Institute of Experimental Morphology, Pathology and Anthropology with Museum
Bulgarian Anatomical Society

Acta Morphologica et Anthropologica, 33 (1-2)
Sofia ● 2026

Angiogenesis in Bone Healing Stimulated by Simvastatin: A Review

Nadezhda Kadreva[1], Nadya Penkova[1], Petar Hrischev[2]*

[1] Department of Anatomy, Histology and Embryology, Faculty of Medicine, Medical University – Plovdiv
[2] Department of Physiology, Faculty of Medicine, Medical University – Plovdiv

*Corresponding author e-mail: nadezhda.kadreva@mu-plovdiv.bg

Simvastatin, a widely used HMG-CoA reductase inhibitor, has pleiotropic effects on bone tissue beyond lipid lowering. It promotes osteoblast proliferation and differentiation through upregulation of BMP-2 and activation of Ras/Smad/Erk signaling, while inhibiting osteoclast activity via MAPK/AKT and Src modulation. Simultaneously, simvastatin enhances angiogenesis by increasing VEGF and VEGFR-2 expression, stimulating endothelial proliferation, migration, tube formation, and pro-angiogenic cytokine release from macrophages. The combined osteogenic and angiogenic effects facilitate coordinated bone regeneration and accelerate healing of skeletal defects. These findings highlight the potential of simvastatin as a pharmacological agent for promoting bone repair and provide a mechanistic framework for developing therapeutic strategies targeting healing of bone defects.

Key words: Simvastatin, Osteogenesis, Angiogenesis, Bone Regeneration, VEGF, BMP-2

Angiogenesis in bone

One of the fundamental principles underlying each layer of tissue regeneration is the physiological mechanism that supplies oxygen and nutrients to the tissue and facilitates the removal of waste products during the process of tissue repair or regeneration. Angiogenesis is a complex process that is driven by a number of mechanisms. It is a process that is subject to a number of factors, including factors that are extrinsic to the organism and factors that are intrinsic to the organism. These factors can be categorised into two groups: promoting the process of angiogenesis and promoting the formation of new vessels (vasculogenesis). In the study by Risau et al., the author presents a pioneering analysis of the formation of the vascular systems during the process of embryogenesis as a dual-stage process [19]. In the initial phase, the formation of angioblastic cells occurs, which subsequently differentiate

into endodermal cells. The second stage is the formation of primitive vascular structures from the angiogenic cells in the vicinity of the site of origin of the tissue. As demonstrated in the preceding section, the process that leads to vascularisation is characterised by the formation of new blood vessels. [20-21]. In adults, the process of vasculogenesis is initiated during the process of wound healing or tumour growth. As stated by Rouwkema et al., endothelium-specific progenitor cells (EPCs) located on the vessel wall undergo a process of differentiation, resulting in the formation of primitive endothelial cells (PEC), which subsequently develop into primitive vascular smooth muscle cells (PVSMC) [22]. Angiogenesis, from another perspective, is the formation of blood vessels in a subject of advanced age. This process is distinct from the formation of blood vessels in a subject of younger age, which are typically characterised by the presence of pathological factors. The process of angiogenesis is driven by various intrinsic and extrinsic factors, including vascular endothelial growth factor (VEGF), an extracellular matrix-derived growth factor, and additional growth factors, as well as cytokines, pre-fibroblast cells, stellate cells, and platelet-derived factors. These factors, in turn, regulate the migration of cells and the formation of new blood vessels, contributing to the development of neoplastic lesions [2, 3, 4]. Specific cell adhesion molecules and transcription factors, as well as mechanical forces and vascular regression and remodeling are involved in the subsequent events of endothelial cell differentiation, apoptosis, and angiogenesis [5, 18].

Alternatively, new blood vessels are formed through a process called vasculogenesis, without a pre-existing vascular component. Vasculogenesis is initiated by local differentiation of endothelial progenitor cells, or alternatively, by the involvement of circulating endothelial progenitor cells (EPCs) in the process [28]. Angiogenesis is maintained and regulated by a variety of growth factors, essentialy vascular endothelial growth factor (VEGF) group, which are products of inflammatory cells and stromal cells to induce blood vessel in-growth. It is the primary process of tissue growth and one of the first processes to occur in tissue repair [9].

