New tool measures strengths of bonds that keep blood clots from washing away

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(PhysOrg.com) -- New work from the Program in Cellular and Molecular Medicine and the Immune Disease Institute at Children's Hospital Boston shows that the bonds that hold wound-healing platelets in place have a special kind of stickiness that keeps them from being swept away in a rush of pulsing blood.

The lab of Timothy Springer, Latham Family Professor of Pathology at Harvard Medical School, found that platelets bind to arterial walls using a unique double-acting bond that Springer calls a "flex-bond." A little like a kitchen cabinet with a child-safe latch, the bond holds in one mode at low forces, and in a different mode at higher forces. The work opens up the potential for a new understanding of bleeding diseases, such as von Willebrand disease, in which the genes that form platelet bonds have clot-preventing mutations. The work appears in the August 9 issue of *Nature*.

The body mends scratches and gashes by plugging holes in small arteries with platelets. Platelets, which flow in the blood stream, stick together and to the walls of blood vessels to form a patch. "They need to bind to one another and they need to resist the force of blood pushing against them," says Springer.

To form the bonds that hold clots together, two proteins interact: One on the platelet surface, called glycoprotein Ib alpha (GPIb-alpha); and the other, called von Willebrand factor (VWF), which binds both to GPIb-alpha on platelets and to collagen in blood vessel walls. VWF protein is

mutated in von Willebrand disease. "This receptor-ligand pair needs to resist a lot of force," says Springer. "So we had a hunch that there might be something special about it."

To explore this hunch, Springer's team employed a novel technique, dubbed ReaLiSM (Receptor and Ligand in a Single Molecule). The technique involves the use of a single molecule that links the protein pair together, separately from the pair's normal bond, to measure how force affects the bond's strength.

The researchers first bind the proteins together as the pair would bind in a clot. Then they attach a flexible molecular chain to the ends of the proteins not involved in the biologically meaningful binding. They mimic the force of blood flow on the protein-protein bond by tugging apart the pair using laser-guided, molecular "tweezers" developed by a research team from the University of California, Berkeley. This tugging applies a force similar to that of a fluid threatening to wash away a bound platelet. The change in extension of the flexible chain measures the strength and lifespan of the bond.

"At low force, it was easy to break the bond," says Springer, the same way it is easy to break the magnetic bond holding a cabinet door shut. "But then, as we applied more force, the second state of this bond got engaged." Like a child safety latch, this second bond kicks in at higher forces and lasts longer than the first. Unlike other well-known bonds, such as slip bonds, which weaken with force, and catch bonds, which strengthen with force, "this receptor-ligand pair is really specialized to resist a broad range of forces."

The discovery sheds light on the physics underlying the process of wound-healing. "Anytime you have a better understanding of biophysics, you can treat patients and treat diseases better," says Springer.

Springer's lab will follow up on this work by exploring how this bond behaves in patients with von Willebrand disease. By using his new measurement technique to test clinical samples that contain mutated forms of the two proteins, Springer hopes to better understand how these mutations give rise to bleeding disorders.

The work also opens the door to examining other receptor-ligand pairs in the body to see how they react to force. "There are forces everywhere in the body. In the intestine, in muscles and tendons, in the skin, there are many, many adhesion molecules that keep your body together that have to resist force."

More information: "A Mechanically Stabilized Receptor-Ligand Flex-Bond Important in the Vasculature" Jongseong Kim, Cheng-Zhong Zhang, Xiaohui Zhang and Timothy A. Springer.

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