Cross-Circulation for Extracorporeal Liver Support in a Swine Model

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Although machine perfusion has gained momentum as an organ preservation technique in liver transplantation, persistent organ shortages and high waitlist mortality highlight unmet needs for improved organ salvage strategies. Beyond preservation, extracorporeal organ support platforms can also aid the development and evaluation of novel therapeutics. Here, we report the use of veno-arterial-venous (V-AV) cross-circulation (XC) with a swine host to provide normothermic support to extracorporeal livers. Functional, biochemical, and morphological analyses of the extracorporeal livers and swine hosts were performed over 12 hours of support. Extracorporeal livers maintained synthetic function through alkaline bile production and metabolic activity through lactate clearance and oxygen consumption. Beyond initial reperfusion, no biochemical evidence of hepatocellular injury was observed. Histopathologic injury scoring showed improvements in sinusoidal dilatation and composite acute injury scores after 12 hours. Swine hosts remained hemodynamically stable throughout XC support. Altogether, these outcomes demonstrate the feasibility of using a novel V-AV XC technique to provide support for extracorporeal livers in a swine model. V-AV XC has potential applications as a translational research platform and clinical biotechnology for donor organ salvage. *ASAIO Journal* 2022; 68;561–570

Key Words: liver transplantation, normothermic machine perfusion, organ preservation, *ex vivo* organ support

Cirrhosis is associated with a global health burden of more than 1.3 million deaths annually.[1,2] Liver transplantation remains the only curative treatment, with over 8,000 transplants performed every year.[3] Despite overcoming impressive technical, immunologic, ethical, and administrative challenges, the shortage of suitable donor organs remains the major hurdle to meeting the demand for organ replacement. Current limitations in organ preservation and recovery strategies impose significant bottlenecks on transplantation, regenerative medicine, and therapeutic development.[4]

Normothermic machine perfusion (NMP) has gained momentum as an alternative method of organ preservation to traditional static cold storage, with the benefits of continuous delivery of oxygen and nutrients, clearance of metabolic waste, and opportunity to monitor graft viability *ex vivo* before transplantation.[5] In addition to the prospect of improving preservation capabilities and donor organ salvage, NMP has been proposed as a research platform for developing *ex vivo* therapies, such as defatting protocols, immunomodulation, RNA interference, and anti-inflammatory agents.[6,7] Early clinical trial and population data demonstrate decreases in graft injury and organ discard, as well as increases in preservation time.[8,9] Despite the provision of oxygen and circulatory support to the donor liver, these isolated single-organ support systems lack the ability to duplicate the myriad hemodynamic, hematologic, metabolic, endocrine, and other biochemical processes that help maintain homeostasis *in vivo*. We hypothesized that a system that can recapitulate a normal physiologic milieu for the extracorporeal liver would better enable organ rescue, recovery, and investigation of advanced therapeutic interventions.

A cross-circulation (XC) platform has the potential to offer physiologic support to an *ex vivo* donor organ within a homeostatic biosystem. Our group recently established a swine model of veno-venous XC for the maintenance and functional recovery of extracorporeal lungs.[10–13] In this study, we aimed to develop a parallel system for the support of *ex vivo* livers.

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In designing a XC configuration with the eventual goal of supporting *ex vivo* human donor livers, we considered the clinical challenge of reperfusion-associated instability in the recipient or host—a phenomenon seen in up to a third of liver transplant patients.[14–16] Thus, in this feasibility trial, we describe a large animal model of XC using a veno-arterial-venous (V-AV) circuit configuration to provide both physiologic support to the extracorporeal liver and concurrent circulatory assistance to the bioreactor host (Figure 1A, B). This is the first study to-date exploring the use of XC for the support of extracorporeal livers.

