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Review Article

Vascularisation Strategies in Stem Cell-Based Bone Tissue Engineering

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Abstract

Background: Constructs larger than the diffusion limit die at their centre, so vascularisation rather than osteogenic induction has been the rate-limiting step in stem cell-based bone tissue engineering.

Objective: To assess the evidence behind vessel-instructive biology, scaffold architecture, growth-factor dose, co-culture prevascularisation, printed channels and surgically axialised vascular beds.

Methods: Narrative review of rodent, rabbit and sheep implantation studies, in vitro co-culture and printing work, human biopsy series and clinical case reports.

Findings: Type H endothelium constitutes 1.77 +/- 0.01% of bone endothelial cells in four-week-old mice (N = 7), yet 82.63 +/- 1.8% of Runx2-positive cells lie adjacent to it. In rabbit femoral condyles, vessel volume fraction at twelve weeks was 13.5 +/- 0.58% with 150 micrometre interconnections against 5.76 +/- 0.52% at 100 micrometres (N = 6).

Conclusions: The evidence supports the coupling of angiogenesis to osteogenesis and the geometric and dose constraints on design, but no strategy at human scale, where the record is case reports.

Keywords: Bone tissue engineering; Vascularisation; Mesenchymal stromal cells; Angiogenesis; Scaffold architecture

Introduction

The engineering of bone from stem cells has a circulation problem. Marrow stromal cells differentiate reproducibly on a ceramic or polymeric carrier and the constructs mineralise; what they do not do, at any clinically interesting size, is stay alive throughout their volume. Nutrient and oxygen exchange in unperfused tissue operates over roughly two hundred micrometres, so cells at the centre of a bulk construct die before host vessels reach them [1]. That is why vascularisation, rather than osteogenic induction, has been the rate-limiting step for two decades [2,3].

The problem is not simply one of supply. Vessels in bone are instructive as well as nutritive: skeletal endothelium secretes factors that recruit and maintain osteoprogenitors, and osteogenic cells reciprocally drive angiogenesis, so the two are mechanistically coupled rather than merely co-located [4,5]. A vascularisation strategy is therefore not an accessory to an osteogenic one; it is part of it.

Two framings have persisted side by side. One treats the construct as a passive recipient the host must invade, optimising the implant so ingrowth is as fast as geometry allows. The other treats it as an active partner arriving with its own vascular anlage, or a pedicle, needing only to connect. The two fail at different scales and for different reasons, and most published constructs are too small for the difference to show.

This review examines the principal approaches — vessel-instructive biology, scaffold architecture, growth-factor delivery, endothelial and stromal co-culture, printed perfusable networks, and surgically axialised vascular beds — and the evidence behind each, which is asymmetric. Almost all the quantitative work comes from mice, rats and rabbits, in calvarial defects, femoral condyle plugs and subcutaneous or thigh chambers; human data are sparse, mostly descriptive,

confined to case reports and small series. The mechanistic story is strong and the translational record thin, and the two should not be conflated.

The Type H Vessel and the Coupling of Angiogenesis to Osteogenesis

Bone endothelium is not uniform — the most consequential recent advance in skeletal vascular biology. In murine long bone, a capillary subtype defined by high CD31 and high endomucin expression — type H — occupies the metaphysis and endosteum surrounded by osteoprogenitors, while the type L sinusoidal network of the diaphysis is not [6]. Type H endothelium constitutes only $1.77 \pm 0.01\%$ of bone endothelial cells in four-week-old mice ($N = 7$, three independent experiments), yet $82.63 \pm 1.8\%$ of Runx2-positive cells, $74 \pm 3.3\%$ of collagen 1α -positive cells and $70 \pm 1.9\%$ of Osterix-positive cells lie directly adjacent to it [6]. Type H abundance falls with age in parallel with osteoprogenitor number, and endothelial Notch signalling drives both its expansion and the associated gain in bone mass [7].

