LIVER TRANSPLANTATION

Supercooling human livers for transplantation

> Storing livers at low temperatures decreases their rate of metabolism

In 2014, a team of scientists presented a liver preservation technique based on supercooling without freezing that enabled the preservation of rat livers ex vivo for up to 4 days. However, the approach proved tricky to translate to humans as the livers froze. Now, an adapted protocol published in *Nature Biotechnology* by the same group has been used to preserve human livers ex vivo for up to 27 hours, three times longer than the clinical average, without any observable change in viability.

Owing to a shortage of viable donor livers for transplantation, there is an urgent need for extended preservation of livers ex vivo. Storing livers at low temperatures decreases their rate of metabolism, thereby reducing organ deterioration, but subzero storage leads to ice formation, which causes extensive injury. "Despites the successes in rat livers, the likelihood of ice formation during supercooling increases with volume," explains lead author Reinier de Vries. This understanding led the authors to question whether this technique could be applicable to human livers, which are 200 times larger than rat livers.

The new protocol was tested using five human livers, all of which were deemed unfit for transplantation. First, the livers were initially maintained under standard hypothermic conditions (4 °C). Next, 3 hours of subnormothermic machine perfusion (SNMP) at 21 °C maintained a low metabolic demand that enabled viability testing. Then, the perfusion temperature was gradually lowered, before approximately 1 hour of hypothermic machine perfusion (4 °C) with a standard preservative solution that was supplemented with cryoprotective agents. The livers were then supercooled and stored ice-free at −4 °C for 20 hours. Finally, the cryoprotective agents were washed out and the livers were recovered by SNMP.

The team made three major adaptations to their 2014 protocol to prevent ice nucleation in the livers. First, they de-aired the storage solution bag before supercooling began — this process eliminated air–liquid interfaces, which are sites of ice nucleation. Second, they added two cryoprotective agents to the supercooling preservation solution that was used for rat livers: trehalose (in addition to polyethylene glycol) and glycerol. Trehalose stabilizes the cell membrane at subzero temperatures, and glycerol supplements 3-O-methyl-D-glucose, another important additive that preserves the intracellular compartment. Finally, they added a hypothermic machine perfusion step to enable the preservation solution to gradually and homogenously distribute throughout the tissue, avoiding liver injury.

To test the post-supercooling viability of the livers, the researchers compared the parameters of the two SNMP steps, one before and one after supercooling. Analysis of adenylate energy charge — considered to be a key metric of liver viability — bile acid production and oxygen uptake rate showed no statistical differences before and after supercooling.

Finally, the team simulated transplantation in three human livers using ex vivo normothermic reperfusion with blood. Post-supercooling reperfusion increased the metabolic rate, bile and urea production and lactate metabolism, and a stable adenlyate energy charge was observed. The authors therefore conclude that the livers withstood the stress of simulated transplantation.

Other liver preservation techniques exist. "The current clinical standard is static storage on ice, and I think our method is much better in terms of preservation duration," says Korkut Uygun, senior author of the study. An alternative approach is warm machine perfusion, in which a donor liver is preserved at physiological temperature (37 °C) and perfused with oxygenated blood. Supercooling and machine perfusion are considered complementary by the authors. "Our approach actually builds on machine perfusion as we have four separate perfusion steps in our protocol", says Uygun.

Going forward, the group hopes to extend the preservation duration, and to test the protocol on other organs. "Our hope and plan is of course to bring it to the clinic," concludes Uygun, "and there is more to be done on that front to develop a device that can be used clinically and easily."

Jordan Hindson

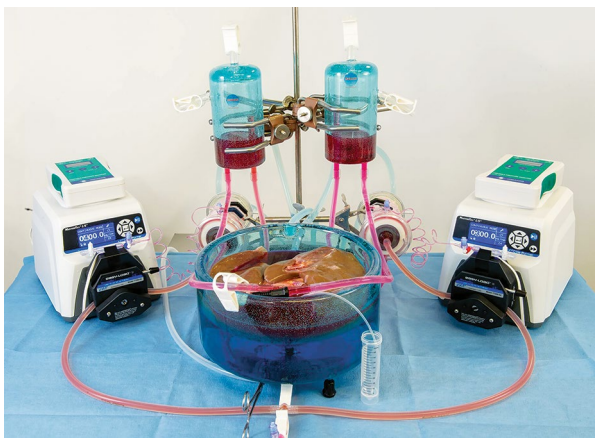

Image courtesy of K. Uygun and R. de Vries, The Center for Engineering in Medicine of Massachusetts General Hospital and Harvard Medical School, USA.

ORIGINAL ARTICLE de Vries, R. J. et al. Supercooling extends preservation time of human livers. *Nat. Biotechnol.* https://doi.org/10.1038/s41587-019-0223-y (2019)
RELATED ARTICLES Berendsen, T. A. et al. Supercooling enables long-term transplantation survival following 4 days of liver preservation. *Nat. Med.* **20**, 790–793 (2014) | Ray, K. Preserving the liver for transplantation. *Nat. Rev. Gastroenterol. Hepatol.* **15**, 327 (2018)