Materials and Methods

Study Design

This feasibility study was designed to assess the ability of the V-AV XC system to maintain the quality and function of explanted swine livers for 12 hours. We hypothesized that the V-AV XC system could provide physiologic support to the donor liver and hemodynamic support to the bioreactor host (Figure 1B). Twelve hours was deemed a sufficient duration to assess the performance of the system since normothermic explanted livers are susceptible to injury, dysregulation, and

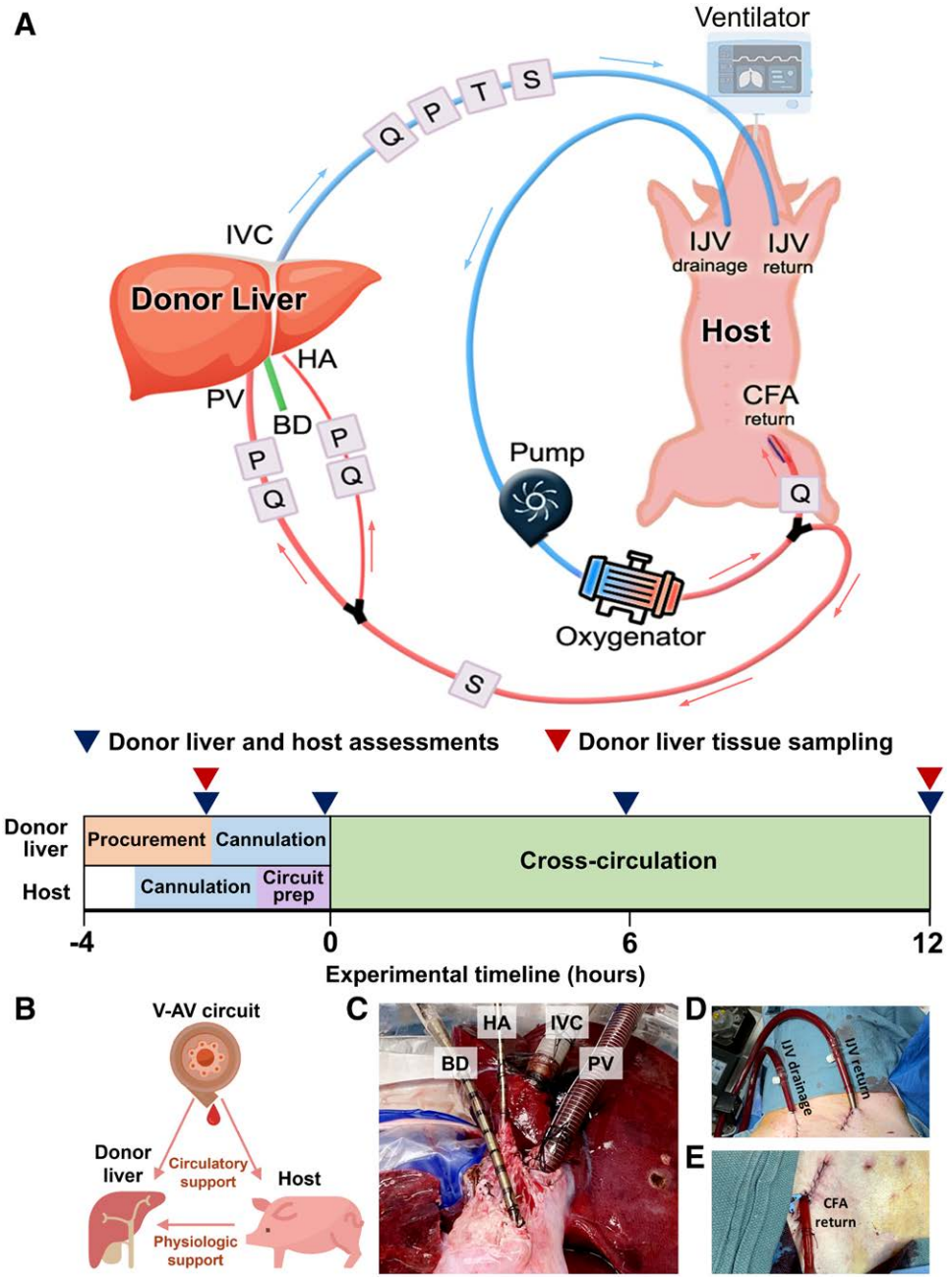

Figure 1. Experimental overview of the liver V-AV cross-circulation system. **A**: Circuit schematic and experimental timeline. **B**: Physiologic support framework. **C**: Donor liver cannulation of HA, PV, IVC, and BD. **D**: Host cannulation of bilateral IJV and (**E**) left or right CFA. BD, bile duct; CFA, common femoral artery; HA, hepatic artery; IJV, internal jugular vein; IVC, inferior vena cava; P, pressure transducer; PV, portal vein; Q, flow probe; S, oxygen saturation probe; T, temperature probe; V-AV, veno-arterial-venous. full color online