It is haemodynamically distinct too. Intravital measurement gives blood flow velocities of 0.98 ± 0.1 mm/s in type H capillaries against 0.16 ± 0.04 mm/s in type L sinusoids ($N = 5$ biological replicates), roughly a sixfold difference, and manipulating flow alters both vascular phenotype and osteogenesis [8]. Coupling also runs the other way: preosteoclasts secrete PDGF-BB that induces type H vasculature, tying remodelling to angiogenesis through a resorptive intermediary [9].

Human confirmation is thinner and cross-sectional. Type H vessel fraction in human bone declines from $32.46 \pm 0.76\%$ (age 20–35, $N = 7$) to $18.93 \pm 0.55\%$ (45–59, $N = 8$) to $13.93 \pm 1.29\%$ (60–75, $N = 24$; $P < 0.0001$), and is lower in osteopenic or osteoporotic bone than in controls ($12.48 \pm 1.28\%$, $N = 18$, versus $18.27 \pm 2.98\%$, $N = 6$; $P < 0.05$), with no separation between osteopenia and osteoporosis ($P = 0.3893$) [10]. These are correlations in biopsy material, not

interventions. No study has yet shown that expanding type H vasculature in a human graft improves union.

Scaffold Architecture as a Vascularisation Variable

The geometry of the void space host vessels must grow into directly determines vascularisation. The classical literature established that pore size governs osteogenesis and vascular ingrowth in ceramic and polymeric scaffolds [11,12], but its numerical thresholds are quoted more often than checked, and are not reproduced here.

The cleanest dataset separates pore size from interconnection size. In β -tricalcium phosphate scaffolds implanted in the rabbit femoral condyle with pore size held at 300–400 μm , blood vessel volume fraction at twelve weeks was $13.5 \pm 0.58\%$ with 150 μm interconnections, against $6.5 \pm 0.63\%$ at 120 μm and $5.76 \pm 0.52\%$ at 100 μm (N = 6 per material per time point) [13]. A 50 μm difference in the throat connecting pores more than doubled vascular volume — the aperture, not the chamber, is limiting — with activation of PI3K/Akt signalling in the ingrowing endothelium.

The corollary is practical: a scaffold specified by a single pore-size number is underdetermined with respect to vascularisation, and constructs matched on porosity can differ severalfold in vessel content. Architecture alone is capped: optimal interconnection only accelerates ingrowth from the periphery, which for a segmental human defect means centimetres of migration.

Growth Factor Delivery and the Problem of Dose

VEGF is the obvious pharmacological lever, with an established role in skeletal development: blockade disrupts hypertrophic cartilage remodelling, ossification and vascular invasion at the growth plate [14], and exogenous VEGF accelerates repair through both angiogenesis and bone turnover [15]. Scaffold delivery improves regeneration in compromised beds, including irradiated defects [16], and VEGF with BMP-4 from

muscle-derived stem cells produces more bone than either alone [17].

Table 1: Type H vessel measurements in mouse long bone and in human bone biopsies, mean plus or minus SEM [6,8,10].			
Measure	Value	N	Model
Type H share of bone endothelium	1.77 +/- 0.01%	7	Mouse
Runx2+ cells adjacent to type H	82.63 +/- 1.8%	4-7	Mouse
Flow velocity, type H capillary	0.98 +/- 0.1 mm/s	5	Mouse
Flow velocity, type L sinusoid	0.16 +/- 0.04 mm/s	5	Mouse
Type H fraction, age 20-35 y	32.46 +/- 0.76%	7	Human
Type H fraction, age 60-75 y	13.93 +/- 1.29%	24	Human

