Targeting Osteogenesis-Angiogenesis Coupling for Bone Repair

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An estimated 6 million long bone fractures occur in the United States each year,[1] and up to 10% of these fractures result in delayed union or nonunion.[2] Although volumetric bone loss and/or inadequate progenitor cell numbers are common causes of nonunion, the most common defining feature of nonunion is impaired vascularization.[3] The current standard of care for fracture nonunion consists of bone grafting and/or distraction osteogenesis; however, limited availability of graft material, pain with graft harvest and distraction, and poor angiogenesis represent significant limitations of these treatment options. Thus, strategies to enhance angiogenesis and subsequent osteogenesis in these most severe cases are urgently needed. Here we discuss osteoprogenitor-endothelial cell crosstalk in bone healing and outline strategies for targeting osteogenesis-angiogenesis coupling for the prevention and management of delayed healing and fracture nonunion.

Osteoprogenitor–Endothelial Cell Crosstalk

During development and in adulthood, osteoprogenitor cells (OPCs) co-localize with endothelial cells (ECs) in the perivascular niche.[4,5] During fracture repair, OPCs invade the injury site with newly forming vasculature,[6] which suggests the existence of a functional codependency between OPCs and ECs via cell-cell contact and paracrine signaling. ECs exert control over osteogenesis by expressing a variety of factors that regulate OPC survival, proliferation, and differentiation, including bone morphogenetic protein (BMP)-2 and -4, Wnt5a, and Notch signaling.[7] Early committed osteoblasts are primarily associated with vessels that highly express CD31 and endomucin; these vessels, which are also referred to as type H vessels, are predominantly located below the growth plate and adjacent to the endosteum in the diaphysis of long bone.[5] The number of type H vessels decreases with age, with a corresponding reduction in OPCs,[8] which may partially explain the reduced healing capacity of aged bone. Although these data suggest that the vascular niche has a role in nurturing osteogenic cells, osteolineage cells at all stages of differentiation express pro-angiogenic factors, including hypoxia-inducible factor-α,[9] vascular endothelial growth factor,[10] BMP-2,[11] and C-X-C motif chemokine ligand 12 (CXCL12),[12] a chemokine involved in stem cell recruitment and differentiation. Our work shows that osteoprogenitor cells regulate EC migration, angiogenic sprouting, and tubule formation,[13] and our unpublished data suggest that release of CXCL12 from osteolineage cells regulates angiogenesis during fracture repair. Ongoing clinical trials are investigating the effects of targeting CXCL12 signaling in conditions such as diabetes and cardiovascular disease, but none has yet focused on bone regeneration.

Targeting Osteogenesis-Angiogenesis Coupling

In animal studies, exogenous vascular endothelial growth factor

(VEGF) treatment has been shown to enhance osteogenesis at low doses[14] and to disrupt osteogenesis at high doses as the result of abnormal angiogenesis and vascular structure.[15] Delivery of CXCL12 and VEGF enhanced recruitment of endothelial progenitors in a hindlimb ischemia model,[16] and fat grafts expressing CXCL12 and BMP-2 enhanced mesenchymal stem cell recruitment to a critical-sized femoral defect in a murine model.[17] More recent delivery strategies have focused on sustained release of osteogenic and angiogenic factors at physiologic levels. For example, fibrin matrices with highly tunable release of $VEGF_{164}$ showed significant functional improvement of hindlimb ischemia ($P < 0.01$).[18] In another approach, three-dimensional–printed β-tricalcium phosphate/calcium silicate scaffolds pre-seeded with human umbilical cord vein endothelial cells and human bone marrow stromal cells stimulated robust angiogenesis and osteogenesis in an ectopic bone formation model.[19] Most recently, collagen-based scaffolds with host cell BMP-2 and VEGF transfection capabilities showed significantly enhanced vessel formation and repair of critical-sized calvarial defects ($P < 0.001$).[20] Mechanical loading is a robust modulator of bone repair,[21,22] and there is evidence that its effects are exerted, in part, through regulation of angiogenesis.[13,23,24] Exogenous mechanical loading during the bone matrix formation phase results in increased bone volume and induces vascular remodeling, resulting in decreased vessel number and connectivity and increased vessel thickness. This effect may be mediated through the release of mechanosensitive paracrine factors from ECs, neighboring cells,[25] and the hematoma.[26]

Summary

The concurrent induction of osteogenesis and angiogenesis using three-dimensional constructs with gene activation capabilities, controlled microarchitecture, and highly tunable protein release profiles is being validated in preclinical animal models. Many of these approaches target endothelial and osteogenic cell coupling through regulation of VEGF, BMP, and CXCL12 signaling. Osteogenesis and angiogenesis are both highly sensitive to mechanical signals, and new approaches must also take into account the mechanical environment at both the macro level (in the form of tissue deformation) and the micro level (in terms of scaffold stiffness).[27] Typically, intramedullary fixation is chosen for reconstruction of critical-sized defects because it allows early weight bearing and increased callus formation. However, additional studies are needed to reveal how mechanical signals regulate osteogenesis-angiogenesis coupling at the cellular and molecular levels.

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References printed in **bold type** are those published within the past 5 years.

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