Angiogenesis during osteogenesis

There is a indissoluble bond between osteogenesis and angiogenesis during the process of bone repair. Neovascularization within the bone defects not only provides nutrients, oxygen, calcium and phosphate but also transports mesenchymal stem cells to facilitate bone regeneration [27]. Therefore, the development of biomaterials and pharmacological substances coupling the enhancement of osteogenesis and angiogenesis has attracted increasing attention in recent years.

Angiogenesis plays a critical role in bone development and regeneration and is closely coordinated with osteogenesis through complex interactions within the bone microenvironment [11]. During skeletal development and fracture healing, the formation of new blood vessels provides both structural support and biochemical signals that facilitate the recruitment, proliferation, and differentiation of osteoblast precursors [10]. Among the principal molecular regulators of this process is vascular endothelial growth factor (VEGF), which promotes endothelial cell proliferation, migration, and

vascular sprouting, while also influencing the osteogenic differentiation of mesenchymal stem cells [29]. Hypoxia-inducible factors, particularly HIF-1α, represent another key regulatory mechanism by enhancing VEGF expression under hypoxic conditions commonly present in developing or healing bone tissue [13]. In addition, endothelial cells actively contribute to bone formation by releasing angiocrine signals, including bone morphogenetic proteins (BMPs), which stimulate osteoblast differentiation and extracellular matrix deposition [16]. This bidirectional communication between vascular endothelial cells and osteogenic cells establishes a coordinated signaling network that ensures the synchronization of vascular development and bone formation during skeletal growth, repair, and regeneration [10].

Angiogenesis and osteogenesis provided by Simvastatin

In recent years, increasing attention has been directed toward the biological effects of statins beyond their well-established role in lowering serum cholesterol levels. Among these additional effects, their potential influence on bone metabolism has attracted particular interest. Early work by Gregory R. Mundy and colleagues demonstrated that statins stimulate bone formation in vivo in rodent models and increase the volume of newly formed bone in cultures derived from mouse calvaria. Subsequent studies have further confirmed the beneficial effects of statins on bone tissue in both in vitro and in vivo experimental settings [15]. The present study investigates the biphasic, dose-dependent effect of HMG-CoA reductase inhibition on angiogenesis. The findings demonstrate that this effect is lipid independent and associated with alterations in endothelial apoptosis and vascular endothelial growth factor signalling. Statins have been observed to exert proangiogenic effects at low therapeutic concentrations, while at higher concentrations, they demonstrate angiostatic properties, which can be reversed by geranylgeranyl pyrophosphate. At clinically relevant doses, statins have been observed to modulate angiogenesis in humans via effects on geranylated proteins [29].

Specifically, investigations examining the effects of simvastatin on osteoblastic differentiation have shown that this statin exerts an anabolic effect on bone, primarily through the promotion of osteoblast differentiation [32].

Statins, inhibitors of the competitive 3-hydroxy-3-methyl coenzyme A (HMG-CoA) reductase, have been widely used to treat hyperlipidemia and hypercholesterolemia. It has been demonstrated that statins, including simvastatin, can induce osteoblastic differentiation of bone marrow mesenchymal stem cells and improve the osteogenesis of human or animal osteoblastic cell lines [11-13, 23-26]. Moreover, statins have been demonstrated to enhance angiogenesis through up-regulation of gene expression of vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (FGF-2) [33]. Statins stimulate VEGF expression in osteoblasts via reduced protein prenylation and the phosphatidylinositide-3 kinase pathway, promoting osteoblastic differentiation [13]. Both VEGF and FGF-2 have been shown to induce BMP-2 expression and stimulate osteoblast differentiation indirectly. Same study reported that locally applied simvastatin promoted critical-sized calvarial defects healing by recruitment of autogenous osteogenic stem cells and endothelial progenitor cells [7].