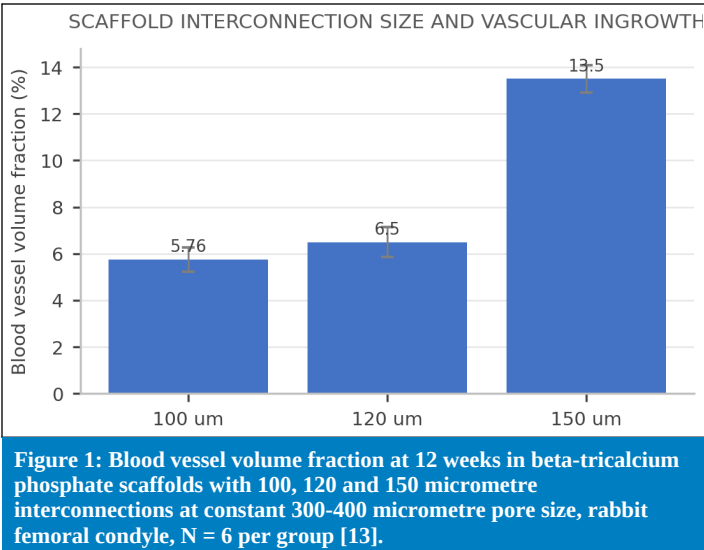


Figure 1: Blood vessel volume fraction at 12 weeks in beta-tricalcium phosphate scaffolds with 100, 120 and 150 micrometre interconnections at constant 300-400 micrometre pore size, rabbit femoral condyle, N = 6 per group [13].

The therapeutic window is narrow and set by local concentration, not total dose. Retrovirally transduced myoblast clones implanted in mouse muscle producing up to approximately 70 ng per million cells per day generated normal capillaries, whereas those at approximately 100 ng and above produced aberrant bulbous structures, and the highest producer (approximately 191 ng per million cells per day) generated frank haemangiomas by 28 days [18]. The parent population averaged 58.5 ng per million cells per day, with

clones spanning roughly 5.5 to 191.2. Within the safe range the effect is real: vessel length density with a low-producing clone reached 87.0 ± 2.9 mm/mm against 55.7 ± 3.8 mm/mm in controls, a 56% increase [18]. The implication is severe: a heterogeneous transduced population holds high-producing outliers even when its mean is safe, so purification by output level, not bulk dosing, separates a capillary bed from a vascular tumour.

Sustained VEGF expression also has a catabolic side: human mesenchymal progenitors engineered to express VEGF vascularised osteogenic grafts but also drove bone resorption, offsetting the vascular gain osteologically [19]. BMP-2 is the cautionary clinical parallel: pooled trial data associate recombinant human BMP-2 in spinal surgery with ectopic bone formation in 70.1% of cases versus 12.9% without, postoperative radiculitis in 14.0% versus 3.0%, retrograde ejaculation in 7.2% versus 0.6%, vertebral bone resorption in 68–82% and graft subsidence in 74% [20]. The same review notes effective concentrations of 0.75–2.0 mg/mL in non-human primates but 0.02–0.4 mg/mL in rodents, a fifty- to hundredfold species gap, with the approved human concentration at 1.5 mg/mL. Rodent dose-finding does not transfer.

Prevascularisation by Endothelial and Stromal Co-culture

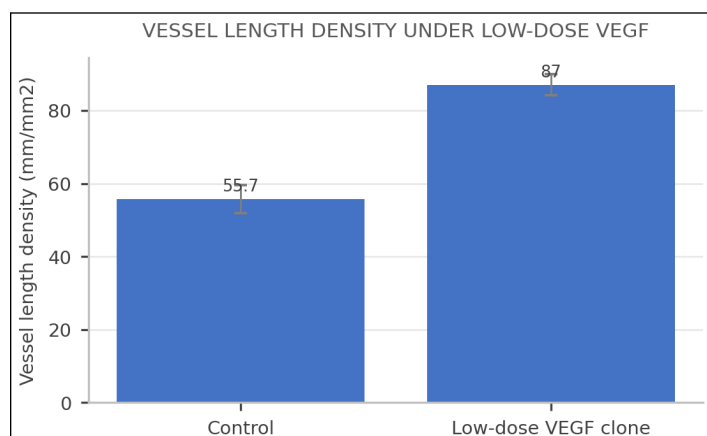


Figure 2: Vessel length density in mouse muscle implanted with a low-VEGF-producing myoblast clone versus control, a 56% increase; clonal populations, exact n per clone not reported [18].

