The bivalent nanobody ALX-0081 inhibits von Willebrand factor (VWF) binding to platelet GPIbα by binding to the VWF A1 domain. V_H indicates heavy chain variable domain; and Ala-Ala-Ala, alanine linker. Professional illustration by Debra T. Dartez.

Ulrichts et al report here that bivalent ALX-0081, but not univalent PMP12A1hi, binds with high affinity binding to VWF and inhibits VWF binding to formalin-fixed platelets.[1] In addition, ALX-0081 inhibits platelet adhesion to collagen-bound VWF in vivo at arterial shear rates; potentiates the inhibitory effects of aspirin, clopidogrel, and unfractionated heparin on platelet adhesion ex vivo using blood from patients undergoing PTCA for acute coronary syndromes; and has activity comparable to abciximab, and somewhat better than clopidogrel, in preventing femoral artery thrombosis in a modified Folts thrombosis model in baboons.[10] A noteworthy finding is that following a standardized wound in these animals, blood loss was less in animals given ALX-0081 than those given clopidogrel or abciximab. Based on these preclinical results, Ulrichts et al suggest that ALX-0081 has high efficacy, as well as an improved therapeu-

tic index, compared with currently marketed anti-thrombotic agents.

ALX-0081 fills a hitherto empty niche in the spectrum of available antithrombotic agents. Nonetheless, it is important to remember that ALX-0081 acts by mimicking the pathophysiology of 2 human hemorrhagic disorders, VWD and the Bernard-Soulier syn-

drome.[11] Thus, it is premature to draw conclusions on its therapeutic index in humans based on the data presented here. Moreover, while Ulrichts et al speculate that ALX-0081 will only have activity in the presence of high shear, like that present in a stenotic artery, patients with VWD and the Bernard-Soulier syndrome bleed at sites where high shear is unlikely to be a factor. Hopefully, ALX-0081 will fulfill the promise its designers expect in clinical trails.

Conflict-of-interest disclosure: The author declares no competing financial interests. ∎

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● ● ● **VASCULAR BIOLOGY**

Innovative blood vessels bring new life

Edward K. Geissler and Peter Angele UNIVERSITY HOSPITAL REGENSBURG

Blood vessels bring life to tissues and their absence signal that something is very wrong. In this issue of *Blood*, Hanjaya-Putra et al report the development of a synthetic matrix that allows the building of functional human microvasculature in vivo, which has great therapeutic potential.[1]
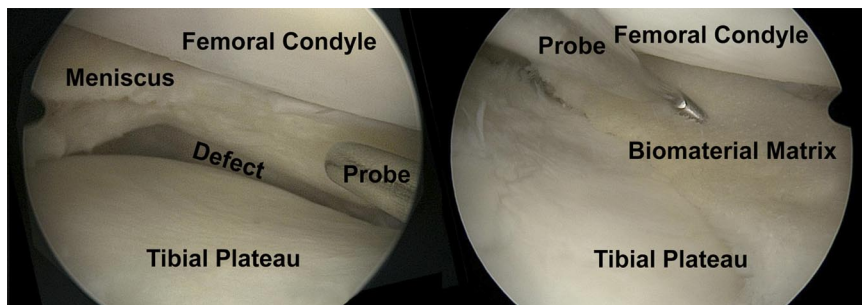
A case of chronic pain postsubtotal meniscectomy after a complex bucket handle tear of the meniscus in a soccer player. (Left) The probe indicates the large size meniscal defect. (Right) This is an arthroscopic view after meniscus transplantation with a cell-free biomaterial. The probe indicates the meniscal defect filling with the cell-free biomaterial. Vascular-forming matrices would improve the integration of the biomaterial and the in-growth of repair cells for regeneration of meniscus tissue.

Building new tissues and organs is no longer science fiction; but it is a very complex process. Like constructing intricate electronic circuits, we must understand all individual components and their interrelationships so that needed functions can be supplied. Essential vessel components include both cellular and structural elements (see figure). From the cellular perspective, stem cell biology is steadily advancing. By better understanding and purifying the stem cells that remain in reserve to replenish our body systems, we theoretically have the ability to replace those systems. Unfortunately, even with the great capacity that these cells hold, it is not as simple as throwing in some stem cells and watching the new tissue or organ grow. To accomplish organ or tissue rebuilding, we must also provide substructures and signaling molecules to guide the correct functional redevelopment of the damaged system. Because of this obstacle, some groups have attempted, with impressive initial success, to recreate new whole organs using a decellularization reconstitution strategy, whereby the cellular component is seeded into a cell-depleted scaffold.[2] Indeed, growing relatively less complex structures (versus whole organs) such as ears, bladder and trachea has been widely publicized.[3,4] Growing new complex organs has yet to be accomplished. Perhaps more tangibly, the fields of regenerative medicine and tissue engineering have also opened the new approaches to tissue repair and healing. In the field of orthopedic surgery, autologous chondrocyte transplantation in sponge-like biomaterials can provide long-term regeneration of large chondral defects.[5,6] Meniscus transplantation with cell-free biomaterials shows promising results in clinical trials.[7] For successful integration of the repair tissue into the surrounding native meniscus, neovascularization is crucial (see figure). Although it will likely be an easier task to repair rather than to completely rebuild a tissue or organ, bringing in new blood vessels is vital to both approaches. The work presented here by Hanjaya-Putra and colleagues is especially important because it offers a first glimpse into how functional vascular networks can be engineered in vivo.[1]