Statins act on the mevalonate pathway in osteoblasts and enhance the expression of the bone morphogenic protein-2 (BMP-2), which is an important growth factor for osteoblast differentiation. It is believed that BMP-2 upregulation mediates the bone forming effect of statins. Additionally, statins act on the same pathway upstream of the bisphosphonates in osteoclasts via the inhibition of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase, leading to decreased protein prenylation which is essential for a normal osteoclast function [1].

Over the last decade, increasing attention has been directed toward the local application of simvastatin due to its pleiotropic effects that extend beyond lipid lowering. Locally administered simvastatin demonstrates pronounced anti-inflammatory, osteogenic, angiogenic, and regenerative properties, making it particularly attractive for applications in tissue engineering, periodontology, bone regeneration, and wound healing. One of the major advantages of local simvastatin delivery over systemic administration is the ability to achieve high drug concentrations directly at the target tissue while minimizing systemic exposure and associated adverse effects. Systemic simvastatin therapy is associated with dose-dependent risks such as hepatotoxicity, elevated liver enzymes, myopathy, and rhabdomyolysis, primarily due to extensive hepatic metabolism and systemic circulation. In contrast, local administration substantially reduces these risks by limiting systemic absorption. Experimental and clinical studies have demonstrated that locally applied simvastatin stimulates bone formation through upregulation of bone morphogenetic protein-2 (BMP-2), vascular endothelial growth factor (VEGF), and osteoblastic differentiation markers. Additionally, simvastatin suppresses inflammatory cytokines and promotes angiogenesis, thereby accelerating tissue repair and regeneration. These effects are particularly beneficial in periodontal defects, extraction sockets, implant osseointegration, and bone healing models. Another important advantage of local delivery is the possibility of controlled and sustained release using biomaterials such as hydrogels, scaffolds, nanoparticles, or collagen membranes. Such systems improve drug retention at the application site and enhance therapeutic efficacy while avoiding first-pass hepatic metabolism [30-33].

Therefore, local simvastatin administration represents a promising therapeutic strategy that preserves the beneficial regenerative and anti-inflammatory effects of the drug while minimizing the systemic adverse reactions commonly associated with oral statin therapy.

It has been demonstrated by Yamashita M et al. that simvastatin exerts its inhibitory effect on the mRNA expression of the key markers for osteoclastic differentiation, including TRAP, RANK and cathepsin-K. A novel inhibitory mechanism of simvastatin on the expression of osteoclast differentiation markers was demonstrated in two different cell lines. The first cell line was osteoclast-like MLC 6 cells, and the second was human osteoclast precursor cells. Both of these cell lines expressed BMP receptors and Smad signalling machinery. The study demonstrated that simvastatin activated MAPK/AKT pathways and inactivated Src phosphorylation in human osteoclasts differentiated by M-CSF and RANKL treatments. Bioassays demonstrated that the inhibition of TRAP and RANK expression by simvastatin was reversed by MAPK/AKT inhibition, whereas Src inhibitor enhanced simvastatin-induced suppression of osteoclast markers [30].

Simvastatin can promote osteoblast viability and differentiation via membrane-bound Ras/Smad/Erk/BMP-2 pathway. In study by Ho et al. Simvastatin promotes osteoblast viability and differentiation via Ras/Smad/Erk/bone morphogenic protein (BMP)-2 signaling pathway. The results of this study demonstrate that simvastatin stimulation promotes membrane localization and GTP loading of Ras, indicating activation of small GTPase signaling molecules involved in Ras regulation [8]. Simvastatin treatment was also associated with increased levels of membrane-bound active phospho-RasGRF1, suggesting that Ras activation may contribute to the regulation of BMP-2 transcription. Furthermore, the activation of Ras signaling was supported by the observed up-regulation of downstream signaling molecules, including phosphorylated Smad (phospho-Smad) and phosphorylated extracellular signal-regulated kinase (phospho-Erk). Together, these findings indicate that simvastatin activates Ras-dependent signaling pathways that may regulate BMP-2 expression and promote osteoblastic activity (**Fig.1**) [6].