The alternative to slow host ingrowth is to build the network before implantation. Endothelial cells co-cultured with osteogenic cells self-assemble into tubular structures, each lineage modifying the other's differentiation programme [5]. Networks formed in vitro on silk fibroin scaffolds by co-culture of human microvascular endothelial cells and osteoblasts anastomose rapidly with host vasculature on implantation [21]; inosculation, not de novo ingrowth, is the fast route to perfusion.

Timing is best characterised in vascularised bone grafts on 5 mm diameter PLGA scaffolds with approximately 1000 μ m pores. Vascular structures formed by day 4 in vitro; the first anastomoses with host circulation, blood cells visible inside human CD31-positive vessels, appeared at day 11; vessels were patent but leaky at two weeks, mature by four weeks; extensive calcium phosphate deposition followed by eight weeks, and some human-derived vessels persisted at five months [22]. Work on decellularised bone plugs 4 mm in diameter and 2 mm high, seeded with HUVECs and mesenchymal stromal cells at a 2:1 ratio and 30×10 cells/mL in 4 mg/mL fibrin and cultured six weeks in vitro and two weeks in vivo (three independent experiments, n = 3 per group), showed that vascular development and osteogenesis can be advanced in the same construct rather than traded off [23].

Co-culture shifts the osteogenic programme rather than merely adding a vascular compartment: endothelial cells modulate stromal differentiation and stromal cells give nascent tubes mural support, making composition a differentiation variable as well as a perfusion one [5].

Scale is the point to hold on to. Four to five millimetres in diameter is where prevascularisation has been shown to work, and roughly where diffusion alone nearly suffices. Anastomosis at day 11 is fast relative to ingrowth but slow relative to survival of a centimetre-scale cell mass.

Printed Channels and Surgically Axialised Vascular

Beds

Two approaches attack the size limit directly. The first builds perfusable channels into the construct. Sacrificial carbohydrate-glass lattices cast into cell-laden gels yield patterned, endothelialised, perfusable networks in monolithic tissue [24], and multi-material extrusion printing has produced vascularised tissue more than 1 cm thick and around 10 cm in volume, perfused for over six weeks, using nozzles of 100–410 μm (200 μm typical) and cell-laden inks at $0.1\text{--}10 \times 10^6$ cells/mL with endothelial ink at 1×10^6 cells/mL [25]. The governing gradient is explicit: viability falls off beyond 1 mm from an embedded vessel, with only 5% of cells viable at 7 mm [25]. Printed channels remain in vitro; their bone-forming performance in a loaded skeletal site is unestablished.

The second imports a vascular axis surgically. An arteriovenous loop in an osteoconductive matrix within an isolation chamber generates axially vascularised, transplantable tissue in the rat [26], and the model scales to sheep [27]. The payoff is clearest when cells and morphogen are combined: in 42 male Lewis rats (14 per group) with an AV loop in the medial thigh and a 60% hydroxyapatite / 40% β -TCP matrix, bone area as a percentage of construct cross-section reached $16.08 \pm 7.3\%$ at six weeks and $21.63 \pm 3.74\%$ at twelve weeks with BMP-2 plus 5×10^6 MSCs, against $2.02 \pm 1.06\%$ and $3.87 \pm 1.64\%$ for BMP-2 alone and $0.05 \pm 0.19\%$ and $2.2 \pm 1.6\%$ for MSCs alone [28]. Vessel counts per cross-section in that study carried standard deviations comparable to their means and were lowest at twelve weeks in the group with the most bone, plausibly because mineralised tissue displaces vascular area, and should not be read as evidence against the combination.

Rodent calvarial work points the same way. In an 8 mm critical-size rat calvarial defect, a nano-calcium-sulfate/alginate scaffold carrying BMP-2-engineered MSCs with endothelial progenitor cells

filled about 70% of the defect with new bone at five weeks and gave significantly higher vessel density than all other groups ($P < 0.05$; $N = 6$ per group for histology) [29].

