Making and storing blood to save lives

Blood can be a scarce resource, but a multitude of approaches are promising new ways to store, create, and deliver blood's component parts to patients in need.

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As soon as a wounded person starts hemorrhaging, the clock is ticking. The quicker an emergency responder can stop the bleeding and replace that blood, the better the chances are for survival. But most bleeding patients do not receive optimal prehospital trauma care, including blood transfusions. That costs lives—up to 30,000 people a year, according to a 2016 report by the National Academies of Sciences, Engineering, and Medicine (1). As noted by the military trauma surgeons who informed that report, the answer to this problem is not necessarily more blood collection, but rather better access to and storage of blood, and, ultimately, production of shelf-stable blood components.

After decades of failed trials to create artificial blood, researchers may finally be starting to meet that challenge. Several are looking to create products that serve as a complement to blood—a sufficient substitute to keep hemorrhaging patients alive until they have access to the real thing. Some researchers are developing freeze-dried versions of blood components; others are looking to create artificial particles that offer replacement components.

New Blood

Hemorrhaging patients rarely receive whole blood; instead, they receive reconstituted blood components.

To save the lives of patients in dire need of blood, hospitals and emergency responders don't necessarily need more blood collection. They need better access to and storage of blood, and, ultimately, production of shelf-stable blood components. Image credit: Shutterstock/Ben Carlson.

First published April 1, 2020.

 www.pnas.org/cgi/doi/10.1073/pnas.2001649117

Donated whole blood is broken down into its parts: Red cells carry oxygen, platelets are responsible for clotting, and plasma is the liquid component that plays a role in coagulation. Breaking down blood into parts allows some of those components to be stored longer. Plasma, for instance, can be stored frozen for months but must be used five days after being thawed. Red cells can be stored refrigerated for up to 42 days, whereas platelets only last five days because they must be stored at room temperature. Whole blood only lasts about a month.

Previous attempts to develop artificial blood focused on designing artificial oxygen carriers. The initial version of these products, tested in the 1950s, used cell-free hemoglobin—the protein in red cells that holds oxygen—purified from either human or animal blood.

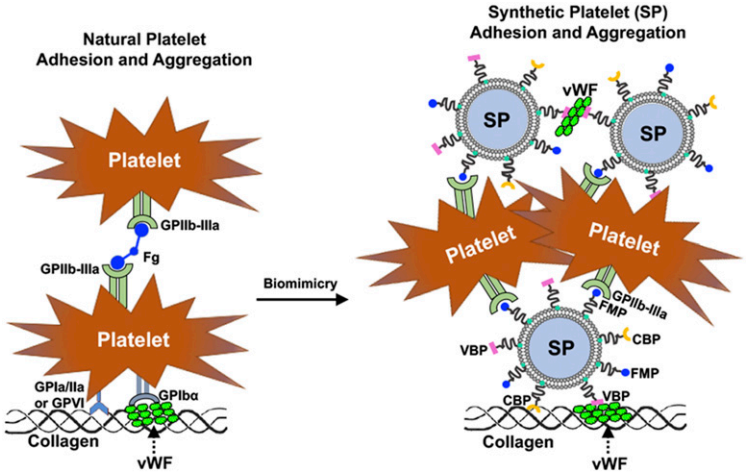
But the use of cell-free hemoglobin led to a number of problems: Hemoglobin that is not inside a cell is unable to effectively deliver oxygen because it rapidly disassembles into smaller fragments, explains Anirban Sen Gupta, a biomedical engineer at Case Western Reserve University in Cleveland, OH. Those fragments are small enough to "leak" into organs such as the kidneys or liver, causing toxicity issues, he adds.

Hemoglobin is also a potent scavenger of nitric oxide, which is produced by the inner lining of blood vessels. Nitric oxide, a vasodilator, keeps blood vessels open. But quickly removing or sequestering nitric oxide, as hemoglobin does, can lead to constriction of vessels, causing cardiovascular distress and blood clots.