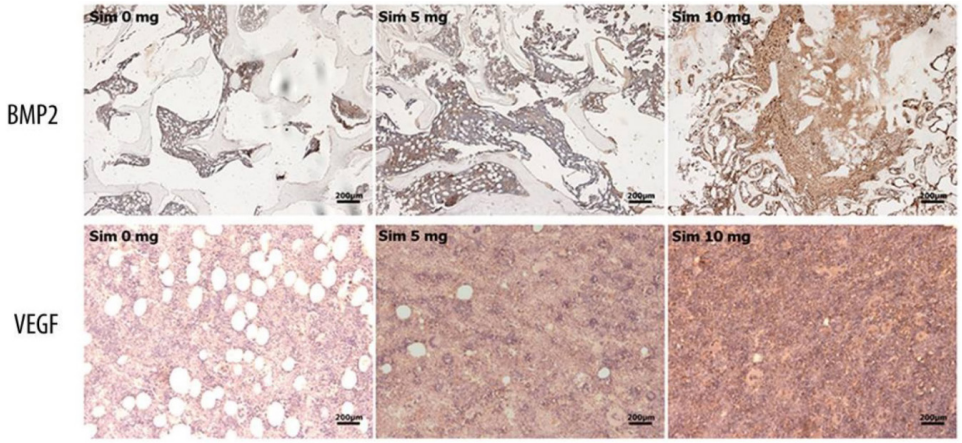

Fig. 1. Immunohistochemistry for BMP-2 and VEGF. Increased BMP-2 and VEGF expression was observed in the simvastatin-treated groups compared with the control group [28].

Simvastatin has been shown to support BMP-induced osteoblast differentiation by antagonizing the cytokine-to-MAPK signaling pathway while simultaneously enhancing BMP–Smad signaling. These mechanisms suggest that simvastatin exerts dual biological functions in bone metabolism, including anti-resorptive effects on osteoclasts as well as stimulatory and anabolic effects on osteoblasts. Consequently, statins with a high affinity for bone tissue may represent promising therapeutic agents for the prevention or treatment of osteoporosis associated with inflammatory cytokines [31].

Recent research has elucidated that simvastatin modulates angiogenic processes that are intrinsically linked to bone regeneration, extending its pleiotropic effects beyond lipid lowering to the vascular compartment of bone healing. Local administration of simvastatin has been shown to significantly enhance neovascularization in osteoporotic

bone, as evidenced by increases in vascular number, volume, and expression of key angiogenic markers such as vascular endothelial growth factor (VEGF) and its receptor VEGFR2 in ovariectomized rat models, concomitant with improvements in bone formation and implant fixation [28]. Moreover, simvastatin's influence on the immune microenvironment has been shown to promote proregenerative cytokine expression, including VEGF, in macrophages, thereby enhancing angiogenic gene expression in endothelial cells and suggesting an immunomodulatory mechanism that supports coordinated angiogenesis and osteogenesis [17]. These proangiogenic effects are consistent with observations in nonskeletal tissues, where simvastatin increases endothelial proliferation and tube formation through activation of signaling pathways such as VEGFR2/Akt/endothelial nitric oxide synthase [14]. Collectively, these findings support a model wherein simvastatin facilitates bone regeneration not only by stimulating osteoblastic differentiation but also by enhancing vascular ingrowth through upregulation of angiogenic signals, thus reinforcing the functional coupling between angiogenesis and osteogenesis that is critical for effective skeletal repair (**Fig.2**).