What Has and Has Not Reached Patients

The human record is two case reports and a handful of small series. A custom vascularised bone graft grown in a patient's latissimus dorsi and transplanted to reconstruct a mandible remains the emblematic demonstration both that the approach can work in a person and how far it is from routine [30]. Clinical AV-loop bone tissue engineering has been reported in two patients, an 8×2.5 cm tibial defect and a 3×4 cm radial defect, both loops patent and both defects completely healed, at 5.5 years of follow-up in the first and 14 and 32 months in the second [31]. These are encouraging and they are two patients.

Set against them is the small-animal evidence above: mouse calvaria, rat thighs, rabbit condyles, constructs of 4 to 8 mm. A rodent critical-size defect is a few millimetres of avascular distance; a human segmental tibial defect is several centimetres, in an injured, often irradiated or infected bed, under mechanical load. Nothing in the coupling biology [6,7], the architecture data [13] or the dose-window data [18] is species-specific in principle, but none has been tested at human scale, and the one place where a growth-factor strategy reached large clinical use produced a durable adverse-event signal [20]. The angiogenic mechanisms are shared with tumour biology, which is why concentration control rather than maximal induction is the operative design principle [32]. Table 2 sets the strategies side by side against the model each was tested in and the scale it reached.

Conclusion

Vascularisation of stem cell-derived bone constructs is solved as a biology problem and unsolved as a clinical one. The instructive role of type H endothelium, the dependence of ingrowth on interconnection geometry rather than nominal pore size, the concentration

threshold dividing capillaries from haemangiomas, and the additive effect of morphogen plus stromal cells on an arteriovenous axis are all supported by primary data with stated N. Every one of those numbers comes from a mouse, rat or rabbit, or from cross-sectional human biopsies with no intervention. The field's next useful contribution is not another rodent calvarial defect but dose-controlled, vessel-instructive constructs tested at centimetre scale in large animals and reported with the same discipline, so the gap between a healed mouse skull and a healed human tibia stops being crossed by assertion.

Table 2: Vascularisation strategies compared by test model, confirmed quantitative result and scale reached, showing that every non-clinical result comes from a rodent, rabbit or in vitro construct [23,18,13,25,28,31].			
Strategy	Model and species	Confirmed result	Scale reached
Co-culture prevascularisation	Human HUVEC + MSC, in vitro, n = 3	Vascular development and osteogenesis advanced in one construct	4 mm dia. x 2 mm plug
Growth factor dose control	Mouse muscle, myoblast clones	Normal capillaries up to ~70 ng VEGF/10 ⁶ cells/day; haemangiomas at ~190	Intramuscular implant, mouse
Scaffold architecture	Rabbit femoral condyle, N = 6, 12 wk	Vessel volume 13.5 +/- 0.58% at 150 um vs 5.76 +/- 0.52% at 100 um interconnections	Condyle defect, rabbit
Printed channels	In vitro construct, no animal model	Perfused more than 6 weeks; nozzles 100-410 um	More than 1 cm thick, ~10 cm ³
Arteriovenous loop + BMP-2 + MSC	Rat, 42 male Lewis rats, 14 per group	Bone area 21.63 +/- 3.74% at 12 wk vs 3.87 +/- 1.64% for BMP-2 alone	Thigh chamber, rat
Arteriovenous loop, clinical	Human, 2 patients	Both loops patent, both defects completely healed	8 x 2.5 cm tibial, 3 x 4 cm radial

References

1. Simunovic, F., Finkenzeller, G. (2021) Vascularization

strategies in bone tissue engineering. Cells, 10(7): 1749.

2. Rouwkema, J., Rivron, N.C., van Blitterswijk, C.A. (2008) Vascularization in tissue engineering. Trends in Biotechnology, 26(8): 434-441.

