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[**How do scientists make artificial blood? How effective is it compared with the real thing?**](http://www.scientificamerican.com/article.cfm?id=how-do-scientists-make-ar&print=true)

**Robert M. Winslow of the University of California at San Diego replies:**

"The concept of 'artificial blood' sounds simple, but it isn't. When William Harvey first described the circulation of blood in 1616, scientists starting thinking about whether blood could be removed and replaced by other liquids, such as wine and milk, for example. They thought that by doing so, diseases could be cured and even that personalities could be changed. Obviously, there were some interesting but disappointing experiments!

"Modern efforts to produce artificial blood were spurred on by the military in World Wars I and II and, more recently, by the discovery in the early 1980s that HIV could be transmitted by blood transfusion. Blood is now safe, thanks to improved collection and screening by blood banks. But it still has to be cross-matched and can be stored for only a few weeks before it has to be discarded. If a solution that could replace blood were immediately available, if it were completely safe, and if it could be stored for long periods, it would be extremely useful in emergencies, disaster and wars--not to mention in countries where blood is not collected and stored as it is in the U.S and western Europe.

"Blood does many things, of course, and artificial blood is designed to do only one of them: carry oxygen and carbon dioxide. No substitutes have yet been invented that can replace the other vital functions of blood: coagulation and immune defense. Therefore, the replacement solutions being developed today are more accurately described as oxygen carriers. There are basically two types of oxygen carriers, which differ in the way they transport oxygen. One is based on perfluorochemicals, the other on hemoglobin.

"Perfluorochemicals are inert materials that can dissolve approximately 50 times more oxygen than blood plasma, the liquid that surrounds the red cells. Perfluorochemicals are cheap to produce and are completely free of biological materials so there is no risk of infectious agents contaminating them. In order to work, however, they must be combined with other materials that enable them to mix in with the bloodstream. These companion materials are fatty compounds known as lipids. They take the form of an emulsion, a suspension of extremely small particles in a liquid that can be injected into a patient. One such lipid product was approved by the Food and Drug Administration, but it has not proved successful, because the amount that can be administered is not enough to achieve a significant benefit. Improved versions of perfluorocarbon emulsions are being developed but have not yet reached the market.

"Hemoglobin-based oxygen carriers (HBOCs) utilize the same oxygen-carrying protein molecule found in blood. Oxygen bonds chemically to the hemoglobin, whereas it dissolves only into the perfluorocarbon emulsions. HBOCs differ from red blood cells in that the hemoglobin is not contained within a membrane. The membrane of a red blood cell contains the antigen molecules that determine the 'type' of the blood (A, B, AB or O). Because HBOCs have no membranes, they do not need to be cross-matched by type and can be given to any patient without previous testing. In addition, these artificial oxygen carriers can be stored for long periods, greatly simplifying the work of the blood bank. Best of all, HBOCs can be used in situations and locations where real blood is not available, as at disaster sites, underdeveloped countries or battle zones.

"Two main problems arise when hemoglobin is removed from the red blood cells; these problems account for the large amount of scientific research that has been conducted so far in this area. First, the red cell membrane protects hemoglobin from degradation and protects tissues from the toxic effects of free hemoglobin. Second, when oxygen is being delivered by a cell-free carrier instead of red blood cells, complex biological mechanisms alter the flow through the smallest blood vessels (the arterioles and capillaries). Major advances have been made in overcoming both of these problems, and several HBOC products are now in advanced human trials. It is anticipated that in the next one to two years the first of these products will become available to physicians for use in patients.

"The second part of the question, regarding the efficacy of oxygen carriers, is difficult to answer. From the discussion above, it is clear that real blood and artificial blood are not strictly comparable, so controlled comparisons are tricky. The Food and Drug Administration and the National Institutes of Health have held two major conferences to address how these new products should be developed. A provisional answer is that if the artificial product can reduce the use of blood, it will achieve a useful goal. But based on animal studies, many of us working in the field believe that HBOCs will perform their specialized function--delivery of oxygen to tissues--even better than blood."