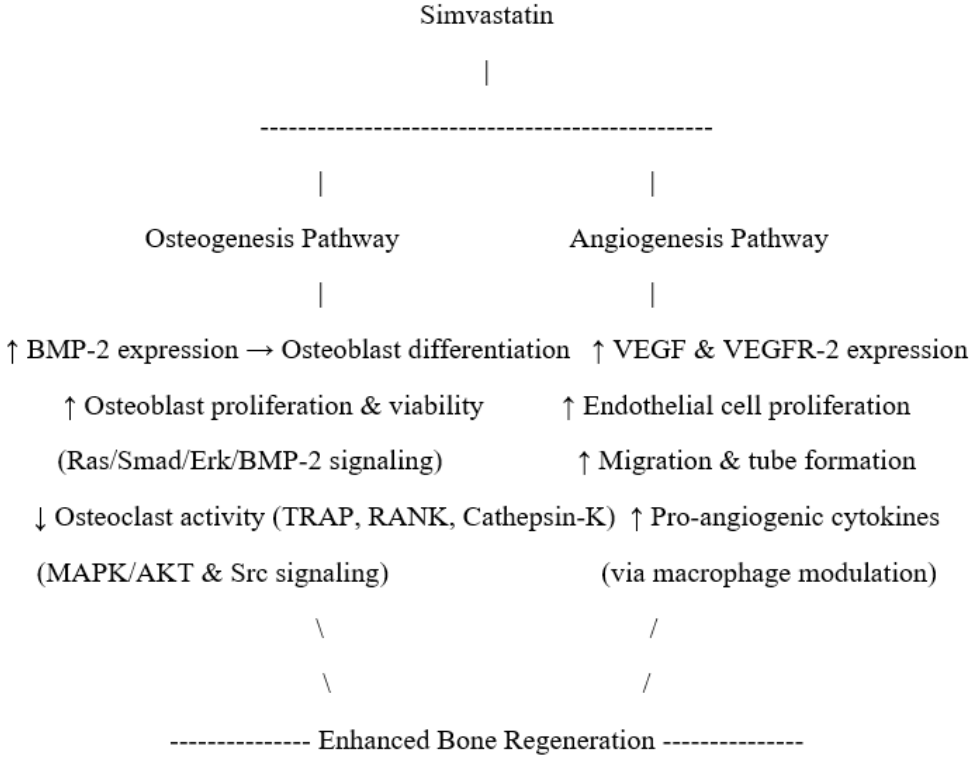

Fig. 2. Simvastatin promotes bone regeneration via dual pathways. On the osteogenic side, it enhances BMP-2 expression, osteoblast proliferation, and inhibits osteoclast activity. On the angiogenic side, it stimulates VEGF/VEGFR-2 signaling, endothelial proliferation and migration, and pro-angiogenic cytokine expression, resulting in coupled angiogenesis and osteogenesis.

Conclusion

Simvastatin exerts dual modulatory effects on bone metabolism by simultaneously promoting osteogenesis and angiogenesis, thereby enhancing bone regeneration. Mechanistically, simvastatin stimulates osteoblast differentiation and proliferation through BMP-2 upregulation and activation of the Ras/Smad/Erk signaling pathway, while inhibiting osteoclast activity via MAPK/AKT and Src signaling modulation. Concurrently, it enhances angiogenic processes by upregulating VEGF/VEGFR-2 signaling, stimulating endothelial proliferation, migration, tube formation, and pro-angiogenic cytokine expression. These interconnected pathways underscore the functional coupling between osteogenesis and angiogenesis, highlighting simvastatin as a promising pharmacological agent for bone healing and regeneration. The integration of osteogenic and angiogenic responses provides a robust biological framework for the development of therapeutic strategies targeting skeletal defects and osteoporosis.