loss of synthetic function without adequate support.[17,18] The study was conducted with the minimum number of animals (n = 4) to demonstrate feasibility and reproducibility between livers and hosts, and across experimental time points.

Animals

Eight closed colony-bred male Yorkshire x Landrace swine (four donor-host pairs; Oak Hill Genetics, Ewing, IL) were utilized in this study. Animals were 3–5 months of age, with a weight range of 52–68 kg for liver donors, and a weight range of 55–88 kg for XC hosts. The study was approved by the Institutional Animal Care and Use Committee at Vanderbilt University Medical Center and conducted in accordance with the US National Research Council of the National Academies "Guide for the Care and Use of Laboratory Animals, Eighth Edition."

Donor Liver Procurement

Anesthetic induction was achieved with ketamine (2.2 mg/kg intramuscular [IM]), tiletamine (4.4 mg/kg IM); Zoetis (Parsippany, NJ), xylazine (2.2 mg/kg IM), and isoflurane (1–3% inhaled). Subjects were intubated and standard anesthetic monitors were placed. Inhaled isoflurane (1–3%) and intravenous (IV) fentanyl (0.03–0.1 mg/kg/h) were used for anesthetic maintenance and analgesia. Animals were prepared and draped in standard fashion and antibiotics were administered (cefazolin, 20 mg/kg; enrofloxacin, 5 mg/kg). Following midline laparotomy, mobilization of the liver, and standard dissection of the porta hepatis, a heparin bolus (30,000 U) was administered IV. The common bile duct, common hepatic artery (HA), portal vein (PV), infrahepatic inferior vena cava (IVC), and suprahepatic IVC were ligated before liver explant. No *in situ* flush was performed as the livers were immediately flushed *ex situ* as described below. Total cold ischemia time (132 ± 18 min) was attributed to back table preparation for *ex vivo* perfusion and initiation of XC support.

Donor Liver Preparation

The liver was topically cooled with ice. The PV was cannulated with a 24 Fr cannula and flushed with 2 L of cold (4°C) Normosol-R. The HA was cannulated with a 10–12 Fr cannula and flushed with 1.5 L of cold Normosol-R. The suprahepatic IVC was ligated. The common bile duct was cannulated with an 8–12 Fr cannula and the infrahepatic IVC was cannulated with a 36 Fr drainage cannula (Figure 1C).

Host Preparation

Host swine (n = 4) underwent induction and preoperative preparation in the same fashion as donor swine. Isoflurane (1–3%), fentanyl (0.03–0.1 mg/kg/h), ketamine (5–15 mg/kg/h), and midazolam (0.1–0.3 mg/kg/h) were used to maintain an appropriate plane of anesthesia. Antibiotics (cefazolin, 20 mg/kg; enrofloxacin, 5 mg/kg) and immunosuppression (tacrolimus, 5 mg; mycophenolate mofetil, 500 mg; methylprednisolone, 1 g) were administered. Open cystostomy and bladder catheterization were performed for urine output monitoring. Exposure of bilateral internal jugular veins (IJV) was accomplished *via* cut-downs (Figure 1D). A heparin bolus (30,000 U) was administered. Activated clotting time (ACT) was targeted to 200 to 300 seconds with a heparin infusion. The right IJV was used for drainage and cannulated with a 19 Fr cannula. The left IJV was used for venous return and cannulated with a 17 Fr cannula. A 12–14 Fr cannula was placed in the common femoral artery (Figure 1E). A central venous catheter was inserted into the common femoral vein. Immediately following recipient cannulation, extracorporeal support was initiated.

