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THERE WILL BE BLOOD

Is mimicking the cells that carry hemoglobin the key to a blood substitute?

By **Andrew Zaleski**
Photography by **Matt Roth**

The candidate blood substitute ErythroMer, shown before freeze drying, could one day be deployed in emergency medical situations.

In 19th century New York City, Theodore Gaillard Thomas enjoyed an unusual level of fame for a gynecologist. The reason, oddly enough, was milk. Between 1873 and 1880, the daring idea of transfusing milk into the body as a substitute for blood was being tested across the United States. Thomas was the most outspoken advocate of the practice.

At the time, severe bleeding was often a death sentence. Blood transfusion was practiced, but it was something of a crapshoot. Medical science was still 3 decades removed from discovering blood types. Patients who received mismatched blood suffered discolored urine, itching, and a sometimes-fatal complication: hemolytic shock, wherein their own immune systems attacked the transfused cells.

Doctors in the U.S. were looking for something less risky to stabilize a hemorrhaging patient. Thomas was sure milk was the answer. In 1875, he injected 175 milliliters of cow's milk into a woman suffering from severe uterine bleeding after an operation to remove her cancerous ovaries. At first, he wrote, the patient "complained that her head felt like bursting." She soon developed a high fever and an abnormally high heart rate, but recovered a week later. Thomas subsequently performed seven separate milk transfusions, publishing his results in several medical journals, and predicted their "brilliant and useful future."

It was not to be: Saline solutions, still used today, were introduced the next decade as a much less dangerous, if imperfect, stopgap measure for emergency bleeding.

The need for blood substitutes, however, survives. And last year in a downtown Baltimore laboratory, a white rabbit embodied the latest hope.

The bunny huddled in a black metal cage, a catheter going straight into its carotid artery. Days before, a portion of its blood had been siphoned out and replaced with an experimental blood substitute called ErythroMer. It is decidedly not milk. Developed by Allan Doctor, a bespectacled 61-year-old physician-researcher at the University of Maryland (UMD) School of Medicine, and colleagues, ErythroMer is made from "recycled" human hemoglobin—the protein in red blood cells that carries oxygen from the lungs to the rest of the body—wrapped in a membrane to mimic a tiny cell. In the rabbit, the transfusion appeared to be working. The animal's heart rate and blood pressure, displayed on a small monitor nearby, looked just fine.

Doctor is as fervent an advocate for hemoglobin-based oxygen carriers (HBOCs), as ErythroMer and its predecessors are more formally known, as Thomas was for lacteal transfusions. Donated blood has a shelf life of just 42 days. There's also not enough, even in developed countries with well-organized blood donation systems: In January 2022, the American Red Cross, which distributes 40% of the country's donor blood, declared the first-ever national blood crisis, as its supply—especially precious O-negative blood, the universal type—dipped dangerously low. Meanwhile, hemorrhagic shock caused by severe blood loss kills some 20,000 people in the U.S., and 2 million globally, every year.

An artificial "blood" could, perhaps, fill the void. In settings where fresh blood is hard to come by, such as battlefields and rural areas (where ambulance wait times are sometimes as high as 45 minutes), ErythroMer could be given on the fly to maintain the vital flow of oxygen to organs until someone reaches a hospital. It's a freeze-dried powder that remains usable for years and can be reconstituted by simply mixing it with widely available saline. And ErythroMer should be safe for any blood type, because its membrane doesn't include the red blood cell surface proteins that cause mismatches.

For now, no human blood substitute is commercially available in the U.S. "There's a real gap here where we don't have access to blood for people bleeding to death outside of the hospital," says Doctor, who co-founded and is chief science officer of KaloCyte, a company hoping to develop ErythroMer into a commercial product.

Last year, the Defense Advanced Research Projects Agency (DARPA) announced a $46 million grant to a UMD-led consortium to develop a shelf-stable, field-deployable whole blood substitute with ErythroMer as its core. "ErythroMer … is notable for its detailed emulation of natural red blood cell function," says Jean-Paul Chretien, program manager in DARPA's Biological Technologies Office and a former Navy medical officer.