3. Rouwkema, J., Khademhosseini, A. (2016) Vascularization and angiogenesis in tissue engineering: beyond creating static networks. Trends in Biotechnology, 34(9): 733-745.

4. Filipowska, J., Tomaszewski, K.A., Niedzwiedzki, L., Walocha, J.A., Niedzwiedzki, T. (2017) The role of vasculature in bone development, regeneration and proper systemic functioning. Angiogenesis, 20(3): 291-302.

5. Grellier, M., Bordenave, L., Amedee, J. (2009) Cell-to-cell communication between osteogenic and endothelial lineages: implications for tissue engineering. Trends in Biotechnology, 27(10): 562-571.

6. Kusumbe, A.P., Ramasamy, S.K., Adams, R.H. (2014) Coupling of angiogenesis and osteogenesis by a specific vessel subtype in bone. Nature, 507(7492): 323-328.

7. Ramasamy, S.K., Kusumbe, A.P., Wang, L., Adams, R.H. (2014) Endothelial Notch activity promotes angiogenesis and osteogenesis in bone. Nature, 507(7492): 376-380.

8. Ramasamy, S.K., Kusumbe, A.P., Schiller, M., Zeuschner, D., Bixel, M.G., Milia, C., et al. (2016) Blood flow controls bone vascular function and osteogenesis. Nature Communications, 7(1): 13601.

9. Xie, H., Cui, Z., Wang, L., Xia, Z., Hu, Y., Xian, L., et al. (2014) PDGF-BB secreted by preosteoclasts induces angiogenesis during coupling with osteogenesis. Nature Medicine, 20(11): 1270-1278.

10. Wang, L., Zhou, F., Zhang, P., Wang, H., Qu, Z., Jia, P., et al. (2017) Human type H vessels are a sensitive biomarker of bone mass. Cell Death & Disease, 8(5): e2760.

11. Karageorgiou, V., Kaplan, D. (2005) Porosity of 3D biomaterial scaffolds and osteogenesis. Biomaterials, 26(27): 5474-5491.