Cross-Circulation and Extracorporeal Liver Support

A list of V-AV XC circuit components is provided in Supplementary Table 1 (http://links.lww.com/ASAIO/A697). After administration of methylprednisolone (1 g) and calcium chloride (1 g), the circuit was connected to the host and extracorporeal liver (Figure 1A) as detailed in the Supplementary Methods (http://links.lww.com/ASAIO/A697), and normothermic V-AV XC was initiated. Circuit flows were titrated to 0.3–0.4 L/min to the HA and 0.65–0.8 L/min to the PV, similar to ranges reported in prior liver NMP studies.[19] Flows were adjusted to achieve 1 L/min of arterial return to the host for veno-arterial (V-A) circulatory support, for anticipated and typical reperfusion-associated instability in transplantation. The height of the liver relative to the host was adjusted to maintain hepatic venous pressure gradient <10 mm Hg. Methylprednisolone (125 mg) was readministered after 8 hours.

XC blood flow, organ inflow and outflow pressures, and host hemodynamics were continuously monitored. Circuit and host temperature were maintained at 37°C using a water heater and oxygenator water jacket. The extracorporeal liver was placed in an organ basin and covered with an isolation bag to prevent desiccation and minimize fluid loss. A sump suction connected to a cardiotomy reservoir was used to collect and recirculate blood loss or ascites volume. After 12 hours of XC, extracorporeal liver perfusion was discontinued, and the liver was flushed with 2 L of Normosol-R. Host animals were euthanized with sodium pentobarbital (125 mg/kg, IV).

Blood Collection and Analyses

Arterial blood samples were collected from the host's auricular line before XC, immediately after start of XC, and every 6 hours thereafter. Blood samples were also collected from the circuit at pre- and post-extracorporeal liver access ports every 6 hours. Oxygen consumption and lactate clearance were computed as described in Supplementary Methods (http://links.lww.com/ASAIO/A697). Blood gas analysis was performed using a point-of-care blood analysis system epoc; Heska, Loveland, CO. Routine complete blood count and biochemical analyses were performed as described in Supplementary Methods (http://links.lww.com/ASAIO/A697).

Bile Collection and Analyses

Bile was passively collected from the common bile duct *via* an 8–12 Fr cannula. Bile volume was quantified, and bile pH was measured using a pH probe (Orion Star; Thermo Fisher Scientific) every 6 hours. Bile acids were measured by liquid chromatography-mass spectrometry as previously described, and as detailed in the Supplementary Methods (http://links.lww.com/ASAIO/A697).[20]

Tissue Collection and Analyses

Baseline tissue biopsies were sharply collected *ex situ* from a randomly selected region of the liver before XC. Endpoint tissue specimens were collected from two randomly selected regions of the extracorporeal donor liver after 12 hours of XC. The location of liver tissue sampling was randomized *a priori* using a random number generator and a map with predetermined, numbered liver regions (Supplementary Figure 1, http://links.lww.com/ASAIO/A697). Donor HA, PV, and bile duct tissue specimens were collected at 12 hours. Tissue specimens from the host swine liver, spleen, kidney, lung, and lymph nodes were also collected. Tissue was fixed in 10% nonbasic formalin for 48 hours at room temperature, paraffin embedded, cut in 5 μm sections, and stained with hematoxylin and eosin (H&E), Gomori's trichrome, and periodic acid-Schiff (PAS) stains. Brightfield microscopy was performed (Axioskop 40; Carl Zeiss, Oberkocken, Germany) and digital images obtained (Axiocam 305; Carl Zeiss). Pathologic review was performed by a liver pathologist. Injury scoring of hepatic parenchymal tissue was performed with blinded histopathologic assessment of four technical replicates (same tissue block) for each experimental timepoint. A novel hepatic injury scoring system was used (Supplementary Table 2, http://links.lww.com/ASAIO/A697). Assessment criteria included scoring of sinusoidal dilatation, hepatocyte congestion, hepatocellular necrosis, neutrophilic infiltration, fibrosis, vacuolation/steatosis, and lymphocytic infiltration. Summation of sinusoidal dilatation, hepatocyte congestion, hepatocellular necrosis, and neutrophilic infiltration scores was used to compute a composite acute injury score.

