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Pulmonary Thrombosis-on-a-Chip provides new avenue for drug development

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An image of a thrombus (blood clot) formed on endothelial tissue in the Pulmonary Thrombosis-on-a-chip, demonstrating the characteristic “teardrop” shape observed in vivo. Credit: Wyss Institute at Harvard University

The average human pair of lungs is permeated by a network of about 164 feet of blood vessels (roughly the width of a football field), including microscopic blood capillaries, which facilitate the diffusion of oxygen into the bloodstream in exchange for carbon dioxide. Damage to any of those vessels can cause a blood clot, or thrombus, to form, which can cause or exacerbate a number of lung diseases, including pneumonia, acute lung injury and acute chest syndrome. The use of some drugs is also limited by their propensity to promote clot formation in lung vessels. Developing and testing drugs to treat or prevent pulmonary thrombosis is difficult because the complex interplay between the many different cell types in the lung hampers efforts to tease out the exact causes of clot formation. A new study conducted by members of the Wyss Institute at Harvard University, Emulate Inc., and Janssen Pharmaceutical Research and Development, published today in the journal *Clinical Pharmacology and Therapeutics*, is the first to successfully recreate a human pulmonary thrombosis within an organ-level model of the lung in vitro.

"It's very difficult to distill out specific mechanisms inside an animal, and a lot of work in toxicology or drug discovery fails when it goes to human clinical trials," says co-first author Abhishek Jain, Ph.D., former Wyss Institute Postdoctoral Fellow and current Assistant Professor of Biomedical Engineering at Texas A&M University. "In vitro models like our Thrombosis-on-a-Chip are made from the ground-up, so you can build them to be exactly as complex as you need for the problem you want to study."

To meet this challenge, the team used Organ-on-a-Chip (Organ Chip) technology developed at the Wyss Institute, which involves engineering microfluidic culture devices with two parallel channels separated by a porous extracellular-matrix-coated membrane. The key innovation in this new design relative to a previously described Lung-on-a-Chip is that the upper surface of the porous membrane is lined by primary human alveolar epithelial cells, and all sides of the lower vascular channel are coated with a layer of lung microvascular endothelium to accurately mimic human lung capillaries. Because thrombosis is perpetrated by platelets and other cells, the team perfused whole human blood through the lower endothelium-lined channel of the chip for the first time, while air was introduced into the upper channel. When an inflammatory stimulus was applied to the endothelial cells followed by perfusing whole blood, platelets clumped and formed blood clots on the surface of the endothelium in a characteristic teardrop shape that has been observed in living animals, but never before in vitro.

"This is the first time we're seeing clots form with the same dynamics and morphology that you see in vivo, which is a major step forward in studying and eventually treating blood clots that cause many life-threatening diseases." says Donald Ingber, M.D., Ph.D., senior author of the study and the Judah Folkman Professor of Vascular Biology at Harvard Medical School (HMS) and the Vascular Biology Program at

Boston Children's Hospital, as well as Professor of Bioengineering at Harvard's School of Engineering and Applied Sciences (SEAS).

The team further tested the chip's functionality by replicating an inflammatory lung injury that originates in the lung's airways - the most likely source of a pathogen or other damaging substance. They introduced lipopolysaccharide endotoxin (LPS), an inflammatory chemical found on the surface of certain types of bacteria and is known to induce clot formation in vivo, into both the upper and lower channels of the chip. They were surprised to find that LPS had no effect on blood clot formation when they added it directly to the endothelium-lined blood channel; but, when added to the air channel, it induced the air-facing epithelium to trigger a cascade of cytokines, a class of inflammatory signaling molecules that initiate blood clot formation, in the underlying endothelium. "Epithelial cells are the guardians of the airways - they need to be sensitive to airborne pathogens and then signal the danger to the rest of the body," says co-author Riccardo Barrile, Ph.D., also a former Wyss Institute Postdoctoral Fellow and current principal investigator at Emulate, Inc. "This study demonstrated that information travels from the epithelium to the endothelium, but I was surprised to see that the entire system is so well-connected."

In addition to facilitating the discovery of crucial insights into the mechanism of how lung injury promotes blood clot formation, the Thrombosis-on-a-Chip allows for the testing of potential drugs on an organ-level system in vitro, an approach that has become highly attractive to pharmaceutical companies. Working with Robert Flaumenhaft, M.D., Ph.D., Associate Professor of Hematology at HMS and Beth Israel Deaconess Medical Center, the team introduced parmodulin-2 (PM2), an inflammation inhibitor, into the vascular channel of the device, and found that it significantly decreased the number of clots on the vessel wall following the addition of LPS to the airway channel. This confirmation of drug activity, as well as the insight

that LPS causes thrombosis only by acting directly on the epithelium, would have been very difficult to achieve in vivo, as blood flow and individual cellular compartments cannot be controlled individually as they can in Organ Chips.

The team plans to continue their pulmonary thrombosis work by introducing mechanical forces that imitate breathing to the Chip and analyzing the role that immune cells such as neutrophils play in blood clot formation. "By including whole blood, we're reaching a new standard of complexity and precision for mimicking a human body in both health and disease," says Barrile. "This study affirms that we are recapitulating organ-level responses to lung injury, emphasizing that this is a true Organ-on-a-Chip, not just a tissue-on-a-chip," adds Ingber.

More information: Abhishek Jain et al, A primary human lung alveolus-on-a-chip model of intravascular thrombosis for assessment of therapeutics, *Clinical Pharmacology & Therapeutics* (2017). DOI: 10.1002/cpt.742

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