Karina Yazdanbakhsh, vice president and director of research at New York Blood Center Enterprises, agrees, calling the candidate blood substitute "promising" for use in trauma and other emergency settings. Indeed, the nonprofit blood bank, which serves and collaborates on research with hundreds of hospitals, in 2022 invested

Better than nature?

Decades of efforts have failed to develop a good substitute for oxygen-carrying red blood cells. A new candidate, ErythroMer, is still in preclinical testing but could be more durable and versatile than the real thing.

Red blood cells

SHELF LIFE
42 days

COMPATIBILITY
By blood type

SIZE
7–8 µm

ErythroMer

SHELF LIFE
2 years

COMPATIBILITY
Universal

SIZE
~0.2 µm

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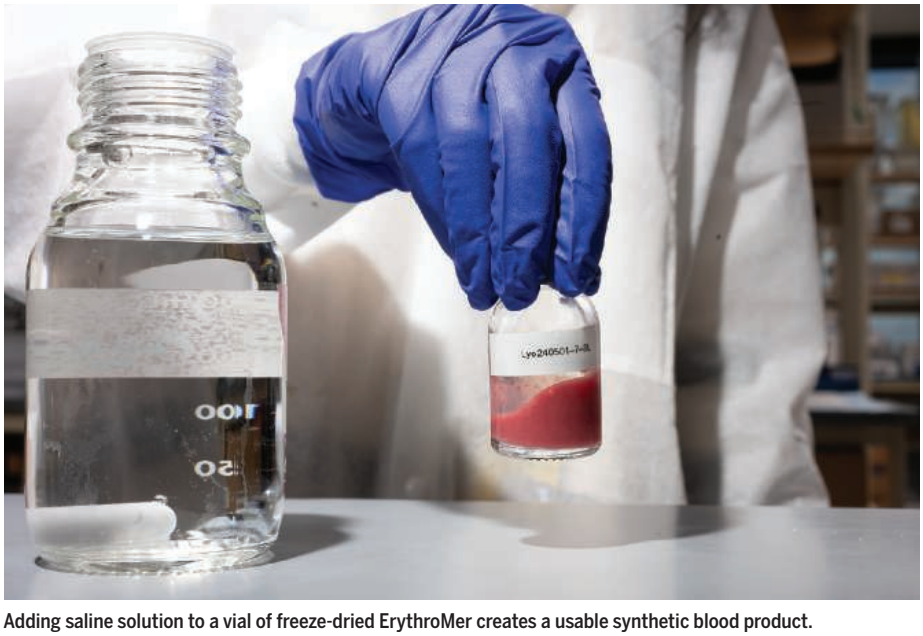
KaloCyte scientist Elizabeth Zheleznyakova uses a filtration machine to get rid of excess hemoglobin not encapsulated into ErythroMer.

ErythoMer would be shipped in a freeze-dried form with a 2-year shelf life.



Adding saline solution to a vial of freeze-dried ErythroMer creates a usable synthetic blood product.

an undisclosed amount in KaloCyte.

So far Doctor's creation remains in animal testing, but it isn't the only effort to package hemoglobin inside lipids to fashion a viable blood substitute. A rival product in Japan has already been tested in a few people and generally appears safe.

But the success of these new products is far from guaranteed. Barely 2 decades ago, earlier formulations of HBOCs were scuttled or sidelined after trial participants died. Subsequent attempts haven't fared much better. The most advanced HBOC to date, approved for people in South Africa and Russia, has struggled amid concerns about side effects.

THERE'S GOOD REASON imitating blood is hard—it's a complex mixture of free molecules and cells. A little more than half of your blood is plasma, yellow-tinted liquid made up of water, proteins, and salts. The rest is cellular matter, mainly platelets, essential for clotting after a cut or wound; white blood cells, to fight infection; and red blood cells, which not only give blood its tomato coloring, but also ferry oxygen-giving hemoglobin.