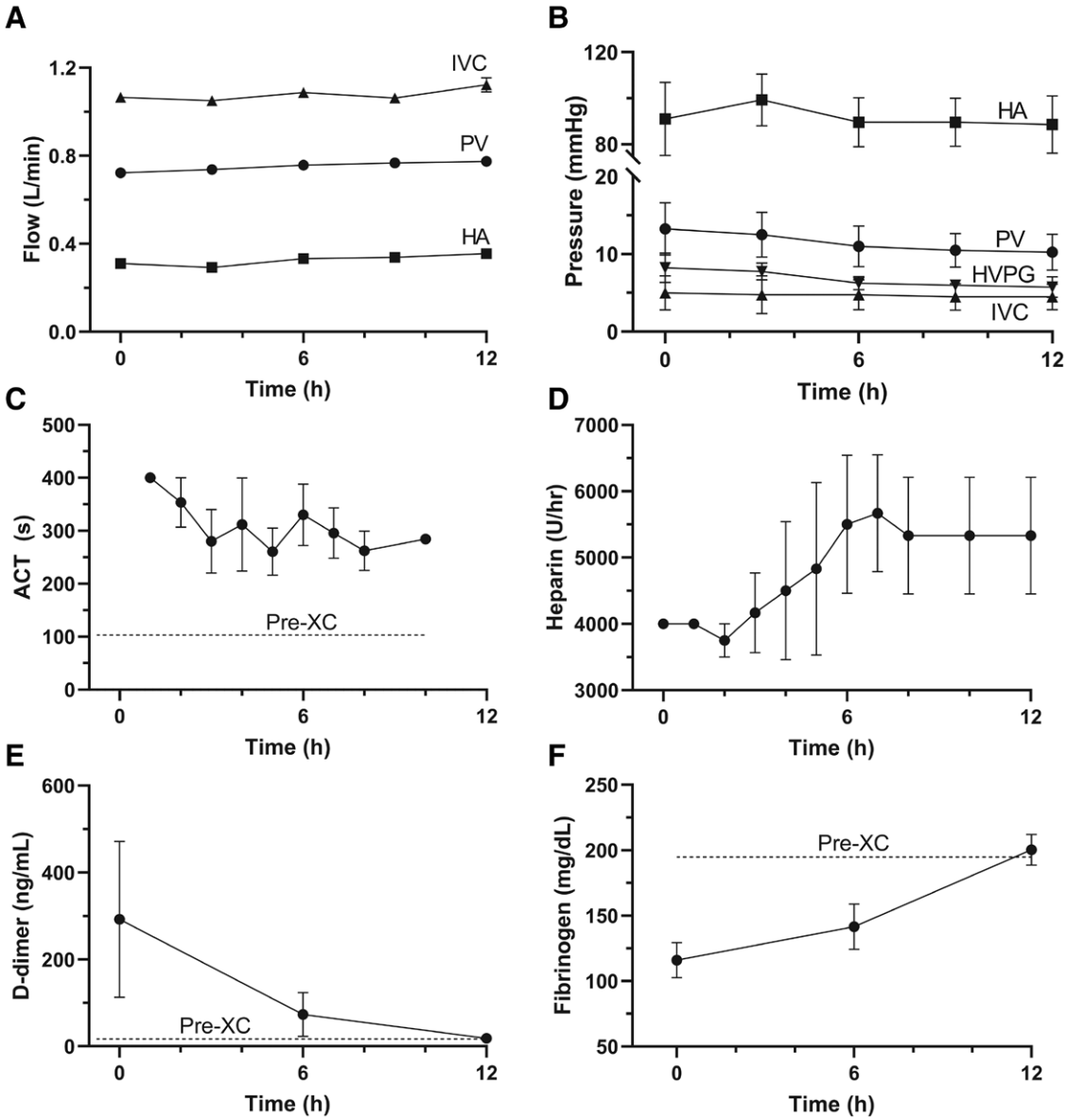

Figure 2. Stability of circuit parameters. **A**: Flows. **B**: Pressures. **C**: ACT. **D**: Heparin infusion rate. **E**: D-dimer. **F**: Fibrinogen. Dotted line represents baseline assessment before heparin administration and cross-circulation. Values are presented as mean ± SEM. ACT, activated clotting time; HA, hepatic artery; HVPG, hepatic venous pressure gradient; IVC, inferior vena cava; PV, portal vein; SEM, standard error of the mean; XC, cross-circulation.

