Just add water

Thanks to a sugar found in yeast, it may be possible to provide 'freeze-dried' blood cells to treat injured soldiers. The technique could also find applications in the cell-biology lab. Geoff Brumfiel reports.

J. VELLACOTT/AP

The US military is one of the most bloodthirsty organizations on Earth. The troops hold regular blood drives to keep a required 70,000 units on hand at all times; and a veritable small army is needed to transport this blood to remote battle zones in Iraq or Afghanistan. It can take more than a week for refrigerated supplies to reach the field. That's a critical delay, explains Joe Bielitzki, a programme manager at the Defense Advanced Research Projects Agency (DARPA), which oversees speculative research for the Pentagon. "Typically, by that point nobody's bleeding," he says.

Ideally, the military needs blood supplies that can be stored and moved easily. So DARPA has assembled a team of US researchers to develop technology that will allow blood to be freeze-dried, rather like instant coffee, and stored at room temperature for years rather than days or weeks. It might seem an impossible task, but in just three years the group has achieved an impressive result — it has extended the shelf-life of human blood platelets, cells critical to wound healing, from a week to almost two years[1].

Medical applications aside, members of the DARPA team claim that their work could have wider uses in the laboratory. For example, it may be possible to store experimental cell lines for years at a time on a shelf, rather than in expensive liquid-nitrogen freezers. And it could become easier to ship cells of all types, including precious embryonic stem cells, to and from labs around the world.

Cells and tissues can potentially be stored for long periods by freezing them, which slows their metabolism, and by dehydrating them, which removes the one ingredient essential to all biological processes — water. But more often than not, these techniques can also kill a substantial number of the cells. On a remote battlefield, freeze-dried packets of blood would be ideal — stable, lightweight and just requiring water to be reconstituted. But this means that the cells must survive the freezing and drying steps.

Kill or cure

Putting cells into a freezer exposes them to all sorts of danger. As water inside and outside the cells cools, ice crystals form and their jagged edges can rip the cells apart. Partial dehydration is a side effect of cooling, and if it is not controlled it causes the cells' membranes to shrivel and stick together. Rapidly cooling cells to liquid-nitrogen temperatures can prevent lethal ice crystals from forming by transforming the watery cytoplasm into an amorphous glass. But even if the cells survive freezing, the deathblow often occurs during thawing and rehydration, when they are subjected to new stresses.

Improving these techniques is as much art as science, according to Juan de Pablo, a chemical engineer at the University of Wisconsin at Madison. Researchers typically treat their samples with chemicals, such as dimethyl sulphoxide, which help to stabilize the cell membrane and contents at low temperatures but have no protective effect during drying. The results are mixed, de Pablo says, and depend largely on the type of cell being preserved and the particulars of the technique, including cooling and warming rates. The chemicals, most of which are toxic, can trigger cell death themselves, and have to be washed away before the cells can be used.

Enter trehalose, a simple sugar found in organisms such as baker's yeast (*Saccharomyces cerevisiae*) and brine shrimps (*Artemia* species) that allows them to survive severe dehydration. Its properties are almost miraculous, says John Crowe, co-director of the Center for Biostabilization at the University of California, Davis, who has devoted most of his career to its study. "We've spent a lot of time looking at how it works," he says. It also has the virtue of being naturally non-toxic.

In the early 1980s, curiosity led Crowe and his team to begin probing the biophysical properties of trehalose. Then in 1994, the US Department of Defense offered funding

K. GULDBRANDSEN/SPL

G. PALACIO/GETTY IMAGES

Sweet success: treated with a sugar called trehalose, some blood cells can be freeze-dried and kept for months or even years. This could make preservation of fresh blood (left) in liquid nitrogen (above) a thing of the past.

to Crowe and another researcher at Davis, Fern Tablin, for new studies into trehalose and blood platelets.

"Platelets had never been available on the battlefield," says Tablin, who now co-directs the biostabilization centre with Crowe. Platelets cause blood to clot — essential for wound healing — but this means that they have to be stored separately from blood plasma at room temperature. Away from the battlefield, platelets normally have to be discarded after five days because of the risk of bacterial contamination.

