3-D engineered bone marrow makes functioning platelets

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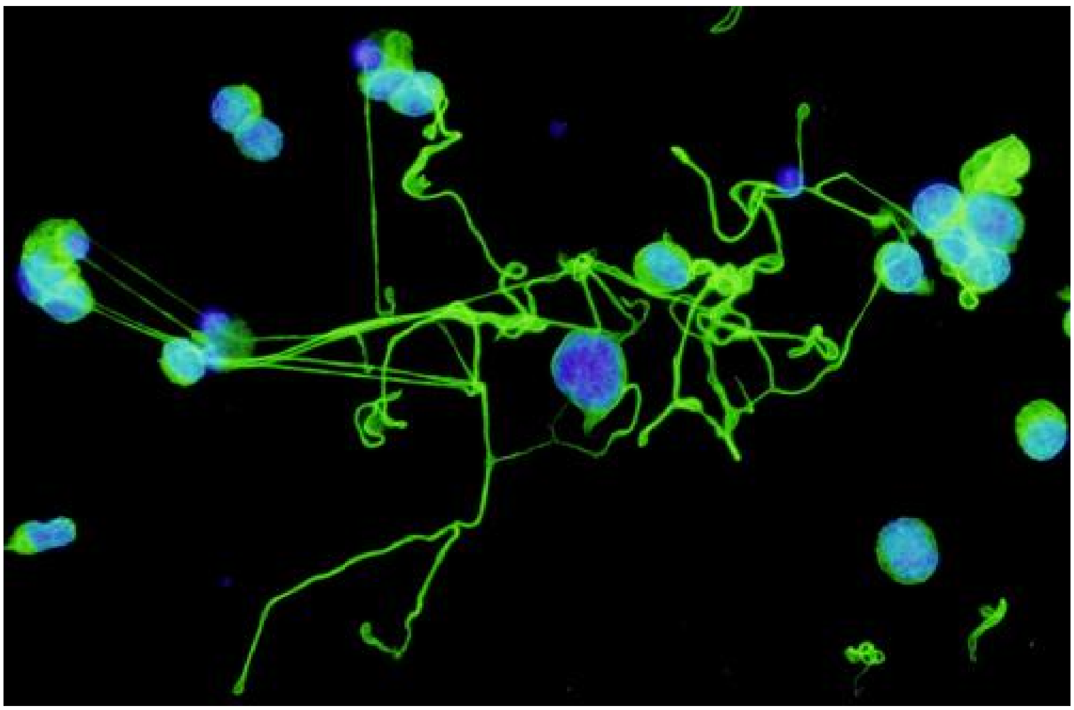

A team led by researchers at the Tufts University School of Engineering and the University of Pavia has reported development of the first three-dimensional tissue system that reproduces the complex structure and physiology of human bone marrow and successfully generates functional human platelets. Using a biomaterial matrix of porous silk, the new, scalable system is capable of producing platelets for future clinical use and also provides a laboratory tissue system to advance study of blood platelet diseases. Shown above are platelet-producing blood cells called megakaryocytes (blue) releasing filament-like Credit: Tufts University

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"There are many diseases where platelet production or function is impaired," says Alessandra Balduini, M.D., research associate professor in the Department of Biomedical Engineering at Tufts, associate professor at the Department of Molecular Medicine at the University of Pavia and co-corresponding author on the paper. "New insight into the formation of platelets would have a major impact on patients and healthcare. In this tissue system, we can culture patient-derived megakaryocytes—the bone marrow cells that make platelets—and also endothelial cells, which are found in bone marrow and promote platelet production, to design patient-specific drug administration regimes."

The new system can also provide an in vitro laboratory tissue system with which to study mechanisms of blood disease and to predict efficacy of new drugs—providing a more precise and less costly alternative to in vivo animal models.

"The need for platelet production systems to treat patients with related diseases is significant. This patient-specific system could provide new insight and options for clinical treatments," says David Kaplan, Ph.D., chair of biomedical engineering and Stern Family professor at Tufts and co-corresponding author. "Further the platelets can be generated on demand, avoiding the complications of storage problems, and in greater quantities and with better quality and control in terms of morphology and function."

The work is pre-published online in the journal *Blood* prior to print publication.

Producing Platelets in Silk Bioreactor

Platelets can be life-saving or life-threatening. These cells keep us from bleeding to death from injuries by enabling our blood to clot, but they also play a pathological role in heart attacks and strokes, inflammation and cancer. Contained in spongy bones, the bone marrow microenvironment and its "niches" support production of platelets and other blood cells. Mature blood cells pass between the bone marrow and the blood stream through blood vessels containing endothelial cells and extracellular matrix components (ECM) that are important for healthy blood cell production.

The special properties of silk protein were essential to successfully mimicking this microenvironment, explains Kaplan, a leading researcher on silk and other novel biomaterials whose laboratory has bioengineered silk-based models for the brain and other tissue.

"Silk protein possesses a unique molecular structure that enables it to be modeled in a wide variety of forms and stiffnesses, characteristics that have been shown to affect platelet formation and release. Furthermore, silk is biocompatible and has the ability to stabilize bioactive agents at normal temperatures; therefore we can 'functionalize' it by adding such agents," he said. Importantly, the silk is nonactivating to platelets, meaning that it does not trigger clotting, thereby allowing the collection of functional platelets from the bioreactor.

The new system combined microtubes spun of silk, collagen and fibronectin surrounded by a porous silk sponge. Megakaryocytes - some of which were derived from patients - were seeded into the engineered microvasculature. The researchers were able to increase platelet production in the bioreactor by embedding the silk with active endothelial cells and endothelial-related molecular proteins that support platelet formation.

Laboratory tests showed that the platelets being generated and recovered from the tissue system were able to aggregate and clot. While the number of platelets produced per megakaryocyte was lower than normally made in the body, the researchers note that the system represents a significant advance over previous models. The scalable nature of the bioreactor system provides engineering options to increase yields of platelets in ongoing studies.

In addition to providing a platform for studying the processes that regulate platelet production and related diseases, the researchers hope the platelets produced can be used as a source of growth factors for wound healing in regenerative medicine, including healing of ulcers and burns, and stimulation of bone tissue regeneration in dentistry and maxillofacial plastic surgery.

Global Collaboration

The new system was developed in Italy and the United States, and research is the result of ongoing collaboration among an international research team. Six years ago, Balduini visited Tufts to discuss her ongoing studies on blood cells and matrix factors that are important in controlling blood cell functions.

"During our discussion we realized there was potentially great synergy between her expertise in blood cells and diseases and our work on 3D tissue-engineered systems, including ongoing work on silk tubular systems for blood vessels," said Kaplan. "This led to joint studies, an early model and the current system, which not only yields more platelets but is also more scalable."

Collaborative research building on the strengths of multiple investigators is critical to making this type of progress, according to Kaplan and Balduini. They also agree that involvement of a talented and motivated group of graduate students and postdoctoral researchers makes a big contribution.

More information: "Programmable 3D silk bone marrow niche for platelet generation ex vivo and modeling of megakaryopoiesis pathologies," Christian A. Di Buduo, Lindsay S. Wray, Lorenzo Tozzi, Alessandro Malara, Ying Chen, Chiara E. Ghazi, Daniel Smoot, Carla Sfara, Antonella Antonelli, Elise Spedden, Giovanna Bruni, Cristian Staii, Luigi De Marco, Mauro Magnani, David L. Kaplan, Alessandra Balduini, *Blood*, pre-published online January 9, 2015; DOI: 10.1182/blood-2014-08-595561

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