No other cell is more abundant than the red blood cell, a disk that appears squashed in the middle. Constantly produced by the bone marrow at a rate of 2 million per second, these cells regenerate every 120 days. At any given time, there are 30 trillion of the cells doing a cannonball run through the body's 20,000 kilometers of blood vessels.

The DARPA project also aims to develop synthetic platelets and freeze-dried plasma. But the jackpot is to mimic blood's mighty oxygen carrier. Open up a red blood cell and you'll find an estimated 260 million hemoglobin molecules. Each globular protein has an iron-bearing complex called heme at the center. The heme complexes capture oxygen, turning bright red and endowing blood cells with their color.

An earlier class of candidate blood substitutes tried to replace hemoglobin with oxygen-bearing chemicals called perfluorocarbons, widely used in refrigerants and fire extinguishers. One was even approved by the U.S. Food and Drug Administration (FDA) in 1989 for use in surgeries. But the complexities of making and administering it, along with various side effects, undermined that product, which was ultimately withdrawn.

That largely left the field to HBOCs. Hemoglobin proteins inside red blood cells link together in groups of four, forming structures called tetramers. Early HBOCs often tried to copy this polymeric construction, minus a membrane. Yet hemoglobin is a tricky molecule, toxic to tissues and vessels. For one thing, it carries oxygen, which itself is an oxidizing agent and can be destructive in the wrong place. (Think of how a slice of apple

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PHOTOS: MATT ROTH

turns brown.) "You can't just squirt [hemoglobin] into the bloodstream," Doctor says.

Over the past century, patients given blood substitutes made of unprotected hemoglobin developed hypertension, high metabolic rates, and quickened pulses. In the worst cases the substitutes caused heart attacks and renal failure, thought to result from a narrowing of blood vessels triggered by the free hemoglobin. But there have also been glimmers of success.

The most successful unencapsulated HBOC to date is Hemopure, developed in the 1990s. It's made by taking red blood cells from cows, extracting their hemoglobin, purifying it to remove pathogens, and chemically bonding four of the proteins together as a tetramer. Hemopure won early favor, including approval in South Africa in 2001—where the HIV/AIDS crisis made blood transfusions high risk—to treat perioperative anemia. Most of its usage to date, though, has been in situations where normal transfusions were not an option.

But Hemopure's prospects took a turn in 2008 when *The Journal of the American Medical Association* (*JAMA*) published a meta-analysis of it and four other HBOCs. The authors concluded all of the products were intrinsically toxic to the heart, and that patients treated with them were 30% more likely to die than if they got conventional transfusions.

Trials were halted, investors panicked, and firms either went bankrupt or stopped developing HBOCs entirely. Biopure, Hemopure's developer, was sold a year later to a different biotech company. Zaf Zafirelis, a feisty South African of almost 80 years and former CEO of Biopure, believes Hemopure got a raw deal.

"The product, like everything else, has a small side effect profile," he says. "But it's certainly not toxic." By comparing the effectiveness of blood substitutes against the routine standard of care, a transfusion of packed red blood cells, the meta-analysis missed the point, Zafirelis insists.

"The first mistake that was made was to call [HBOCs] a blood substitute," he says. "There is no artificial product that can ever, ever match the real thing. The best we can do is have something that can temporarily help the patient until the bone marrow is able to produce more red cells."

"We know enough now where we can prevent the risk associated with these products to use as a beneficial unmet medical need," adds Kim Vandegriff, chief science officer at Vivosang, Inc. The company is developing its own HBOC, made of human hemoglobin linked to the polymer polyethylene glycol, which is often used to help drug delivery.

Jonathan Waters, director of the Patient Blood Management Program at the University of Pittsburgh Medical Center, has used Hemopure for almost a decade for people who refuse transfusions for religious reasons or those with sickle cell disease who develop reactions to their frequent blood transfusions. "I can think of probably 10 patients that would be dead if it wasn't for Hemopure," he says.