Winslow suggests the following for further reading:

*Hemoglobin-Based Red Cell Substitutes*. Robert M. Winslow. Johns Hopkins University Press, 1992.

Blood Substitutes--A Moving Target. Robert M. Winslow in *Nature Medicine,* Vol. 1, No. 11, pages1212-1215; 1995.

Blood Substitutes. Robert M. Winslow in *Science & Medicine,* Vol. 4, No. 2, pages 54-63; 1996.

**T.M.S. Chang is the director of the Artificial Cells and Organs Research Centre and is professor of Physiology, Medicine and Biomedical Engineering at McGill University in Montreal. He offers another perspective on the question:**

"Hemoglobin is the protein in red blood cells that is responsible for carrying oxygen from the lung to the other tissues. Therefore, the present approach for making blood substitutes is to use hemoglobin extracted from red blood cells. Raw hemoglobin extracted from red blood cells cannot be used as a blood substitute, however. Each hemoglobin molecule consists of four subunits, known as tetramers. When infused into the body, a hemoglobin molecule breaks down into potentially toxic half molecules, or dimers. There are also other problems related to hemoglobin in free solution. The challenge is to modify hemoglobin to allow it for use as blood substitutes.

"The first-generation hemoglobin blood substitutes rely on molecular modifications of hemoglobin, either by chemically cross-linking the molecules or by modifying them using recombinant DNA technology. So-called bifunctional agents can cross-link the hemoglobin molecules to one another to form polyhemoglobin. The cross-linked hemoglobin molecules are stable and do not break down. Some bifunctional agents can also cross-link each hemoglobin molecule internally to prevent its breakdown into dimers. Recombinant technology applied to the bacterium *E. coli* can produce altered hemoglobin molecules that do not break down into half molecules. Hemoglobin can also be cross-linked to soluble polymers to form so-called conjugated hemoglobin. All the above modifications also result in blood substitutes that have a greater ability to release oxygen to the tissues than do red blood cells.

"Unlike red blood cells, blood substitutes can be pasteurized, filtered and chemical-cleansed to make them sterile. These procedures remove microorganisms responsible for diseases such as AIDS and hepatitis. Because the substitutes do not have cell membranes with blood-group antigens, cross-matching and typing are not required before use. This saves time and facilities and allows on-the-spot transfusion. Furthermore, blood substitutes can be stored for more than one year, as compared with about one month for donor blood stored using standard methods.

"On the other hand, these first-generation blood substitutes can stay in the body's circulation only for about 20 to 30 hours (a typical red blood cell lasts about 100 days). Thus, their present role is restricted to short-term applications. For example, substitutes are being tested in humans for replacing blood lost during some cardiac, cancer, orthopedic and trauma surgeries. Another promising application is to ameliorate the effects of severe bleeding in traumatic injuries from accidents, disasters or wars.

"Clinical trials in humans are ongoing using products from a number of companies. In the case of polyhemoglobin, Northfield is now in Phase III (large-scale efficacy) clinical trials that infuse up to 5,000 milliliters of blood substitutes into surgical patients. The company is using pyridoxalated glutaraldehyde cross-linked human hemoglobin. Biopure is in Phase II (small-scale efficacy) clinical trials using pyridoxalated glutaraldehyde cross-linked bovine hemoglobin. Hemosol is in Phase II clinical trials in surgical patients, using a new cross-linker to form a molecule known as o-raffinose cross-linked human polyhemoglobin. In the case of intramolecularly cross-linked hemoglobin, Baxter is now in Phase III clinical trials in a large number of surgical patients; the company is using Diaspirin cross-linked human hemoglobin. Somatogen is now deep into their Phase II clinical trials with their recombinant human hemoglobin. In conjugated hemoglobin, Enzon is now in Phase II clinical trials, and Apex is now in Phase I (safety) clinical trials.