References

1. **Baek, K. H., W. Lee, K. Oh, H. Tae, J. Lee, E. Lee, J. Han, M. Kang, B. Cha, K. Lee, H. Son, S. Kang.** The effect of simvastatin on the proliferation and differentiation of human bone marrow stromal cells. – *J. Korean Med. Sci.*, **20**, 2005, 438-444.
2. **Bryan, B. A., P. D'Amore.** What tangled webs they weave: Rho-GTPase control of angiogenesis. – *Cell. Mol. Life Sci.*, **64**, 2007, 2053-2065.
3. **Carmeliet, P.** Angiogenesis in health and disease. – *Nat. Med.*, **9**, 2003, 653-660.
4. **Carmeliet, P.** Angiogenesis in life, disease and medicine. – *Nature*, **438**, 2005, 932-936.
5. **Carmeliet, P.** Manipulating angiogenesis in medicine. – *J. Intern. Med.*, **255**, 2004, 538-561.
6. **Chen, P. Y., J. Sun, Y. Tsuang, M. Chen, P. Weng, F. Lin**. Simvastatin promotes osteoblast viability and differentiation via Ras/Smad/Erk/BMP-2 signaling pathway. – *Nutr. Res.*, **30**, 2010, 191-199.
7. **Choi, K. Y., H. Kim, M. Lee, T. Kwon, H. Nah, T. Furuichi, T. Komori, S. Nam, Y. Kim, H. Kim, H. Ryoo.** Runx2 regulates FGF2-induced Bmp2 expression during cranial bone development. – *Dev. Dyn.*, **233**, 2005, 115-121.
8. **Ho, M. L., Y. Chen, H. Liao, C. Chen, S. Hung, M. Lee, Y. Fu, Y. Wang, G. Wang, J. Chang.** Simvastatin increases osteoblasts and osteogenic proteins in ovariectomized rats. – *Eur. J. Clin. Invest.*, **39**, 2009, 296-303.
9. **Hu, K., B. Olsen.** Vascular endothelial growth factor control mechanisms in skeletal growth and repair. – *Dev. Dyn.*, **246**, 2016, 227-234.
10. **Kusumbe, A. P., S. Ramasamy, R. Adams.** Coupling of angiogenesis and osteogenesis by a specific vessel subtype in bone. – *Nature*, **507**, 2014, 323-328.
11. **Maeda, T., A. Matsunuma, I. Kurahashi, T. Yanagawa, H. Yoshida, N. Horiuchi.** Induction of osteoblast differentiation indices by statins in MC3T3-E1 cells. – *J. Cell. Biochem.*, **92**, 2004, 458-471.
12. **Maeda, T., A. Matsunuma, T. Kawane, N. Horiuchi.** Simvastatin promotes osteoblast differentiation and mineralization in MC3T3-E1 cells. – *Biochem. Biophys. Res. Commun.*, **280**, 2001, 874-877.

