Press Release

Keio University

October 2, 2019
Keio University School of Medicine

Resuscitation of Marginal Donor Organs Following Circulatory Arrest Development of Hydrogen-Rich Organ Preservation Solution Using Hydrogen-absorbing Alloy Canisters

A research team at the Keio University School of Medicine, led by Project Professor Eiji Kobayashi and Associate Professor of Cardiology Motoaki Sano, through joint research with Doctorsman Co., Ltd. (President So Hashimoto), has shown that by quickly injecting hydrogen gas into an organ preservation solution[1], an injured organ removed from an older miniature pig (donor) can be resuscitated and used as a transplant organ, even after circulation has ceased for a certain period of time.

The research team has developed a new method for safely producing, in a matter of minutes, a hydrogen-rich organ preservation solution that contains dissolved hydrogen gas by instantly injecting the solution with hydrogen gas from a canister containing a hydrogen storage alloy[2].

The transplantation of organs from marginal donors[3], including donation after circulatory death, is an important countermeasure to make up for donor shortages and reduce waiting times for transplant recipients. With marginal organs, however, in addition to warm ischemia[4], cold preservation failure while in a preservation solution can cause pronounced ischemia-reperfusion injury(IRI)[5] upon transplantation, and there is a high probability of an organ going into a non-functional state (primary non-function) after transplantation. It was anticipated that developing a simple method for hydrogen supplementation to organ preservation solution could facilitate the recovery of damaged organ function ahead of transplantation as well as improve prognosis after a transplantation is complete.

The research team's newly developed method brings portability to hydrogen gas sources so that, in the event of an emergency, hydrogen gas can be quickly injected into an organ preservation solution at the site of donor organ procurement.

The results of this study are expected to play a role in expanding the use of donor organs that could not otherwise be used in conventional transplants.

The results of this study were published on October 1 (EST) in the online open access scientific journal *PLOS ONE*.

1. Background and outline of research

Improving the success rate of transplantation using marginal organs procured from donation after circulatory death is an important issue to make up for organ donor shortages and to reduce transplant waiting times.

Organs begin to undergo necrosis if they remain in the body for a certain period of time following circulatory arrest and often become unsuitable for transplantation in the period between procurement and transplantation. To prevent this, organs are cooled and stored in an organ preservation solution immediately following procurement from a donor, but with resumption of blood flow after transplantation, cryogenically preserved marginal organs are susceptible to damage from ischemia-reperfusion injury and have a higher incidence of primary dysfunction. This has been a significant barrier

to using marginal organs for transplantation.

Current organ preservation solutions are not able to reanimate damaged donor organs to a state where they can be used as transplantable organs. In recent years, hydrogen gas has been shown to have various biological effects, and clinical trials are being conducted to verify their effectiveness (Tamura et al., Circ. J., 2016, Katsumata et al., Circ. J., 2017; Tamura et al., Trials, 2017). Particularly in the field of organ transplantation, animal testing has shown that hydrogen gas improves the recovery of organ functionality following transplantation (Haam et al., J. Heart Lung Transplant., 2018; Ishikawa et al., Surg. Today, 2018; Tamaki et al., Liver Transpl., 2018; Uto et al., BMC Gastroenterol., 2019, etc.).

With the exception of the lungs, organs can be exposed to hydrogen gas by introducing hydrogen gas into a preservation solution. Since organs must be preserved immediately following their procurement, donor hospitals needed a simple technique for quickly producing a hydrogen-rich preservation solution.

Previously, hydrogen tanks, electrolyzers, and hydrogen generating agents were proposed as potential sources of hydrogen gas generation. But as organ procurement is often sudden and unexpected, it is impractical to keep hydrogen gas cylinders at the ready. Further, producing a hydrogen-rich organ preservation solution with an electrolyzer or hydrogen generating agent is a complicated process and takes between 24 and 48 hours to reach a hydrogen gas concentration of 1 ppm, rendering it unsuitable for emergency transplantation in a clinical setting.

In this study, we have developed a new device that instantaneously injects high-concentration hydrogen gas into a container of organ preservation solution. This device uses a small, portable hydrogen storage canister (see Fig. 1).

Using this technique, we have shown that a kidney previously removed from an older miniature pig following circulatory arrest and placed in a hydrogen-rich organ preservation solution can be resuscitated to a level that can maintain urinary excretory function when transplanted into another miniature pig.