Hanjaya-Putra et al devised their blood vessel–producing synthetic matrix using a biocompatible tunable hyaluronic acid hydrogel possessing key properties, including a high water content to promote cell viability, and flexible degradability for vessel maturation through a system of peptide cross-linkers that can be broken via matrix metalloproteinases.[1] The latter feature is particularly critical to allow for seeded human endothelial colony-forming cells to undergo the process of vacuolization, coalescence, and finally, vascular morphogenesis where endothelial cells branch and sprout to form a nascent vascular network. Important also to their matrix system was the incorporation of cell adhesive (via $\alpha_5\beta_1$ and $\alpha_V\beta_3$ integrins) RGD peptides that regulate the initial process of endothelial cell vacuolization and lumen formation.[8] Therefore, using RGD peptides in the matrix, and through expression of metalloproteinases by endothelial cells, vasculogenesis could be properly initiated and advanced to the point of forming proper interconnecting blood microvessels. Most importantly, the formation of human blood vessels was demonstrated after matrices impregnated with human colony-forming cells were implanted into mice, with evidence that these vessels were carrying blood cells; moreover, the vessels appear to integrate and even anastomose with adjacent mouse tissues, as evidenced by a mixture of human and mouse cells found in these areas. This result suggests that migrating cells supportive of vessel formation and stability (eg, smooth muscle cells, pericytes) could further promote vascular morphogenesis initiated by the synthetic matrix. Indeed, it would be most desirable if an orthotopically placed synthetic matrix could mesh with existing damaged tissue, thus providing a new source of nutrients and immune cells to aid in repair processes; interestingly, macrophages were found in large numbers in the area of the implanted synthetic matrix in this study. Of course, several questions still remain unanswered. Do other cells need to be directly incorporated into the matrix, such as pericytes or smooth muscle cells? Is the vessel formation in vivo well enough organized to be functional? Will allogeneic matrix components be attacked by the immune system? Nonetheless, this study is extraordinary in that it shows in vivo vasculogenesis is possible from a synthetic matrix.

If synthetic matrix products for vascular morphogenesis do become available in the future, one can envision a wide variety of applications that could have a major impact on medicine. For example, limbs where there is peripheral vascular disease (eg, diabetes) could be treated with injections of this material to promote healing as could patients with other wound-repair problems. With regard to growing organs or tissue for replacement therapy, such a vascular-forming matrix would be valuable in bringing this science forward. We envision from our own experience with the use of mesenchymal stem cell therapy for meniscal tissue repair[9,10] that a combination therapy with vascular-promoting matrices could be of great benefit to patients with joint disease. In our experiments, stem cells deliver repair cells and chemokines to the biomaterials and could therefore optimize regeneration. However smart biomaterials like the ones presented by Hanjaya-Putra and colleagues could achieve similar results in meniscus regeneration without cost-intensive cell culture procedures and could improve the regeneration process.[1]

There is no question that blood brings life, and opportunities to restore life. The work presented by Hanjaya-Putra et al is a significant scientific and translational step forward that brings new hope of improving vascular disease treatment and repairing, or even replacing, damaged human tissues.

Conflict-of-interest disclosure: The authors declare no competing financial interests. ■

