NEWS

New Prospects for Putting Organs on Ice

After a lull, scientists are again exploring vitrification and other techniques for deep-freezing tissues and organs

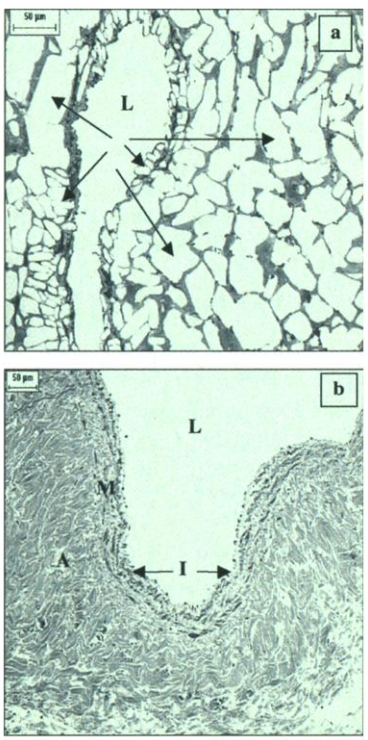
In the movie *Vanilla Sky*, Tom Cruise has his broken body frozen in the hope that he'll someday be revived and healed. In reality, cryonics, as this practice is known, remains the most speculative science fiction. Researchers have failed for decades to deep-freeze and thaw most tissues—let alone organs or animals—without damaging them, often seriously. But recently, a few cryobiologists have celebrated successes with ovaries and complex tissues like vascular grafts.

The work has sparked hope that donated organs can eventually be banked for longer than the current few days, which would buy time for distributing them and finding recipients who are good immunological matches. And as progress is made engineering artificial livers, bladders, and other tissues containing living cells, the need for better preservation techniques is expected to grow (see Viewpoint on p. 1009). "Each company at some point will need to store and transport their product," says Mike Taylor of Organ Recovery Systems in Charleston, South Carolina.

Currently, transplant surgeons perfuse whole organs with a special solution that enables them to be banked just above 0°C for up to a few days. Ideally, they would like to preserve organs at –196°C, the boiling point of liquid nitrogen—so cold that molecular motion virtually stops and tissues cease to decay. Half a century ago, that seemed within easy reach. Scientists found that blood and sperm could survive such deep freezing if mixed with glycerol. The glycerol lowers the cells' freezing point and keeps them from getting lethally salty when they do freeze and water diffuses out. And since 1972, embryos have been frozen with liquid nitrogen and later successfully implanted.

Organs, however, don't hold up so well below the freezing point. Water leaked from cells during freezing forms ice crystals in the space between cells, and this ice destroys fragile structures such as ducts and blood vessels. Sometimes pieces of organs, such as pancreatic islet cells, work adequately after freezing. But larger, more complex organs such as kidneys don't function properly when sufficiently ravaged.

As a way around this problem, some researchers turned to vitrification, or "ice-free" cryopreservation. The idea is to fill the organ with a viscous fluid that turns into a glassy (not crystalline) solid at low temperatures. This reduces the problems of ice, but toxicity of the cryopreservant can still damage organs. And ice crystals tend to form as vitrified tissue—especially large pieces—is warmed. Partial success came in the 1980s, when Red Cross scientist Greg Fahy showed that rabbit kidneys could withstand the high concentrations of cryoprotectants needed to vitrify these organs. They worked when reimplanted, but Fahy had only cooled them to –3°C. Many experiments later, it's clear that "vitrification is very, very complicated," says cryobiologist David Pegg of the University of York, U.K., and in most labs vitrification of large organs has been on hold.



Vanquishing ice. Damaging ice crystals form in a rabbit jugular vein that's frozen (*top*), but they're absent from a vein that was vitrified.

CREDITS: Y. C. SONG *ET AL.*, *NATURE BIOTECHNOLOGY* (18 MARCH 2000)

Recently, cryobiologists have had better luck studying vitrification on a smaller scale. For example, fine-tuning the solutions that are injected into the organs, as well as heating and cooling rates, minimized injury to 2- to 3-centimeter-long pieces of rabbit veins, Taylor's team at Organ Recovery Systems reported in the 18 March 2000 issue of *Nature Biotechnology*. The vessels retained 80% of their function when dosed with drugs that cause them to contract, compared to 20% following simple freezing. When implanted in rabbits, the grafts appeared to work normally. "We're the first to demonstrate [that vitrification works better than freezing] in reasonably complex tissue," Taylor says. Two other groups have recently shown that they can vitrify corneas, getting much less damage than from freezing. But the scientists have yet to show whether these corneas function in vivo.

Roger Gosden and colleagues at McGill University in Montreal have had success even with conventional freezing with a small organ, they reported last month in *Nature*. They froze rat ovaries, fallopian tubes, and attached blood vessels in liquid nitrogen and transplanted them into genetically identical rats whose ovaries had been removed. The animals ovulated. Some ice damage occurred, so vitrification might be even more successful, suggests Gosden, now at the Jones Institute for Reproductive Medicine in Norfolk, Virginia.

Several groups are tackling problems that thwart both vitrification and conventional freezing. Pegg's group at York, for instance, is working on how to thaw tissues and avoid ice crystal formation. The team has developed a technique that Pegg says can evenly and quickly heat pingpong ball–sized clumps of cells embedded in gelatin to simulate large tissue. Taylor's group, meanwhile, is collaborating with Carnegie Mellon scientists on dosing tissues with iron compounds that are then excited with magnets to generate heat.

Taking a cue from nature, researchers are also using natural antifreeze proteins to help mop up crystals formed during freezing and thawing. Many organisms, from carrots to fish to beetles, produce proteins that latch onto and isolate growing ice crystals. The one drawback is that at temperatures well below 0°C, this system can backfire by causing ice crystals to form spikes that disrupt cells. Several companies are developing improved synthetic versions of these "ice blockers"; for example, Taylor's company hopes to produce smaller molecules that attach to ice crystals at the base as well as the face, which could prevent spike formation.

Fahy, meanwhile, has never given up trying to vitrify large organs. "He has soldiered the way on this for years and years," Pegg says. Now at a company called 21st Century Medicine in Rancho Cucamonga, California, Fahy has tested hundreds of vitrification solutions and patented the most promising ones. "Greg has always been tantalizingly close to getting it to work," says cryobiologist William Rall of the National Institutes of Health. The company Fahy works for receives funding from the Life Extension Foundation, which supports cryonics. Fahy says his work is strictly limited to cryopreserving organs. But he adds, "If I'm successful, perhaps it will remove some of the [cryonics] controversy."

–JOCELYN KAISER