12. Klenke, F.M., Liu, Y., Yuan, H., Hunziker, E.B., Siebenrock,

- K.A., Hofstetter, W. (2008) Impact of pore size on the vascularization and osseointegration of ceramic bone substitutes in vivo. *Journal of Biomedical Materials Research Part A*, 85A(3): 777-786.
13. Xiao, X., Wang, W., Liu, D., Zhang, H., Gao, P., Geng, L., et al. (2015) The promotion of angiogenesis induced by three-dimensional porous beta-tricalcium phosphate scaffold with different interconnection sizes via activation of PI3K/Akt pathways. *Scientific Reports*, 5: 9409.
14. Gerber, H.P., Vu, T.H., Ryan, A.M., Kowalski, J., Werb, Z., Ferrara, N. (1999) VEGF couples hypertrophic cartilage remodeling, ossification and angiogenesis during endochondral bone formation. *Nature Medicine*, 5(6): 623-628.
15. Street, J., Bao, M., deGuzman, L., Bunting, S., Peale, F.V., Ferrara, N., et al. (2002) Vascular endothelial growth factor stimulates bone repair by promoting angiogenesis and bone turnover. *Proceedings of the National Academy of Sciences*, 99(15): 9656-9661.
16. Kaigler, D., Wang, Z., Horger, K., Mooney, D.J., Krebsbach, P.H. (2006) VEGF scaffolds enhance angiogenesis and bone regeneration in irradiated osseous defects. *Journal of Bone and Mineral Research*, 21(5): 735-744.
17. Peng, H., Wright, V., Usas, A., Gearhart, B., Shen, H.C., Cummins, J., et al. (2002) Synergistic enhancement of bone formation and healing by stem cell-expressed VEGF and bone morphogenetic protein-4. *Journal of Clinical Investigation*, 110(6): 751-759.
18. Ozawa, C.R., Banfi, A., Glazer, N.L., Thurston, G., Springer, M.L., Kraft, P.E., et al. (2004) Microenvironmental VEGF concentration, not total dose, determines a threshold between normal and aberrant angiogenesis. *Journal of Clinical Investigation*, 113(4): 516-527.
19. Helmrich, U., Di Maggio, N., Guven, S., Groppa, E., Melly, L., Largo, R.D., et al. (2013) Osteogenic graft vascularization and bone resorption by VEGF-expressing human mesenchymal progenitors. *Biomaterials*, 34(21): 5025-5035.
20. James, A.W., LaChaud, G., Shen, J., Asatrian, G., Nguyen, V., Zhang, X., et al. (2016) A review of the clinical side effects of bone morphogenetic protein-2. *Tissue Engineering Part B: Reviews*, 22(4): 284-297.
21. Unger, R.E., Ghanaati, S., Orth, C., Sartoris, A., Barbeck, M., Halstenberg, S., et al. (2010) The rapid anastomosis between prevascularized networks on silk fibroin scaffolds generated in vitro with cocultures of human microvascular endothelial and osteoblast cells and the host vasculature. *Biomaterials*, 31(27): 6959-6967.
22. Tsigkou, O., Pomerantseva, I., Spencer, J.A., Redondo, P.A., Hart, A.R., O'Doherty, E., et al. (2010) Engineered vascularized bone grafts. *Proceedings of the National Academy of Sciences*, 107(8): 3311-3316.
23. Correia, C., Grayson, W.L., Park, M., Hutton, D., Zhou, B., Guo, X.E., et al. (2011) In vitro model of vascularized bone: synergizing vascular development and osteogenesis. *PLoS ONE*, 6(12): e28352.
24. Miller, J.S., Stevens, K.R., Yang, M.T., Baker, B.M., Nguyen, D.H., Cohen, D.M., et al. (2012) Rapid casting of patterned vascular networks for perfusable engineered three-dimensional tissues. *Nature Materials*, 11(9): 768-774.
25. Kolesky, D.B., Homan, K.A., Skylar-Scott, M.A., Lewis, J.A. (2016) Three-dimensional bioprinting of thick vascularized tissues. *Proceedings of the National Academy of Sciences*, 113(12): 3179-3184.
26. Kneser, U., Polykandriotis, E., Ohnolz, J., Heidner, K., Grabinger, L., Euler, S., et al. (2006) Engineering of vascularized transplantable bone tissues: induction of axial vascularization in an osteoconductive matrix using an arteriovenous loop. *Tissue Engineering*, 12(7): 1721-1731.
27. Boos, A.M., Loew, J.S., Weigand, A., Deschler, G., Klumpp, D., Arkudas, A., et al. (2013) Engineering axially vascularized bone in the sheep arteriovenous-loop model. *Journal of Tissue Engineering and Regenerative Medicine*, 7(8): 654-664.
28. Buehrer, G., Balzer, A., Arnold, I., Beier, J.P., Koerner, C.,

- Bleiziffer, O., et al. (2015) Combination of BMP2 and MSCs significantly increases bone formation in the rat arterio-venous loop model. *Tissue Engineering Part A*, 21(1-2): 96-105.
29. He, X., Dziak, R., Yuan, X., Mao, K., Genco, R., Swihart, M., et al. (2013) BMP2 genetically engineered MSCs and EPCs promote vascularized bone regeneration in rat critical-sized calvarial bone defects. *PLoS ONE*, 8(4): e60473.
30. Warnke, P.H., Springer, I.N.G., Wiltfang, J., Acil, Y., Eufinger, H., Wehmoller, M., et al. (2004) Growth and transplantation of a custom vascularised bone graft in a man. *The Lancet*, 364(9436): 766-770.
31. Horch, R.E., Beier, J.P., Kneser, U., Arkudas, A. (2014) Successful human long-term application of in situ bone tissue engineering. *Journal of Cellular and Molecular Medicine*, 18(7): 1478-1485.
32. Carmeliet, P., Jain, R.K. (2000) Angiogenesis in cancer and other diseases. *Nature*, 407(6801): 249-257.