In February, a case report in *Transfusion* described how doctors were unable to find suitable blood for a 54-year-old woman with leukemia whose hemoglobin levels had dropped to 2.5 grams per deciliter, less than 20% of healthy levels. They treated her with 17 units—4.25 liters—of Hemopure, and watched her hemoglobin levels stabilize to 9.7 grams per deciliter.

"I've used it on four or five Jehovah's Witness patients," adds Jed Gorlin, a clinical pathologist at the University of Minnesota and chief medical officer of America's Blood Centers, which supplies about 60% of U.S. blood donations. "I had a lady come in with a hemoglobin under 3. Ten units later, this lady wakes up and says, 'See? God takes care of us.'"

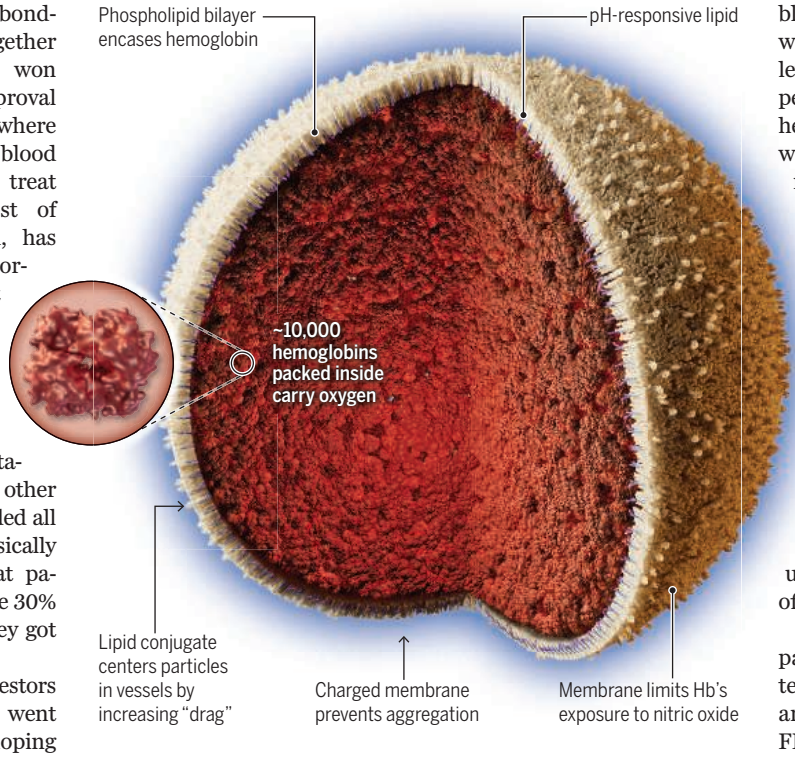
Zafirelis started a new company in 2014 that licensed the technology behind Hemopure and has been trying to gain full FDA approval. But all the agency has agreed to is its use as an "investigational new drug" for patients who have no other alternative or refuse donated human blood, like Waters's and Gorlin's. (A nearly identical sister product, Oxyglobin, is approved for use in pets in the U.S. and other countries.)

Zafirelis, who routinely ships bags of Hemopure to doctors himself, was as of May still trying to raise the money to restock the manufacturing facility of his new company, Hemoglobin Oxygen Therapeutics LLC.

