**Why is this research about?**
When a blood vessel is injured, a clot forms at the vessel wall to plug the injury. This is necessary to stop bleeding. However, clots may also form within vessels, for example when an atherosclerotic deposit (plaque) ruptures. These clots can become life-threatening, causing a heart attack or stroke. Maintaining a balance between preventing blood loss (called hemostasis) and preventing unnecessary clotting (called thrombosis) is critical. While much is known about how hemostasis/thrombosis is controlled in the body, the process is not fully understood. Platelets, which circulate in the blood ready to respond to injury, and fibrinogen, a plasma protein, are important in blood clot formation. To plug the injured vessel, platelets stick together (aggregate) at the injury site, and fibrinogen forms a tough mesh of fibres called fibrin. Another plasma protein, fibronectin, has long been thought to play a role in clotting, but there was no direct evidence of a link between fibronectin and hemostasis. This study aimed to explore the unique contribution of plasma fibronectin to blood clotting.

**What did the researchers do?**
This laboratory-based study used several types of gene knockout mice - mice that are bred so that they lack a specific gene in their DNA. Genes responsible for making a certain protein (e.g. fibronectin) or a group of proteins (e.g. fibronectin and fibrinogen) were absent from these mice. This allowed the researchers to understand the role of fibronectin in blood clotting by:

- Examining bleeding and clotting in mice missing the fibronectin gene and other genes.
- Using high resolution microscopy to see when and where fibronectin is deposited in clots as they form.
- Testing the strength of the clot and looking at the effect of platelets and other plasma proteins on fibronectin’s role in hemostasis.

**What did the researchers find?**

- **Plasma fibronectin is essential to control bleeding in the absence of fibrinogen.**
- Similarly, in mice that were treated with drugs to prevent clotting (anticoagulants), plasma fibronectin is needed to control bleeding. Plasma fibronectin is also important to control bleeding when another plasma protein involved in clotting, called von Willebrand factor, is missing.
- **At the site of blood vessel injury, fibronectin is deposited rapidly, even before platelets.** Platelet build-up at the injury site has long been considered the first event in hemostasis. This result suggests that fibronectin comes first and may even initiate hemostasis before platelets arrive at the site of injury.
- In the clot, fibronectin fuses with fibrin fibres. This makes the clot stronger and more stable by increasing the diameter of the fibres.
- High resolution images of clots as they form showed that fibronectin is mostly found at the base of the clot, near the blood vessel wall. Fibronectin becomes less concentrated as the clot grows and extends into the interior of the vessel.
- When fused with fibrin, fibronectin promotes platelet stickiness (aggregation).

Plasma fibronectin: keeping the delicate balance between bleeding and clotting
Together, these findings suggest that plasma fibronectin quickly deposits on the injured vessel wall and interacts with fibrin to stop bleeding. Afterwards, fibronectin gradually switches from supporting hemostasis to inhibiting clotting (thrombosis). Fibronectin plays a uniquely self-limiting role in thrombosis/clot-formation. By interacting with fibrin, fibronectin limits the growth of the clot away from the site of injury, preventing it from growing too large and blocking the vessel.

How can you use this research?
These findings redefine our basic understanding of how blood clots form. They establish a clear link between fibronectin, often-overlooked but always suspected of playing a role in hemostasis, and clot formation. Plasma fibronectin may be the body's first response to the site of vessel injury. Not only is it the 'glue' that initiates clot formation, fibronectin also appears to prevent clots from becoming too large and blocking the vessel. This is thought to be the first demonstration of a plasma protein that both supports hemostasis and inhibits thrombosis. Fibronectin's role in controlling bleeding is particularly important when formation of fibrin fibres is not working, that is when fibrinogen is absent or following treatment with anticoagulant drugs such as heparin.

This study opens up many new avenues of basic and clinical research. Although more research and investigations with human patients are needed, the implications of these findings are wide-ranging. Finding the right balance between hemostasis and thrombosis is a constant battle, particularly for surgery and trauma patients. How to best control bleeding is a matter of debate. Many treatments that help stop bleeding (including blood component transfusions), may also increase the risk of a life-threatening clot. Conversely, treatments that prevent clot formation may increase the risk of a life threatening bleed. This research shows that, uniquely, plasma fibronectin may be instrumental in stopping bleeding while also preventing life-threatening blood clots. This suggests that plasma fibronectin infusions may be important to control bleeding, particularly in patients who are also on anticoagulants.

About the research team: Dr. Heyu Ni is a Canadian Blood Services scientist and a professor in the departments of laboratory medicine and pathobiology, physiology and medicine at the University of Toronto. Dr. Ni is also a member of the Toronto platelet immunology group at the Keenan Research Centre for Biomedical Science at St. Michael's Hospital, Toronto. He is the platform director for Hematology, Cancer, and Immunologic Diseases at St. Michael's Hospital. Yiming Wang is a graduate student in Dr. Ni's laboratory at the University of Toronto, and has a Canadian Blood Services fellowship. The research team included other members of Dr. Ni's laboratories as well as collaborators from the University of North Carolina at Chapel Hill, NC, from McMaster University, Hamilton, ON, from the University of Wisconsin, Madison, WI and from the Hospital for Sick Children, Toronto, ON.

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