelial progenitor cell sprouting in spheroid cultures is resistant to inhibition by osteoblasts: a model for bone replacement grafts. *FEBS Lett*, 579(24): 5338-5342.
- Sun, H.C.; Qu, Z.; Guo, Y.; Zang, G.X.; Yang, B. (2007) In vitro and in vivo effects of rat kidney vascular endothelial cells on osteogenesis of rat bone marrow mesenchymal stem cells growing on polylactide-glycolic acid (PLGA) scaffolds. *Biomed. Eng. Online*, 6: 41.
- Sun, G.; Shen, Y.I.; Kusuma, S.; Fox-Talbot, K.; Steenbergen, C.J.; Gerecht, S. (2011) Functional neovascularization of biodegradable dextran hydrogels with multiple angiogenic growth factors. *Biomaterials*, 32(1): 95-106.

- Tengood, J.E.; Kovach, K.M.; Vescovi, P.E.; Russell, A.J.; Little, S.R. (2010) Sequential delivery of vascular endothelial growth factor and sphingosine 1-phosphate for angiogenesis. *Biomaterials*, 31 (30): 7805-7812.
- Tremblay, P.L.; Hudon, V.; Berthod, F.; Germain, L.; Auger, F.A. (2005) Inosculation of tissue-engineered capillaries with the host's vasculature in a reconstructed skin transplanted on mice. *Am J Transplant*, 5 (5): 1002-1010.
- Unger, R.E.; Sartoris, A.; Peters, K.; Motta, A.; Migliaresi, C.; Kunkel, M.; Bulnheim, U.; Rychly, J.; Kirkpatrick, C.J. (2007) Tissue-like self-assembly in cocultures of endothelial cells and osteoblasts and the formation of microcapillary-like structures on three-dimensional porous biomaterials *Biomaterials*, 28(27): 3965-3976.
- Uriel, S.; Brey, E.M.; Greisler, H.P. (2006) Sustained low levels of fibroblast growth factor-1 promote persistent microvascular network formation. *Am J Surg*, 192(5): 604-609.
- Villars, F.; Guillotin, B.; Amedee, T.; Dutoya, S.; Bordenave, L.; Bareille, R.; Amedee, J. (2002) Effect of HUVEC on human osteoprogenitor cell differentiation needs heterotypic gap junction communication *Am J Physiol Cell Physiol*. 282(4): C775-C785.
- Wenger, A.; Stahl, A.; Weber, H.; Finkenzeller, G.; Augustin, H.G.; Stark, G.B.; Kneser, U. (2004) Modulation of in vitro angiogenesis in a three dimensional spheroidal coculture model for bone tissue engineering. *Tissue eng*, 10(9-10): 1536-1547.
- Yeh, H.I.; Lee, P.Y.; Su, C.H.; Tian, T.Y.; Ko, Y.S.; Tsai, C.H. (2006) Reduced expression of endothelial connexins 43 and 37 in hypertensive rats is rectified after 7-day carvedilol treatment. *Am J Hypertens*, 19(2): 129-135.
- Zhu, X.H.; Tabata, Y.; Wang, C.H.; Tong, Y.W. (2008) Delivery of Basic Fibroblast Growth Factor from Gelatin Microsphere Scaffold for the Growth of Human Umbilical Vein Endothelial Cells. *Tissue Eng A*, 14(12): 1939-1947.
- Zhao, M.D.; Zhou, J.; Li, X.L.; Fang, T.L.; Dai, W.D.; Yin, W.P.; Dong, J. (2011) Repair of Bone Defect with Vascularized Tissue Engineered Bone Graft Seeded with Mesenchymal Stem Cells in Rabbits. *Microsurgery*, 31 (2): 130-137.
- Zhou, J.; Lin, H.; Fang, T.L.; Li, X.L.; Dai, W.D.; Uemura, T.; Dong, J. (2010) The repair of large segmental bone defects in the rabbit with vascularized tissue engineered bone. *Biomaterials*, 31 (6): 1171-1179.
- Zhou, M.; Peng, X.; Mao, C.; Xu, F.; Hu, M.; Yu, G.Y. (2010) Primate mandibular reconstruction with prefabricated, vascularized tissue-engineered bone flaps and recombinant human bone morphogenetic protein-2 implanted in situ. *Biomaterials*, 31 (18): 4935-4943.
- Zisch, A.H.; Lutolf, M.P.; Hubbell, J.A. (2003) Biopolymeric delivery matrices for angiogenic growth factors. *Cardiovasc. Pathol*. 12 (6): 295-310.



## **Osteogenesis**

Edited by Prof. Yunfeng Lin

ISBN 978-953-51-0030-0

Hard cover, 296 pages

**Publisher** InTech

**Published online** 10, February, 2012

**Published in print edition** February, 2012

This book provides an in-depth overview of current knowledge about Osteogenesis, including molecular mechanisms, transcriptional regulators, scaffolds, cell biology, mechanical stimuli, vascularization and osteogenesis related diseases. Hopefully, the publication of this book will help researchers in this field to decide where to focus their future efforts, and provide an overview for surgeons and clinicians who wish to be directed in the developments related to this fascinating subject.

### **How to reference**

In order to correctly reference this scholarly work, feel free to copy and paste the following:

Jian Zhou and Jian Dong (2012). Vascularization in the Bone Repair, Osteogenesis, Prof. Yunfeng Lin (Ed.), ISBN: 978-953-51-0030-0, InTech, Available from:  
<http://www.intechopen.com/books/osteogenesis/vascularization-in-the-bone-repair>

**INTECH**  
open science | open minds

### **InTech Europe**

University Campus STeP Ri  
Slavka Krautzeka 83/A  
51000 Rijeka, Croatia  
Phone: +385 (51) 770 447  
Fax: +385 (51) 686 166  
[www.intechopen.com](http://www.intechopen.com)

### **InTech China**

Unit 405, Office Block, Hotel Equatorial Shanghai  
No.65, Yan An Road (West), Shanghai, 200040, China  
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元  
Phone: +86-21-62489820  
Fax: +86-21-62489821

© 2012 The Author(s). Licensee IntechOpen. This is an open access article distributed under the terms of the [Creative Commons Attribution 3.0 License](https://creativecommons.org/licenses/by/3.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

IntechOpen

IntechOpen