Statistical Analysis

Two-tailed, paired Student's *t* tests and one-way analysis of variance (ANOVA) with repeated measures (with Tukey's post hoc analysis) were performed using statistical analysis software (Prism 9.0.0; GraphPad Software, San Diego, CA), and $p < 0.05$ was considered statistically significant. Continuous variables are reported as mean ± standard error of the mean (SEM).

Results

Extracorporeal Circuit Stability

Circuit parameters were maintained within target liver-protective ranges throughout extracorporeal support.[21] Host circulatory support *via* femoral arterial return was maintained at a flow of 0.9–1.1 L/min. HA flow was maintained at 0.33 ± 0.02 L/min (0 hours: 0.31 ± 0.02 L/min; 12 hours: 0.36 ± 0.02 L/min), portal venous flow was maintained at 0.75 ± 0.02 L/min (0 hours: 0.72 ± 0.01 L/min; 12 hours: 0.77 ± 0.01 L/min), and total caval flow was maintained at 1.08 ± 0.02 L/min (0 hours: 1.06 ± 0.02 L/min; 12 hours: 1.13 ± 0.03 L/min) (Figure 2A). HA pressure was maintained below 120 mm Hg. Hepatic venous pressure gradient, the difference between portal and caval pressures, was maintained within the target range below 10 mm Hg (Figure 2B). ACT was targeted to 200 to 300 seconds with a heparin infusion (Figure 2C, D). D-dimer peaked (Figure 2E) while fibrinogen nadired (Figure 2F) at the onset of XC, but both subsequently returned to baseline by 12 hours of XC.

Host Swine Safety and Stability

Host safety and stability were assessed by hemodynamic monitoring and blood gas analysis. Parameters remained within normal ranges after transient instability with initiation of XC (Figure 3A–D, mean heart rate, 91 ± 2 beats per minute [bpm]; mean systolic pressure, 92 ± 2 mm Hg; mean pH, 7.47 ± 0.03). Hemoglobin remained without statistically significant change from baseline over 12 hours of XC (Figure 3D, pre-XC: 10.0 ± 0.3 g/dl; 12 hours: 8.5 ± 0.5 g/dl; $p = 0.12$). Additional blood gas, blood counts, serum chemistries, and serum coagulation studies indicate global preservation of normal host physiology (Supplementary Table 3, http://links.lww.com/ASAIO/A697). Endpoint histologic evaluation of host liver, spleen, kidney, lung, and lymph nodes demonstrated no significant abnormalities (Supplementary Figure 3, http://links.lww.com/ASAIO/A697 and Supplementary Table 4, http://links.lww.com/ASAIO/A697).

Gross and Histologic Assessment

Gross imaging of extracorporeal livers demonstrated normal appearance of hepatic surfaces, maintenance of