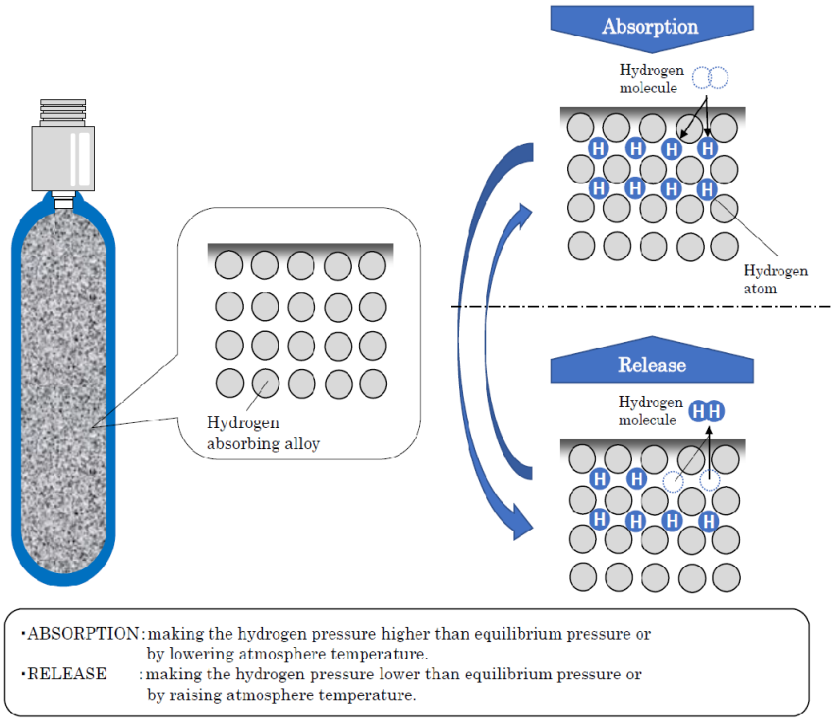

Fig. 1: How the hydrogen-absorbing alloy canister works
Hydrogen storage canisters absorb hydrogen when cooled or pressurized and release hydrogen when heated or decompressed.

2. Research Significance and Future Development

Using a hydrogen-injection device equipped with a hydrogen-absorbing alloy canister, we added hydrogen gas to an organ preservation solution (ET-Kyoto solution) that was cooled to 4°C. When the internal pressure of the container reached 0.06 MPa, the plastic bag containing the organ preservation solution was disconnected from the hydrogen storage canister and pressure control device. We then took the soft plastic bag in hand and shook it vigorously (for over 30 seconds), and opened the lid to return it to normal atmospheric pressure (see Fig. 2).

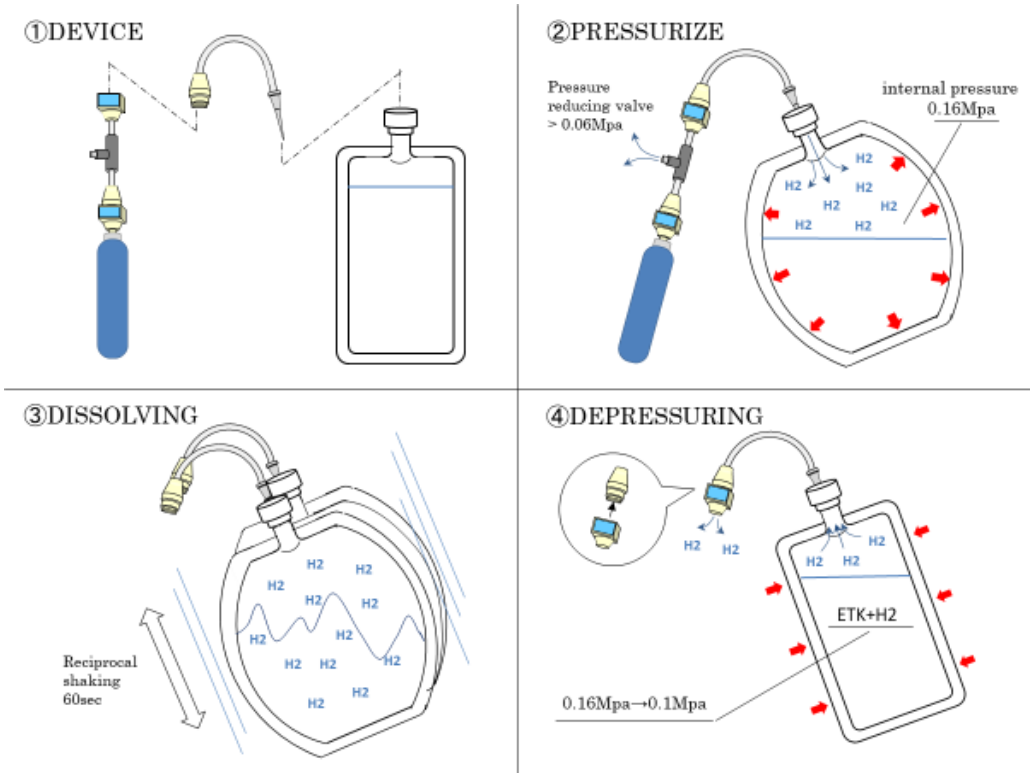

Fig. 2: Method for using a hydrogen-absorbing alloy canister to prepare a hydrogen-rich organ preservation solution

It takes just 4–5 minutes from initial assembly of this prefabricated device to produce a hydrogen-rich organ preservation solution. Decrease of the hydrogen gas concentration was extremely gradual in a preservation solution stored at 4°C under normal pressure, and a high concentration of at least 1 ppm could be maintained for up to 4 hours after injecting the hydrogen gas.