To mitigate such effects in patients treated with hemoglobin, a second generation of artificial oxygen carriers attempted to chemically tether or crosslink hemoglobin molecules so they would not disassemble into organs (2). But these types of hemoglobin-based oxygen carriers, or HBOCs, fell out of favor a decade ago when a meta-analysis (3) found that patients treated with these products had a 30% increased risk of death and more than twice the risk of having a heart attack—likely owing to the nitric oxide sequestration side effect that some products had.

But Sen Gupta says that this second generation of HBOCs also fell short because they failed to properly control oxygen levels. Real red blood cells can suck up oxygen in the lung and then drop it off throughout the rest of the body. Red blood cell "effector molecules," such as 2,3-diphosphoglycerate, tightly regulate the process, explains Sen Gupta. Without such molecules, the HBOCs become saturated with oxygen but can't provide oxygen to where it's needed in the tissue.

The third generation of HBOCs in development are designed to solve that problem by including effector molecules with hemoglobin that has been encapsulated in some sort of particle, such as synthetic lipid vesicles (4). A product called ErythroMer, developed by bioengineer Dipanjan Pan and pediatric critical care doctor Allan Doctor, both at the University of Maryland in Baltimore, is designed not just to deliver hemoglobin but also to mimic the actions of red blood cells (5). Doctor says that ErythroMer has a high affinity for oxygen in the lung, an affinity that lessens when the artificial red cell goes out into the body.



The latest generation of synthetic platelets is designed to mimic the adhesive and aggregatory mechanisms of natural platelets. Peptides on the outside of the particle direct the synthetic platelets to only stick to other platelets. In principle, doing so will limit clot formation to areas where natural platelets are already congregating, and hence help prevent stroke. Image credit: Anirban Sen Gupta (Case Western Reserve University, Cleveland, OH).

"Like an electromagnet, that picks up a car in one spot and drops it in another," he says. "That's closer to how hemoglobin works."

ErythroMer, which is being tested in rabbits, also has a synthetic polymer shell that selectively reduces interaction between hemoglobin and nitric oxide, which would also reduce blood vessel constriction issues. Doctor hopes to begin phase one clinical trials by 2022.

Products such as ErythroMer could be stored in powder form, allowing for a shelf life of several years—and shelf stability is key to stockpiling such bridge therapies for patients in remote locales. Even if an emergency responder can't access blood right away, at least they'd have access to a product that mimics some aspect of whole blood. "If it is good enough, it might actually be valuable to save lives," says Sen Gupta.

Artificial Platelets

Development of artificial or dried platelets is even more pressing because of the platelets' short shelf life. Although researchers had thought that cold storage of platelets would impair function, recent work suggests otherwise, revealing that cold platelets actually produce greater clot strength (6). Room-temperature platelets expire after five days, whereas refrigerated platelets could last up to 21 days. But although cold-stored platelets would mitigate supply limits in hospitals, shelf-stable platelets are still needed to equip remote locations and ambulances providers. Those products are also in development.

Closest to market may be freeze-dried platelets. Rockville, MD–based biotechnology company Cellphire, Inc. is gearing up for a phase two trial of its product, Thrombosomes. The company pools and dries donated platelets, which then can be reconstituted with water when ready to be used. According to president

In an attempt to develop a product that mimics aspects of blood, researchers are testing a product called ErythroMer. Stored in powder form, it has a shelf life of several years, which could help serve critically ill patients in remote locales. Image credit: University of Maryland School of Medicine/Mark Teske.

G. Michael Fitzpatrick, Thrombosomes could potentially last more than three years at room temperature.

The challenge with dried products is ensuring that they retain functionality when they are resuspended in fluid, notes Erin Lavik, a professor of biochemical engineering at the University of Maryland in Baltimore. "A lot of times, when these things get resuspended, they're not quite the same," says Lavik. Fitzpatrick claims that tests on dogs and rabbits show that Thrombosome dried platelets work just as well as fresh liquid ones. A canine version of their product is already approved for use. "We're seeing very good results with that product in stopping bleeding in dogs," he says.