After two decades of probing the structure of trehalose and how it interacts with cellular components, Crowe's team has worked out the main ways in which the sugar protects cells during drying and freezing. First, it replaces some of the water in the cell so that, as the temperature drops, trehalose prevents uncontrolled dehydration. Second, the sugar stabilizes the cell's membrane and stops it from disintegrating. Then, as the temperature falls below water's freezing point, trehalose forms an amorphous glass inside the cell, which prevents ice crystals from forming. If the cell is subsequently fully dehydrated, the glass becomes stable, and the cell can be kept at room temperature for long periods of time.

The main obstacle is that cell membranes are typically impermeable to sugars. "The big hurdle in the field is getting these molecules into the cells," says Mehmet Toner, a biomedical engineer at Harvard Medical School in Cambridge, Massachusetts. Toner's solution is to use pore-like proteins that dissolve in the cell membrane and can be opened and closed using chemical signals. Trehalose flows easily through these large pores, and Toner's group preserved 70% or more of human skin and connective-tissue cells using this technique[2]. Another option is to genetically modify the cells to manufacture trehalose on their own[3]. But genetically modified cells have yet to be conclusively shown to work, according to Crowe.

"Stem cells are very finicky — when they come under stress they usually kill themselves."

Back to life

A simpler option is to warm the cells slightly to encourage endocytosis, a process that allows them to 'eat' the trehalose, before freezing the cells in a mixture of trehalose and proteins from blood plasma and drying them under vacuum. Using this technique, Crowe and Tablin have achieved 90% recovery of the platelets after rehydration — even after two years in storage[1]. They have also scaled up the technique to produce whole bags of platelets at concentrations required for real transfusions.

Bielitzki is convinced that these techniques will, within a matter of years, allow soldiers to carry their own supply of dried platelets into battle for use in an emergency. He also sees therapeutic uses for the cells for patients undergoing chemotherapy who have vastly reduced platelet counts. Being able to store bags of your own platelets in advance of treatment — and for longer periods — would ease transfusion problems later.

But using trehalose to preserve other cell types could prove much more difficult. Platelets are fairly simple cells that lack nuclei. Efforts to preserve more complex cells have so far had limited results. "There is still a way to go before we can desiccate these cells," says Toner. Key issues to be resolved are whether trehalose can get inside and stabilize the nucleus, and if it plays the same role inside the densely packed nucleus as it does in the cell's cytoplasm. Another important question is whether trehalose can prevent cell suicide occurring in more complex cells as a 'programmed' response to any mechanical or physical stress.

Crowe's group has so far been able to store red blood cells, which also lack nuclei, for about a week. "And even that is a big accomplishment," he adds. At the University of Wisconsin, de Pablo and his colleague Sean Palecek have been trying to store desiccated versions of the university's lines of embryonic stem cells, produced in the lab of James Thomson. "Stem cells are very finicky," says Palecek. "When they come under stress they usually kill themselves." To date, they have been able to preserve only about 10% of the stem cells they freeze. But the researchers believe that computer modelling might help them to understand how trehalose improves cell viability, and so refine their methods.

Human egg cells are also targets for the trehalose treatment. Eggs are much harder to freeze and recover than sperm or even embryos, so better survival rates would help women undergoing *in vitro* fertilization. In 2002, Toner and his colleagues showed that using trehalose both inside and outside a frozen egg greatly increased the chances of recovery[4].

Ultimately, Crowe and Tablin believe that trehalose may revolutionize the techniques used to store conventional cell lines in the lab. Cumbersome vats of liquid nitrogen would no longer be needed to preserve samples and, in many cases, it would be possible to send cell lines between labs with ease, says Tablin. "It sure would be nice to ship cells cross-country in a envelope," Bielitzki muses. ■

Geoff Brumfiel is *Nature*'s Washington physical sciences correspondent.

1. Wolkers, W. F., Walker, N. J., Tamari, Y., Tablin, F. & Crowe, J. H. *Cell Preservation Technol.* **1,** 175–188 (2003).
2. Eroglu, A. *et al. Nature Biotechnol.* **18,** 163–167 (2000).
3. Guo, N., Puhlev, I., Brown, D. R., Mansbridge, J. & Levine, F. *Nature Biotechnol.* **18,** 168–171 (2000).
4. Eroglu, A., Toner, M. & Toth, T. L. *Fertil. Steril.* **77,** 152–158 (2002).