# Bone vascularization: its role in the bone-organ

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All the functions of bone, including calcium/phosphate metabolism, endocrine secretions, locomotion and hematopoiesis, depend on a common blood supply. Not only bone blood vessels bring oxygen, nutrients and regulatory factors and remove metabolic waste products, as in any other organ, they also transport or bear bone precursor cells. Throughout life, these functional relationships are supported by a complex network of reciprocal signals in which vascular cells stimulate bone cells and bone cells, in turn, elicit cues to modulate blood vessels. The role of bone vessels is rather well described in bone modeling situations such as development, growth or fracture repair. In contrast, their functions in bone *re*modeling remain poorly understood.

In trabecular bone, Bone Multicellular Units BMU are separated from the marrow and the vessels by a thin canopy of cells which delineates the bone remodeling compartment (BRC). In cortical bone, remodeling secondary osteons constituting the Haversian canals are also centered by vessels Thus, remodeling BMUs are anatomically linked to a microvessel. Bone vessels bring osteoclast precursors at the front of the BMU while the osteoprogenitors, which give rise to the osteoblastic lineage, are recruited at the rear from circulating cells or differentiate from perivascular cells. The mechanisms which control the functional coupling between bone remodeling and vessels are not well known. For instance, we do not know whether the birth of BMU always necessitates sprouting of a new vessel (ie angiogenesis) or whether preexisting vessels are able to remodel their position in relation to the activated bone surface. Interestingly, JM Délaissé's team observed that the presence and spatial orientation of the vessels neighboring the BRC depended on the remodeling activity of the bone surface ([1]). Furthermore, few information related to the tight spatial and temporal regulations that allow adequate *guidance and regression* of the capillary that accompanies the BMU throughout its lifespan, is available.

Kusumbe et al ([2]) showed that osteoprogenitors are borne on the wall of a subtype of bone vessels which express both CD31 and endomucin. These vessels, close to the bone surfaces, are located at the interface between the arterial capillary and the venous sinusoid networks and are therefore referred to as "transitional vessels"([3]). Flow cytometry analyses showed that the population of bone endothelial cells that express high levels of CD31 and endomucin, namely Type H cells, declines with age while no overall loss of endothelial cells occurs during ageing in the bone marrow. The H cell population can be expanded by activating endothelial HIF-1α or downregulating their Hippo/YAP/TAZ signaling pathways. Most interestingly, these genetic manipulations *in endothelial cells only*, increased transitional vessel density together with perivascular osteoprogenitors number, bone formation and bone mass. Furthermore, Cao's team demonstrated that PDGF-BB released by pre-osteoclasts also induced growth of type H vessels and stimulated bone formation in ovariectomised mice ([4]), emphasising the coupling role of bone vessels in bone remodeling.

We used the intermittent administration of Parathyroid Hormone (int PTH) as a model to analyse vessel behaviour during bone anabolism. Blocking VEGF, a potent angiogenic agent, during int PTH treatment blunted PTH osteoanabolic properties in rodents. However, we found that int PTH relocated small bone vessels closer to the bone forming surfaces without significant changes in bone vessel density ([5]) and induced microvessel morphological changes, suggesting that post angiogenesis, also referred to as "vascular maturation" may occur during bone anabolism ([6]). Post angiogenesis entails an integrated series of vascular events including arteriovenous specification, vessel pruning and vessel coverage by mature pericytes. Bone marrow pericytes constitute a heterogeneous population which can express a number of markers including Nestin, PDGFRβ, SDF-1/ CXCL12 (CAR cells), Leptin receptor or NG2. Lineage tracing analyses revealed that the proportion of osteoblasts arising from the differentiation of Leptin receptor positive (Leptin $R^+$) cells, found at the bone surface, increases with age ([7]). Interestingly, it was recently shown that the expression of the transcription factor Ebf3 ([8]) by Leptin $R^+$ cells, dedicated these cells to supporting the hematopoietic niche and prevented them from differentiating into osteoblasts. Using Angiogenesis microarray, vessel immunofluorescence

quantification, and Flow cytometry analyses at various time points, we found that int PTH did not expand the type H endothelial cell population but impacted the transitional vessels by reducing their coverage by Leptin $R^+$ pericytes while increasing the number of "free" Leptin $R^+$ detached from vessels. Int PTH upregulated transitional vessel expression of Collagen Type 18/Endostatin, while a same daily dose of continuously infused PTH did not. We also detected, under int PTH, a significant increase in bone expression of Pigment Epithelial Derived Factor (PEDF), an extracellular matrix-linked molecule which, as Collagen type 18 ,may exert potent antiangiogenic properties ([9]).

In summary, a subset of vessels among the bone vasculature seem to be specifically involved in bone remodeling. These vessels may respond differentially according to the type of anabolic stimulus. A fine tuning of pro and antiangiogenic cues is probably critical in a time- and location-dependent manner to insure normal bone remodeling. Whether it is the nature of the vessel/pericyte cross-talk which determines the fate of pericytes remains to be further elucidated. Better understanding of the role of vessels in bone, and especially during bone anabolism in adults should help us to improve designing better drugs for treatment of bone fragility.

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