One downside of freeze-dried platelets: They still need access to donated blood. Hence, researchers are also looking to develop synthetic platelets that wouldn't tap into the blood supply. In the early to mid-1990s, researchers attempted to make synthetic platelets by taking synthetic particles, such as liposomes, and coating those particles in fibrinogen to make a sort of "super structure" to cement the platelets. The problem: They weren't creating platelets, they were creating a "platelet cement," says Sen Gupta. The mixture didn't have the ability of natural platelets to clump together only at the site of a wound. That's important—if activated platelets don't stay near the bleeding, the risk of stroke increases.

The newest generation of synthetic platelets is intended to solve that problem. For a product called SynthoPlate, Sen Gupta created a liposome decorated with different peptides that direct the synthetic platelets to only stick to other platelets. This limits clot formation to where natural platelets are already congregating, such as at an open wound. Tests with pigs have not revealed any adverse clotting events and have shown significantly improved survival in pigs with femoral artery injury (7). Researchers at North Carolina State University in Raleigh, meanwhile, are testing a version of synthetic platelets that they claim avoids excessive clotting by targeting fully formed fibrin polymers that only appear at the site of a wound, according to North Carolina State bioengineer Ashley Brown.

Immune responses are a concern when creating any sort of artificial particle to be used in the body, notes Lavik. Overactive immune responses can lead to inflammation at the site of a wound, causing a widening of blood vessels. In other words, "it makes it really hard to stop the bleeding," says Lavik. Trauma itself can trigger an immune reaction, she adds. "Now you've got multiprong things going on that can all potentially exacerbate bleeding." Even if you succeed in stemming the bleeding with some artificial platelet, sepsis can be a problem. "You're not out of the woods when the bleeding stops," she says.

Sen Gupta sees room for a variety of approaches. "There will never be a one size fits all solution," he says. "You need all of them as complementary components in the toolbox of transfusion medicine because the logistics vary widely between locations and countries and scenarios."

First Plasma

Before artificial red cells, synthetic platelets, or even freeze-dried platelets, the first shelf-stable blood product to hit the market will likely be dried plasma. It's already approved for use in combat situations and is farthest along in clinical trials compared with other blood products.

Plasma is the liquid component of blood but it also factors into coagulation, providing key proteins that aid in clotting. Dried plasma has been tested over the past decade in military and, in some cases, civilian settings. For instance, a small study in Norway in 2015 found that it was feasible and safe to reconstitute and administer dried plasma to patients being picked up by helicopter emergency medical services.

The US military is catching up, having partnered with medical device company Teleflex of Wayne, PA, to start a phase one trial of freeze-dried plasma in 2017 (8). Other companies, such as Velico Medical in Beverly, MA, are developing platforms to allow blood collection centers to dry platelets on site. Velico is gearing up a phase one clinical trial of that technology this year. Unlike platelets, which can work with any blood type, plasma to be used in emergency settings needs to be universal AB blood type, an added challenge.

Another challenge is logistical: the fragmented for-profit nature of the emergency response industry in the United States. Some emergency medical service providers are run by hospitals, some private business, notes Lisa Buckley, a regenerative medicine researcher at Oregon Health & Science University in Portland, who has consulted with companies developing freeze-dried plasma. In many cases, these providers are not reimbursed based on care provided but rather only if they transport a patient to the hospital. This "makes it hard to sell any product, whether it's hemorrhage control, resuscitation fluid, tourniquet, it's not an easy distribution pathway," she says.

If researchers and physicians can overcome the bureaucracy, the results could be impressive (9). A

study (10) conducted with air ambulances at Pittsburgh Medical Center, PA, demonstrated a 10-percent reduction in mortality in hemorrhaging patients receiving prehospital plasma compared with a control group of hemorrhaging patients not receiving plasma, says Matt Neal, a trauma surgeon in Pittsburgh who collaborates with Sen Gupta. "We haven't done anything in 30 years in trauma that has reduced mortality by 10%."

Ideally, physicians would have a better stock of blood products in hospitals, especially in rural areas. For the short term, that means they need FDA approval for use of dried plasma and cold-stored platelets, says Neal. "We are absolutely at a critical juncture where we have to get approval for cold-stored platelets used in trauma."

Over the long term, Neal adds, researchers need to continue development of synthetic platelets and dried red cells. "The goal here," he says, "should be to advance the needle on the science so we are able to have a freeze-dried whole blood."

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