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COMMENTARY

Down in a hole: a new laser ablation model of hemostasis

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The data generated from animal models of disease continue to improve in parallel with advances in intravital microscopy, thereby allowing real-time imaging at the resolution of individual cells. In animal models of hemostasis and thrombosis, there is an additional challenge of inflicting vascular injuries that are well defined and repeatable. Laser-induced injuries partially meet these criteria because the laser power and pulse duration can control the injury size with high accuracy, especially in comparison to some chemically and mechanically induced injuries [1,2]. Laser-induced injuries also have the advantage of seamlessly integrating into intravital microscopy apparatuses. The laser-injury model in the arterioles of the murine cremaster muscle has recently been used to fine-tune our view of the spatial–temporal evolution of thrombus structure to include biophysical mechanisms, such as hindered transport of platelet agonists and coagulation factors, resulting in gradients of platelet activation [3]. Previous studies have shown that the nature and depth of a laser-induced injury can result in thrombi that are more or less sensitive to different platelet agonists [4]. These models simulate a vessel injury that results in a thrombus that grows from the vessel surface into the lumen and, under certain conditions, can occlude the vessel. However, few models recapitulate hemostasis, the process by which a hole in a vessel is sealed to prevent the loss of blood into extravascular space.

In this issue of *JTH*, Getz *et al.* [5] introduce a new murine model of hemostasis that uses a laser to ablate a hole through the saphenous vein. The transient accumulation of fluorescently labeled platelets and fibrin is monitored in a top-down view of the lesion. A bleeding time—defined as the amount of time required for a thrombus to form and seal an injury for at least 15 s—was reported as a metric for hemostasis. The initial thrombus evolves via a familiar sequence: initial adhesion and aggregation of platelets within seconds, followed by fibrin deposition over the time scale of minutes. Fibrin appears to anchor the thrombus to the extracellular matrix as shown by its ring-like appearance along the periphery of the injury. Little fibrin is observed in the center of the thrombus. The initial thrombus is then subjected to additional laser insults to model reinjury. The secondary and tertiary injuries have prolonged bleeding times and are more fibrin rich compared with the initial injury, but fibrin is still localized to a ring adjacent to the vessel wall. Five separate injury sites can be created in one vessel, significantly improving the statistical power over models limited to single-injury models.

The authors performed a series of experiments with genetically modified mice showing the relative roles of platelets and the intrinsic and extrinsic pathways of coagulation on thrombus formation for the initial and repeated injuries. Platelets play the dominant role in forming the initial occlusive thrombus. Mice deficient in *talin1* could not achieve hemostasis, while those deficient in the extracellular domain of GP1bα showed significantly longer bleeding times. Mice expressing low levels of human tissue factor (TF) or deficient in factor IX (FIX) had similar bleeding times as wild-type mice in the initial injury. However, prolonged bleeding was observed for secondary and tertiary injuries, suggesting that the primary role of coagulation in this model is thrombus stabilization. While both platelet and fibrin accumulation were reduced for low-TF mice, fibrin accumulation actually increased in FIX-deficient mice. The authors speculate that deficiencies in the intrinsic pathway may lead to reduced platelet activation, which is then compensated for by further TF-dependent fibrin generation. Inhibition of P2Y12 by clopidogrel gave modest increases in bleeding

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time for all three injuries in wild-type mice but, when coupled with low TF expression results, significantly prolonged bleeding times. These data show that both ADP and thrombin generation are required for an optimal hemostatic response in the model.

The laser-ablation technique offers advantages over existing hemostatic models. The most common hemostatic model is the tail-clip assay, which, while having the advantage of simplicity, is not particularly sensitive to coagulation defects because of vascular spasms and does not allow for real-time imaging [6]. The micropipette injury model is probably the closest analog to the laser ablation model [7]. Here, the mesenteric arteriole of a rabbit is punctured with a micropipette with a 6- to 8-μm tip. The initial hemostatic plug of this model is independent of ADP and thrombin, although they both play a role in thrombus stabilization [8]. In contrast, the relatively larger hole (50–65 μm) in the laser ablation model shows that bleeding times are prolonged when ADP-mediated activation is inhibited by clopidogrel.

Despite the fascinating data emerging from this laser ablation and laser-induced injury models, important questions remain with respect to their relevance to human bleeding and thrombotic disorders and the possibility of artifacts due to laser damage of tissue. The laser power used to ablate the hole in the vessel causes local heating, which, even if brief, could denature extracellular matrix proteins and suddenly interrupt cellular processes, resulting in cell death [9]. Heating collagens and von Willebrand factor can lead to both increased and decreased platelet adhesion depending on temperature [10]. The physics of laser ablation is complex, and the levels of radiation during ablation can damage tissue via thermal, mechanical, and photochemical mechanisms [11]. Certainly more work is needed to quantify tissue damage in laser–vessel wall interactions in all laser-induced hemostasis and thrombosis models. Furthermore, the heating and/or reactive oxide species generated by the laser injury model may propagate into the surrounding endothelium. For example, Ivanciu *et al*. [12] showed that endothelial cells activate and promote coagulation well beyond the laser injury site. To better describe the nature of the ablation injury, careful histology is needed similar to that of the excellent work by Hechler *et al*. on the influence of laser power on injury depth and the relative dependence on collagen, von Willebrand factor (VWF), and thrombin in the murine mesenteric arterioles [4].

There are important differences in the hemorheology for a thrombus growing on a surface versus filling a hole. In surface growth, the primary forces experienced by platelets and the thrombus are shear forces, which is why we typically report shear rates and shear stresses in both *in vivo* and *in vitro* flow-based models. In hole filling, shear forces are present, but so are extensional forces. Extensional forces are those caused by the squeezing of a fluid when it passes through a contraction, in this case the ablated hole. These extensional forces pull on blood cells and plasma proteins and, in the case of VWF, may be important for exposing A1 and A2 domains to GP1bα and ADAMTS-13, respectively [13]. In the ablation model, the authors found that mice deficient in the extracellular domain of GP1bα have prolonged bleeding following the initial injury, suggesting that VWF may play a role in hemostasis even in vessels where the shear stress of the intact vessel is relatively low.

The laser ablation model along with well-established shallow and deep vessel wall laser–induced injuries provides a spectrum of lesion types in both the microvasculature and now larger vessels. These different lesion types could be particularly useful in supporting claims that a given therapeutic strategy prevents thrombosis without affecting hemostasis, which is typically done with a sophisticated thrombosis model and a crude hemostasis model. Now we have a hemostasis model that will provide for a more faithful comparison to thrombosis models.

Disclosures of Conflict of Interest

The author states that he has no conflict of interest.

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