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● ● ● **VASCULAR BIOLOGY**

Comment on Ballmer-Hofer at al, page 816

Different sorts for different sprouts

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Vascular endothelial growth factor A (VEGF-A) regulates many aspects of blood vessel physiology including angiogenesis. In this issue of *Blood*, Ballmer-Hofer et al report that the VEGFR2 receptor tyrosine kinase binding to different VEGF-A isoforms and neuropilin-1 can program different endothelial responses such as angiogenesis.[1]
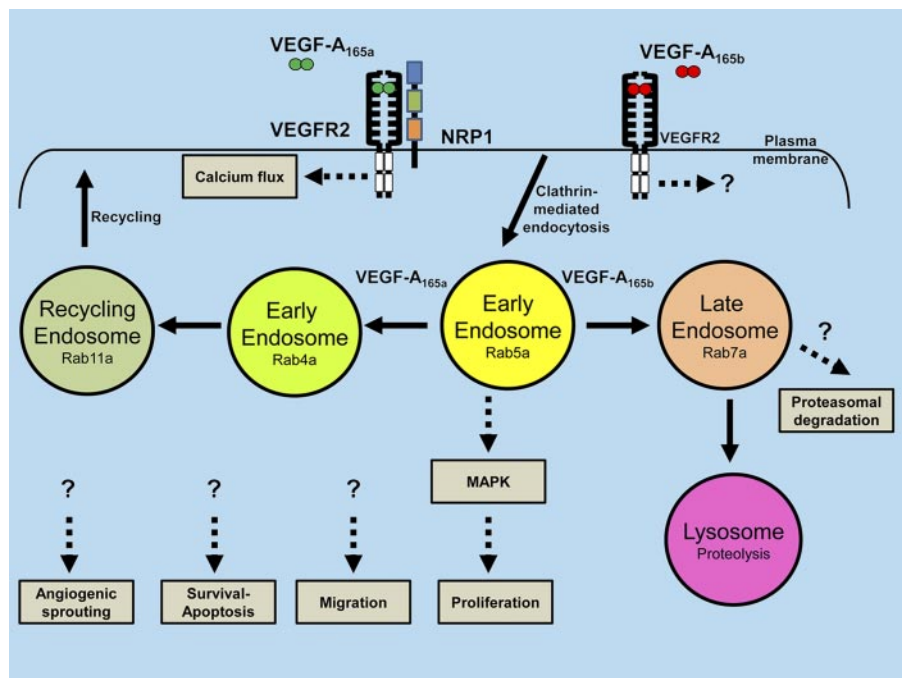
Alternative pathways of VEGFR2 signaling, trafficking, and proteolysis influence decisions on cell migration, proliferation, survival, apoptosis, and vessel sprouting.

In multicellular organisms that use a vascular network, spatial and temporal control of blood vessel sprouting is needed to mobilize cells, molecules, and lipid particles for normal and pathophysiologic responses. Vascular endothelial growth factor receptors (VEGFR1-3) and neuropilins (NRP1, NRP2) bind VEGF-related ligands to respond to the changing extracellular milieu. In this way, an organism is able to build a 3-D vascular network, regulate physiology, and respond to pathologic insults, for example, injury or hypoxia. The existence of neuropilins as VEGF coreceptors and the abundance of VEGF-A splice isoforms (more than 8) provide additional levels of mechanistic complexity.

VEGF-A binding to endothelial VEGFR2 triggers proangiogenic signals that activate mitogen-activated protein kinase (MAPK), the key serine/threonine protein kinase c-Akt, and endothelial nitric oxide synthase (eNOS). Such signaling modulates cell migration, survival, proliferation, apoptosis, and new blood vessel sprouting (angiogenesis).[2] How can these different outcomes be fine-tuned by a single interaction between VEGFR2 and VEGF-A? This mechanism is further complicated by the finding that VEGFR2 can form homo- and heteromeric complexes with VEGFR1 and NRP1 that can mediate signaling to regulate prostacyclin synthesis, which in turn regulates vasodilation.[3]

Ballmer-Hofer and colleagues now provide evidence that endothelial VEGFR2 binding to either the VEGF-A$_{165a}$ or VEGF-A$_{165b}$ isoforms triggers different trafficking, signaling, and cellular outcomes.[1] Trafficking within the endocytic pathway is regulated by the Ras-related small GTP-hydrolysing enzymes such as Rab4a, Rab5a, Rab7a, and Rab11a. Depending on the VEGF-A isoform and coreceptor (NRP1) bound to VEGFR2, different signals are generated. VEGFR2 complexes that bind to the more abundant VEGF-A$_{165a}$ isoform triggers assembly of a VEGF-A$_{165a}$–VEGFR2-NRP1 heteromeric complex that undergoes trafficking through Rab4a-, Rab5a-, and Rab11a-associated endosomes. Importantly, the VEGF-A$_{165b}$ isoform failed to recruit NRP1 to VEGFR2 but promoted trafficking via a late endosome-to-lysosome route regulated by the Rab7a GTPase. Depending on the expression of wild-type or mutant NRP1, VEGFR2 activation, signaling, proteolysis, and endothelial sprouting in vitro were differentially affected.

Quiescent VEGFR2 undergoes clathrin-mediated endocytosis and recycling via endosomes but activated VEGFR2 undergoes proteolysis and terminal degradation in lysosomes.[4,5] In the schematic diagram (see

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