"The basic ideas of cross-linked hemoglobin and encapsulated hemoglobin date back to the 1960s (see T.M.S. Chang in *Science,* Vol. 146, page 524; 1964, and H. F. Bunn and J. H. Jandl in the *Transactions of the Association of American Physicians,* Vol. 81, page 147; 1968). Concentrated efforts to develop blood substitutes for public use only seriously started after 1986 because of public concerns regarding HIV in donor blood. Unfortunately, a product must undergo years of research and development followed by clinical trials before it is ready for use in patients. It will take at least another one to two years for blood substitutes to be available for routine use. Had there been a serious development effort in the 1960s, blood substitutes would have already been available in 1986. As it is, the public has continued to be exposed to the potential, though extremely rare, hazard of HIV in donor blood.

"The present, first-generation blood substitutes are mainly effective for short-term uses because of their brief circulation time. They also do not have the enzymes needed to protect the body against oxidants such as oxygen radicals. Unchecked, oxygen radicals may cause reperfusion injuries and other problems. Enzymes are also important in preventing hemoglobin from being oxidized to methemoglobin, which cannot carry oxygen. Researchers are studying ways to solve this problem, including cross-linking the required enzymes to hemoglobin or further modifying the molecular structure of hemoglobin. These advances will appear in second-generation blood substitutes.

"Even in those second-generation substitutes, the hemoglobin molecules will not be protected by a red blood cell membrane. Thus, researchers are working on more complicated, third-generation blood substitutes that will encapsulate hemoglobin and the required enzymes inside artificial red blood cells. One method is to encapsulate hemoglobin inside lipid vesicles about 0.2 microns (millionths of a meter) in diameter. This technique also increases the circulation time. A more recent approach is to use nanotechnology to encapsulate hemoglobin and enzymes inside biodegradable polylactic acid membrane nanocapsules some 0.15 microns in diameter.

"The first modified-hemoglobin blood substitutes should soon be ready for use in clinical applications. Yet researchers are now facing many of the same problems as in the 1960s and 1970s. Granting agencies focus their resources to national priorities; blood substitutes research is not generally considered an urgent item. At the same time, private industry does not normally support the kind of long-term R&D needed to improve the present, imperfect substitutes. Developers of blood substitutes have formed an international network--the International Society for Artificial Cells, Blood Substitutes and Immobilization Biotechnology (www.physio.mcgill.ca/artcell/isabi.htm)--to promote their effort and encourage national committees and commissions around the world to include blood substitutes as a priority area in national medical policies.

"In addition to the hemoglobin substitutes discussed so far, there is another type of blood substitutes based on perfluorochemicals, synthetic fluids in which oxygen can dissolve. Perfluorochemicals are made into fine emulsions for use as oxygen carriers. Their biggest advantage is that they are synthetic materials and so can be produced in large amounts; also, their purity can be more easily controlled. On the other hand, perfluorochemicals have a much lower capacity for carrying oxygen than does hemoglobin, so the patient must breathe an oxygen-rich air mixture.

"Improved fluorochemicals have recently made it possible to use a higher concentration of perfluorochemicals without causing medical complications. Alliance has developed a blood substitute based on perfluoroctylbromide (C8F17Br) with egg yolk lecithin as the surfactant. The company is now carrying out Phase II clinical trials to delay the need for blood transfusion in surgery, especially when used with autologous blood transfusions (restoring blood previously taken from the patient). At present, the safe volume of blood substitute is limited to 500 to 1,000 milliliters. A group at HemoGen has developed perfluoro-dichoroctane (C8F16Cl2) with triglyceride and egg yolk lecithin as surfactant. One of the potential uses of perfluorochemicals is for those patients whose religious beliefsreligion does not allow them to use donor blood or product prepared from donor blood.

"Further information about blood substitutes is available on the Web from Artificial Cells & Organs Research Centre."