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CORRESPONDENCE

In search for *in vivo* methods to visualize clot forming in cut vessels and interrupted flow

C. Solomon[1,2,3,*], N. J. White[4], G. Hochleitner[3], M. Hermann[3] and D. Fries[3,5]

[1]Marburg, Germany, [2]Salzburg, Austria, [3]Vienna, Austria, [4]Seattle, USA, and [5]Innsbruck, Austria

*E-mail: solomon.cristina@googlemail.com

Editor—A key aspect of improving care for patients suffering from life-threatening haemorrhage as a result of trauma or major perioperative bleeding, is a better understanding of mechanisms involved in coagulation and haemostasis around the injury site. Prevailing models of coagulation, like the cascade or waterfall model,[1] are limited, describing haemostasis as a set of complex interactions between coagulation factors and other blood components, but not taking into account the context in which haemostasis takes place at the injury site. However, decades ago it was established that other mechanisms, such as vasoconstriction and the formation of extraluminal soft clots, are key mechanisms involved in the initiation of haemostasis in ruptured vessels (see Table 1 for a selection of evidence from relevant studies).[2–5]

The initial experiments hinting at the importance of injury-site vasoconstriction and formation of extraluminal clots are more than 50 yr old. Although we recently demonstrated the importance of injury site clot formation in haemostasis using ultrasound elastography (see also Table 1),[6] it seems that these mechanisms, despite their obvious importance, have been largely overlooked in recent research. We believe that the lack of appropriate models may be the main reason why these mechanisms have not received more attention. One method of particular interest for studying thrombus formation *in vivo* is intravital confocal microscopy.[7] Advances in real-time live confocal microscopy have made it possible to visualize thrombus formation as it occurs *in vivo*. Until now, this method has been helpful for increasing our understanding of certain aspects of coagulation, such as the interaction between fibrin generation and platelet deposition during the process of thrombus formation.[8] However, a major shortcoming of current *in vivo* models is that intraluminal thrombus formation is generally induced with ferric chloride injections or laser beams targeted at blood vessels.[8] The fact that vessels are not cut and local blood flow and pressure are not also considered, means it is impossible to investigate haemostatic mechanisms specifically involved in perioperative bleeding.

We believe that coagulation following cut-vessel injury differs significantly from what is observed with present intravital thrombus models,[7 9] where the thrombus forms under normal flow conditions. Conversely, in cut vessels, the thrombus instead forms in the extraluminal space. We therefore believe that an intravital model for confocal microscopic assessment of cut-vessel outflow is needed to study these mechanisms in greater detail. Such a model would allow for a thorough investigation of mechanisms specific to perioperative bleeding, such as injury-site vasoconstriction and formation of extraluminal soft clots described previously. Challenges in developing this model include the creation and validation of molecular tools for visualizing fibrin formation and other coagulation processes under these conditions with high sensitivity and specificity. Furthermore, as cut vessels are not static because of contraction, retraction and pulsation,[4] obtaining high-quality confocal imagery under these conditions represents a challenge.

Although developing an intravital model of cut-vessel outflow will be challenging, the rewards in terms of increased understanding of bleeding could be major. Therefore, preparations for the development of a method that will allow for real-time live confocal microscopic assessment of cut-vessel outflow in a rodent model are underway. The utility of intravital microscopy for imaging biopsies to assess organ function was recently demonstrated,[10] and it should be feasible to adapt this method to image cut-vessel outflow. Such a model, with adaptations for arterial or venous blood flow simulation, has the potential to have a large impact on our understanding of perioperative bleeding that could translate into improved treatments. Considering the risks associated with severe bleeding and the importance of properly managing coagulopathy, such a model is way overdue.

Table 1. Selected evidence of injury-site mediated haemostatic mechanisms

Authors	Methods	Results/observations	Conclusions
Chen and Tsai (1948)[3]	In rabbits (normal and heparinized), ear arteries were punctured using a cutting needle and observed under a microscope	Haemostasis following puncture coincided with arterial constriction/retraction. Even in heparinized rabbits no serious or prolonged bleeding occurred	Arterial vasoconstriction plays a key role in haemostasis following cut vessel injury
Shaftan and colleagues (1964, 1965)[4 5]	In dogs, bp and haemostasis was assessed following transection-induced arterial haemorrhage. Subsequently, bp was increased by epinephrine injection/blood transfusion	A significant drop in bp from blood loss was required before spontaneous haemostasis. Investigation of the injury site revealed that the area surrounding the arterial ends were covered by a newly developed blood clot. Removing the clot or increasing BP resulted in resumption of bleeding	Haemorrhage control is also mediated by the formation of extraluminal soft clots that contain rather than thrombose bleeding arteries and are susceptible to rupture by increasing BP
White and colleagues (2015)[6]	In a swine model, arterial bleeding was induced and ultrasound elastography was used to visualize clot formation *in vivo*	Elastography and visual inspection of the haematoma revealed that haemostasis coincided with the formation of a single large clot around the injured artery	The results confirm that clot formation within the peri-wound haematoma is an integral component of haemostasis

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Declaration of interest

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References

1. Hoffman M, Monroe DM. Coagulation 2006: a modern view of hemostasis. *Hematology/Oncology Clinics of North America* 2007; **21**: 1–11
2. Zucker MB. Platelet agglutination and vasoconstriction as factors in spontaneous hemostasis in normal, thrombocytopenic, heparinized and hypoprothrombinemic rats. *Am J Physiol* 1947; **148**: 275–88
3. Chen TI, Tsai C. The mechanism of haemostasis in peripheral vessels. *J Physiol* 1948; **107**: 280–8
4. Shaftan GW, Chiu CJ, Dennis C, Harris B. Fundamentals of physiologic control of arterial hemorrhage. *Surgery* 1965; **58**: 851–6
5. Shaftan GW, Chiu CJ, Grosz CS, Dennis C. The Effect of Transfusion and of Certain Hemodynamic Factors on the Spontaneous Control of Arterial Hemorrhage. *J Cardiovasc Surg (Torino)* 1964; **5**: 251–6
6. White NJ, Mehic E, Wang X, *et al*. Rediscovering the wound hematoma as a site of hemostasis during major arterial hemorrhage. *J Thromb Haemost* 2015; **13**: 2202–9
7. Falati S, Gross P, Merrill-Skoloff G, Furie BC, Furie B. Real-time in vivo imaging of platelets, tissue factor and fibrin during arterial thrombus formation in the mouse. *Nat Med* 2002; **8**: 1175–81
8. Furie B, Furie BC. Thrombus formation in vivo. *J Clin Invest* 2005; **115**: 3355–62
9. Day SM, Reeve JL, Myers DD, Fay WP. Murine thrombosis models. *Thromb Haemost* 2004; **92**: 486–94
10. Ashraf MI, Fries D, Streif W, *et al*. Biopsychronology: live confocal imaging of biopsies to assess organ function. *Transpl Int* 2014; **27**: 868–76

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