ERYTHROMER DOESN'T HAVE clinical success stories yet, but it now

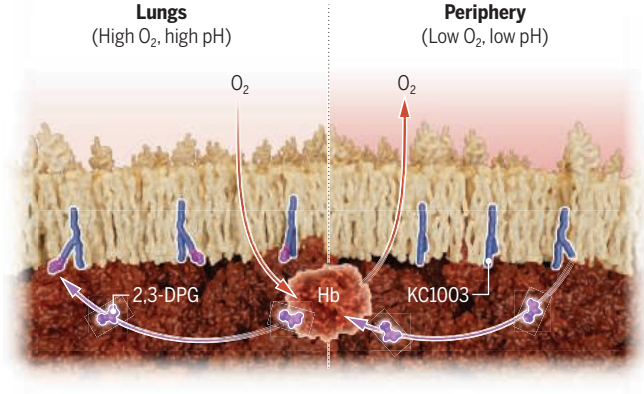
Blood cell mimic

Blood substitutes composed of free hemoglobin (Hb), the blood's oxygen carrier, can be toxic. So some scientists are enclosing the oxygen carrier in a membrane, like a minicell. One candidate blood substitute, ErythroMer, has a membrane engineered to aid its flow in blood vessels and prevent Hb from scavenging nitric oxide, a gas that keeps blood vessels open.



Breathe in, breathe out

Like red blood cells, ErythroMer uses a molecule called 2,3-DPG to regulate Hb's affinity for oxygen. In lungs, 2,3-DPG binds to a synthetic pH-sensing molecule, KC1003, in ErythroMer's membrane, enabling Hb to take up oxygen. In more acidic tissues, 2,3-DPG is released and attaches to Hb, promoting oxygen release.



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has DARPA's millions. Doctor believes it can avoid the toxicity of pure hemoglobin products—by even more closely imitating nature and enclosing the molecules like red blood cells do.

Doctor never set out to design a blood substitute. In the early 2000s, while a junior faculty member at the University of Virginia, he was studying the relationship between hemoglobin and nitric oxide, a gas released into the blood by the lining of blood vessels. Vessels dilate in its presence, and contract without it. And red blood cells control its levels because, like oxygen, nitric oxide can bind to hemoglobin. The cells either sequester or export nitric oxide, depending on oxygen traffic between them and tissue in the microenvironment.

Think about a person bench-pressing weights. First, muscle oxygen consumption increases; then blood flow increases to sustain the tissue's activity and slowly returns to normal after it ceases. As red blood cells deliver oxygen to working muscles, they also release nitric oxide, which dilates regional blood vessels, increasing local blood flow. When the workout is over, red cells are no longer releasing lots of oxygen, and their relationship with nitric oxide reverses—they begin taking in the gas, which binds to the cell's hemoglobin, and vessels contract.

This intricate biochemical choreography has dogged the world of artificial oxygen carriers, accounting for many of the side effects of unencapsulated HBOCs. Floating around in plasma, free hemoglobin can gobble up too much nitric oxide, causing vasoconstriction, which in turn can lead to hypertension, and even a heart attack or a stroke. The *JAMA* meta-analysis published in 2008 singled out this interplay between hemoglobin and nitric oxide as a problem.

Researchers surmised that an HBOC that encapsulated hemoglobin could avoid the vasoconstriction problem, but no one knew how to design one. Around 2010, however, Dipanjan Pan, now a nanomedicine specialist at Pennsylvania State University, was a chemical engineer at Washington University in St. Louis (WUSTL) designing lipid nanoparticles, microscopic membranes made of fat, for innovative imaging applications.

As Pan later recounted, not only did the nanoparticles resemble the structure of red blood cells, but "they could carry a very high payload of hemoglobin." By this point, Doctor had arrived at the same university and established a lab studying red blood cells in various diseases and how well the cells maintained their function after being kept in storage. Aware of Doctor's interests, Pan cold called him to discuss the nanoparticle work.

"Once I understood what they were doing, I became excited," Doctor says. "I knew that the problems with the old HBOCs were that they were free in plasma and that we needed to find a way to sheath [the hemoglobin]. No one had ever done that very well."

The pair teamed up and began to develop their artificial red blood cell. Eventually they pulled in Philip Spinella, an expert in military transfusion medicine at WUSTL. In 2016, the three of them co-founded KaloCyte—a portmanteau of the Greek word for beautiful and the suffix cyte, which means cell.