Further, the effectiveness of the hydrogen-rich organ preservation solution was verified in a porcine kidney transplant model where circulation had been stopped for a period of time. Porcine kidney transplant models have traditionally used young livestock pigs, but for this study, considering the age of the patients being studied, we used older miniature pigs as donors and recipients. After circulation had been stopped for 30 minutes, kidneys were procured from the donors and divided into left and right groups. Using a hydrogen-rich organ preservation solution for one group and a hydrogen-free organ preservation solution for the other, we washed away any blood that had accumulated in the organs over a 5-minute interval using a 1m perfusion solution drip. Compared with the hydrogen-free organ preservation solution, the hydrogen-rich solution made graft rinses faster, and analysis of the tissue showed dilation of the capillaries around the tubules as well as excellent rinsing effect on microthrombi.

Short-term observation following transplantation (through the sixth day following surgery) found that kidneys kept in the hydrogen-free storage solution were in a state of primary nonfunction and showed no sign of urinary excretory function after being stored for just one hour. For kidneys kept in the hydrogen-rich storage solution, urinary excretory function was observed even after being stored for as long as four hours.

These results show that this new method is effective for resuscitating marginal organs procured from donation after circulatory death, thus making them usable for transplantation. It also demonstrates that this method is safe and convenient, without risk of explosion in a clinical setting.

While this study focused on the acute phase following surgery, in the future we expect that it will be possible to increase the success rate of marginal organ transplantation by verifying the long-term preservation of kidney graft function under immunosuppressive treatment.

3. Notes

This study was carried out with support from Doctorsman Co., Ltd.

4. Research Paper

Title：Organ Preservation Solution Containing Dissolved Hydrogen Gas from a Hydrogen-absorbing Alloy Canister Achieves Better Function of Transplanted Ischemic Kidneys in Miniature Pigs
Japanese Title：水素吸蔵合金キャニスターを用いて作成した水素ガス充填臓器保存液は、循環停止ミニブタから摘出した重篤な虚血障害を受けた腎臓の移植後の予後を改善する
Authors：Eiji Kobayashi, Motoaki Sano
Journal：PLOS ONE (online version)

[Glossary]

[1] Organ Preservation Solution
Organ transplantation treatment is being carried out as a radical treatment for organs that have become dysfunctional. It is currently common to immerse a donor organ in a preservation solution and store it at low temperatures. However, a serious problem with this approach is that donor organs maintain healthy conditions only during short-term storage.

[2]Hydrogen Storage Alloy
An alloy that reversibly absorbs hydrogen when cooled or pressurized and releases hydrogen when heated or decompressed.

[3]Marginal Donor
Marginal donors are defined as donors who do not meet standard donor conditions. For living donor transplantation, marginal donors include, in addition to the elderly, anyone with high blood pressure, obesity, or diabetes. Due to a lack of donors, it is sometimes necessary to perform organ transplants using organs from marginal donors. Marginal donors also include donation after circulatory death.

[4] Warm Ischemia
Organ damage can occur if ischemia (cardiac or circulatory arrest) occurs at normal body temperature due to a lack of oxygen and other nutrients required for cell metabolism. This is known as warm ischemia, and its severity is proportional to the amount of time the body is without blood circulation.

[5] Ischemia-Reperfusion Injury (IRI)
Ischemia-reperfusion injury refers to tissue damage caused by the restoration of blood circulation to an ischemic organ. This can also be seen following an organ transplant.

*Please direct any requests or inquiries to the contact information provided below.

【Contact Information】
Keio University School of Medicine
Department of Cardiology
Associate Professor Motoaki Sano
TEL: +81(0)3-5843-6702
FAX: +81(0)3-5363-3875
E-mail: msano@keio.jp
http://www.cpnet.med.keio.ac.jp/

Keio University School of Medicine
Bridgestone Endowed Course for Regenerative Medicine
Project Professor Eiji Kobayashi
TEL: +81(0)3-5315-4090
FAX: +81(0)3-5315-4089
E-mail: organfabri@a2.keio.jp

【Source of this release】
Keio University
Shinanomachi Campus
Office of General Affairs: Suzuki / Yamazaki
35 Shinanomachi, Shinjuku-ku, Tokyo 160-8582
TEL: +81(0)3-5363-3611
FAX: +81(0)3-5363-3612
E-mail: med-koho@adst.keio.ac.jp
http://www.med.keio.ac.jp/en/

*A color version of this press release is available. Please contact the above address for more information.