13. **Maeda, T., T. Kawane, N. Horiuchi.** Statins augment vascular endothelial growth factor expression in osteoblastic cells via inhibition of protein prenylation. – *Endocrinology*, **144**, 2003, 681-692.
14. **Matsuno, H., M. Takei, H. Hayashi, K. Nakajima, A. Ishisaki, O. Kozawa.** Simvastatin enhances the regeneration of endothelial cells via VEGF secretion in injured arteries. – *J. Cardiovasc. Pharmacol.*, **43**, 2004, 333-340.
15. **Mundy, G., R. Garrett, S. Harris, J. Chan, D. Chen, G. Rossini, B. Boyce, M. Zhao, G. Gutierrez.** Stimulation of bone formation in vitro and in rodents by statins. – *Science*, **286**, 1999, 1946-1949.
16. **Murasawa, S., T. Asahara.** Endothelial progenitor cells for vasculogenesis. – *Physiology*, **20**, 2005, 36-42.
17. **Niu, Y., Z. Wang, Y. Shi, L. Dong, C. Wang.** Modulating macrophage activities to promote endogenous bone regeneration: Biological mechanisms and engineering approaches. – *Bioact. Mater.*, **6**, 2020, 244-261.
18. **Ribatti, D., A. Vacca, B. Nico, M. Presta, L. Roncali.** Angiogenesis: basic and clinical aspects. – *Ital. J. Anat. Embryol.*, **108**, 2003, 1–24.
19. **Risau, W.** Angiogenesis is coming of age. – *Circ. Res.*, **82**, 1998, 926-928.
20. **Risau, W.** Mechanisms of angiogenesis. – *Nature*, **386**, 1997, 671-674.
21. **Risau, W., I. Flamme.** Vasculogenesis. – *Annu. Rev. Cell Dev. Biol.*, **11**, 1995, 73-91.
22. **Rouwkema, J., N. Rivron, C. van Blitterswijk.** Vascularization in tissue engineering. – *Trends Biotechnol.*, **26**, 2008, 434-441.
23. **Ruiz-Gaspa, S., X. Nogués, A. Enjuanes, J. Monllau, J. Blanch, R. Carreras, L. Mellibovsky, D. Grinberg, S. Balcells, A. Díez-Pérez, J. Pedro-Botet.** Simvastatin and atorvastatin enhance gene expression of collagen type 1 and osteocalcin in primary human osteoblasts and MG-63 cultures. – *J. Cell. Biochem.*, **101**, 2007, 1430-1438.
24. **Song, C., Z. Guo, Q. Ma, Z. Chen, Z. Liu, H. Jia, G. Dang.** Simvastatin induces osteoblastic differentiation and inhibits adipocytic differentiation in mouse bone marrow stromal cells. – *Biochem. Biophys. Res. Commun.*, **308**, 2003, 458-462.
25. **Sonobe, M., K. Hattori, N. Tomita, T. Yoshikawa, H. Aoki, Y. Takakura, T. Suguro.** Stimulatory effects of statins on bone marrow-derived mesenchymal stem cells. Study of a new therapeutic agent for fracture. – *Bio-Med. Mater. Eng.*, **15**, 2005, 261-267.
26. **Stegen, S., N. van Gastel, G. Carmeliet.** Bringing new life to damaged bone: the importance of angiogenesis in bone repair and regeneration. – *Bone*, **70**, 2015, 19-27.
27. **Street, J., M. Bao, L. deGuzman, S. Bunting, F. Peale Jr., N. Ferrara, H. Steinmetz, J. Hoeffel, J. Cleland, A. Daugherty, N. van Bruggen, H. Redmond, R. Carano, E. Filvaroff.** Vascular endothelial growth factor stimulates bone repair by promoting angiogenesis and bone turnover. – *Proc. Natl. Acad. Sci. USA*, **99**, 2002, 9656-9661.
28. **Tan, J., N. Yang, X. Fu, Y. Cui, Q. Guo, T. Ma, X. Yin, H. Leng, C. Song.** Single-dose local simvastatin injection improves implant fixation via increased angiogenesis and bone formation in an ovariectomized rat model. – *Med. Sci. Monit.*, **21**, 2015, 1428-1439.
29. **Weis, M., C. Heeschen, A. Glassford, J. Cooke.** Statins have biphasic effects on angiogenesis. – *Circulation*, **105**, 2002, 739-745.
30. **Yamashita, M., F. Otsuka, T. Mukai, R. Yamanaka, H. Otani, Y. Matsumoto, E. Nakamura, M. Takano, K. Sada, H. Makino.** Simvastatin inhibits osteoclast differentiation induced by bone morphogenetic protein-2 and RANKL through regulating MAPK, AKT and Src signaling. – *Regul. Pept.*, **162**, 2010, 99-108.

31. Yazawa, H., B. Zimmermann, Y. Asami, J.-P. Bernimoulin. Simvastatin promotes cell metabolism, proliferation, and osteoblastic differentiation in human periodontal ligament cells. – *J. Periodontol.*, **76**, 2005, 295-302.

32. Yu, W. L., T. Sun, C. Qi, H. Zhao, Z. Ding, Z. Zhang, B. Sun, J. Shen, F. Chen, Y. Zhu, D. Chen, Y. He. Enhanced osteogenesis and angiogenesis by mesoporous hydroxyapatite microspheres-derived simvastatin sustained release system for superior bone regeneration. – *Sci. Rep.*, **7**, 2017, 44129.

33. Yueyi, C., X. Hu, J. Wang, Q. Su, J. Tian, X. Fu, Y. Xu, C. Song. Calvarial defect healing by recruitment of autogenous osteogenic stem cells using locally applied simvastatin. – *Biomaterials*, **34**, 2013, 9373-9380.