Like its predecessors, ErythroMer contains hemoglobin, in this case collected from donated human red blood cells past their shelf life. (Doctor concedes that if ErythroMer becomes widely used, supply could run short, but he says his lab plans to explore making hemoglobin in recombinant yeast.) But the research team envelops the recycled hemoglobin in an artificial membrane designed to mimic how a red blood cell controls the capture and release of oxygen.

Developed primarily by Pan, the membrane components—there are five in total—include a proprietary lipid called KC1003 that the team developed to be responsive to local pH. It governs the availability of 2,3-DPG, a small molecule found naturally in red blood cells, inside the artificial hemoglobin package. 2,3-DPG normally binds to hemoglobin and regulates its affinity for oxygen. In the lungs—where pH is high—ErythroMer's KC1003 locks up 2,3-DPG, allowing hemoglobin to capture oxygen; in peripheral tissues—where pH is low—KC1003 sheds 2,3-DPG, promoting oxygen release from hemoglobin (see graphic, p. 19).

> **"You can't just squirt [hemoglobin] into people."**
>
> **Allan Doctor**,
> University of Maryland School of Medicine

"We've imitated the mechanism in normal red cells for optimizing oxygen transport from lungs to tissue," Doctor says. Nitric oxide can still cross the artificial membrane and bind to hemoglobin inside, but it does so very slowly. "The principal idea is not to interfere with the signaling between red blood cells and blood vessels," he says. In other words, the goal is to not interrupt the normal patterns of vasoconstriction and vasodilation.

ErythroMer is still in the early stages of testing. The latest preclinical data demonstrated effective oxygen delivery in mice that had 70% of their blood volume replaced with ErythroMer. And in rabbits, when half of their blood volume is removed, infusing fluid containing ErythroMer resuscitated the animals, as real blood does. Doctor hopes to perform an initial safety test of ErythroMer in healthy humans, perhaps before the DARPA grant ends in 4 years.

KaloCyte's product isn't the only encapsulated hemoglobin around. In Japan, a team led by chemist Hiromi Sakai at Nara Medical University has done something similar by enclosing hemoglobin in lipid vesicles. These HbVs, or hemoglobin vesicles, are simpler than ErythroMer, without its membrane additions, but the product is further along in development.

A phase 1 safety trial in men was cut short by the pandemic coronavirus in 2020, but initial results appeared encouraging. Side effects included rash and fever, but they were temporary, and there were no clinically significant changes in participants' vital signs. "This finding suggests that, compared with the first generation of HBOCs, HbVs have less potential, if any, for myocardial infarction," wrote Sakai and his colleagues in a 2022 research letter.

OTHER INNOVATIVE IDEAS to replace red blood cells are also still being explored. A company called Hemarina is developing a product that makes use of a free hemoglobin found in the blood of a marine worm species.

What are the odds any of these new HBOCs cracks the blood substitute code? At least one expert scarred by the field's failures is skeptical. "A sheathed hemoglobin will probably fill too much space [in a blood vessel] and not carry enough oxygen to be useful," says John Hess, a physician and University of Washington professor of laboratory medicine. A retired U.S. Army colonel, Hess ran the army's blood product development program from 1991 to 2001. He halted the military's work on hemoglobin products in 1997. "I'm not saying there cannot be a safe HBOC," he adds. "I certainly could not see a pathway to one, and shut down the army program on that basis."

Others see more promise. "We are close enough in knowledge with how these products work that with the appropriate financing, one or more will be on the market," Vandegriff says. "It remains a highly unmet medical need."

After all, as physicians have developed new and better ways to help people threatened by severe bleeding, the demand for an emergency blood substitute has only gotten stronger. "There isn't actually enough O-negative blood to just give everybody," Doctor says. "You need blood that's shelf stable and universal donor."

He hopes ErythroMer will prove the doubters like Hess wrong. For now, Doctor monitors the health of his rabbits. ■

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