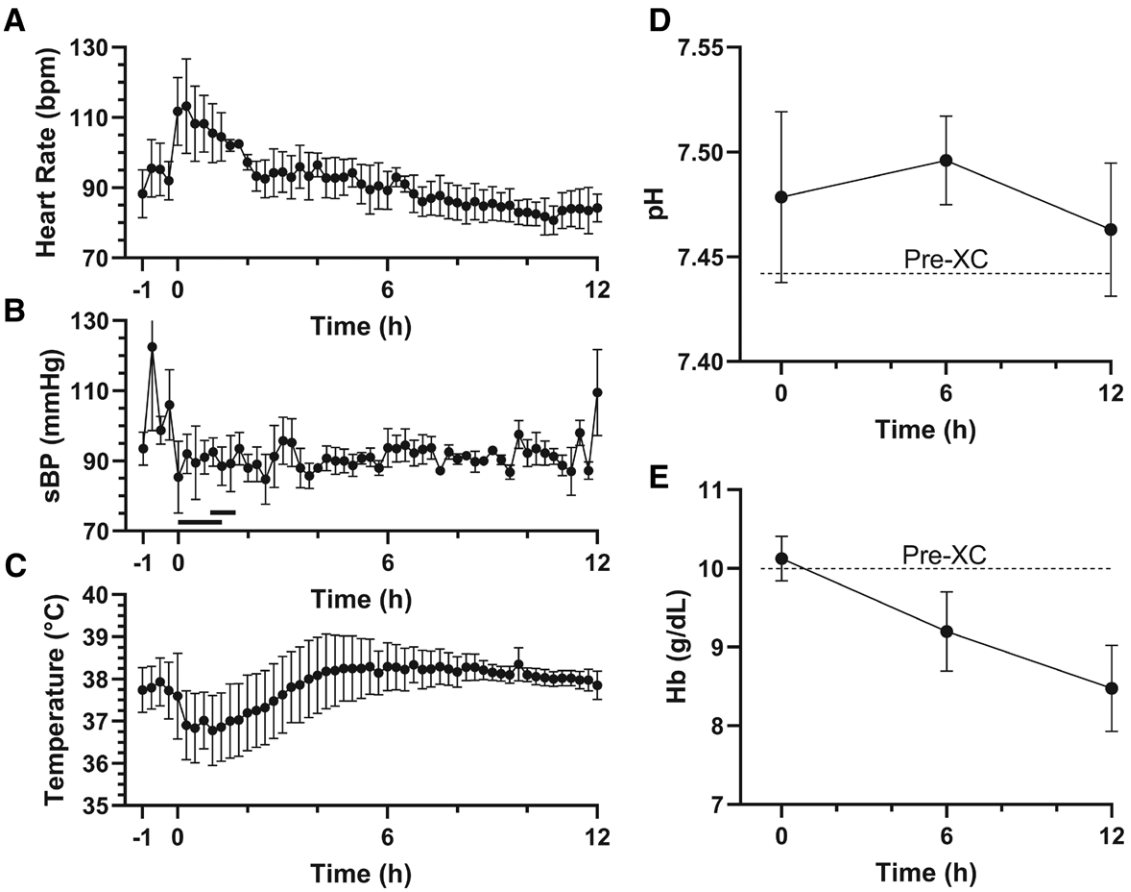

Figure 3. Host physiologic parameters throughout veno-arterial-venous cross-circulation. **A**: Heart rate. **B**: Systolic blood pressure (sBP). Transient vasopressor requirement in two hosts are marked by solid black lines. **C**: Host core temperature. **D**: Host pH. **E**: Host hemoglobin (Hb). Dotted line represents baseline assessment before heparin administration and cross-circulation. Values are presented as mean ± SEM. bpm, beats per minute; Hb, hemoglobin; sBP, systolic blood pressure; SEM, standard error of the mean; XC, cross-circulation.

global hepatic structure, and uniform perfusion (Figure 4A). Histologic evaluation demonstrated preservation of hepatic lobular and sinusoidal structural integrity with no evidence of hepatocellular necrosis or substantial periportal inflammation after 12 hours of extracorporeal liver perfusion (Figure 4B). Trichrome staining revealed maintenance of normal lobular architecture and portal triad structures (Figure 4C). No substantial glycogen accumulation was observed on histologic examination with PAS special stain (Figure 4D).

Histopathologic liver injury scoring demonstrated a statistically significant decrease in sinusoidal dilatation (–0.63 points; 95% confidence interval [CI]: –1.14 to –0.12 points; $p = 0.020$) and composite acute injury score (–0.75 points; 95% CI: –1.41 to –0.09 points; $p = 0.029$) between 0-hour and 12-hour timepoints (Figure 4E). Hepatocellular congestion and necrosis remained low and without significant change. No neutrophilic infiltration, steatosis, or fibrosis was observed in any livers at any timepoint. Lymphocytic infiltration was significantly higher at 12 hours than at 0

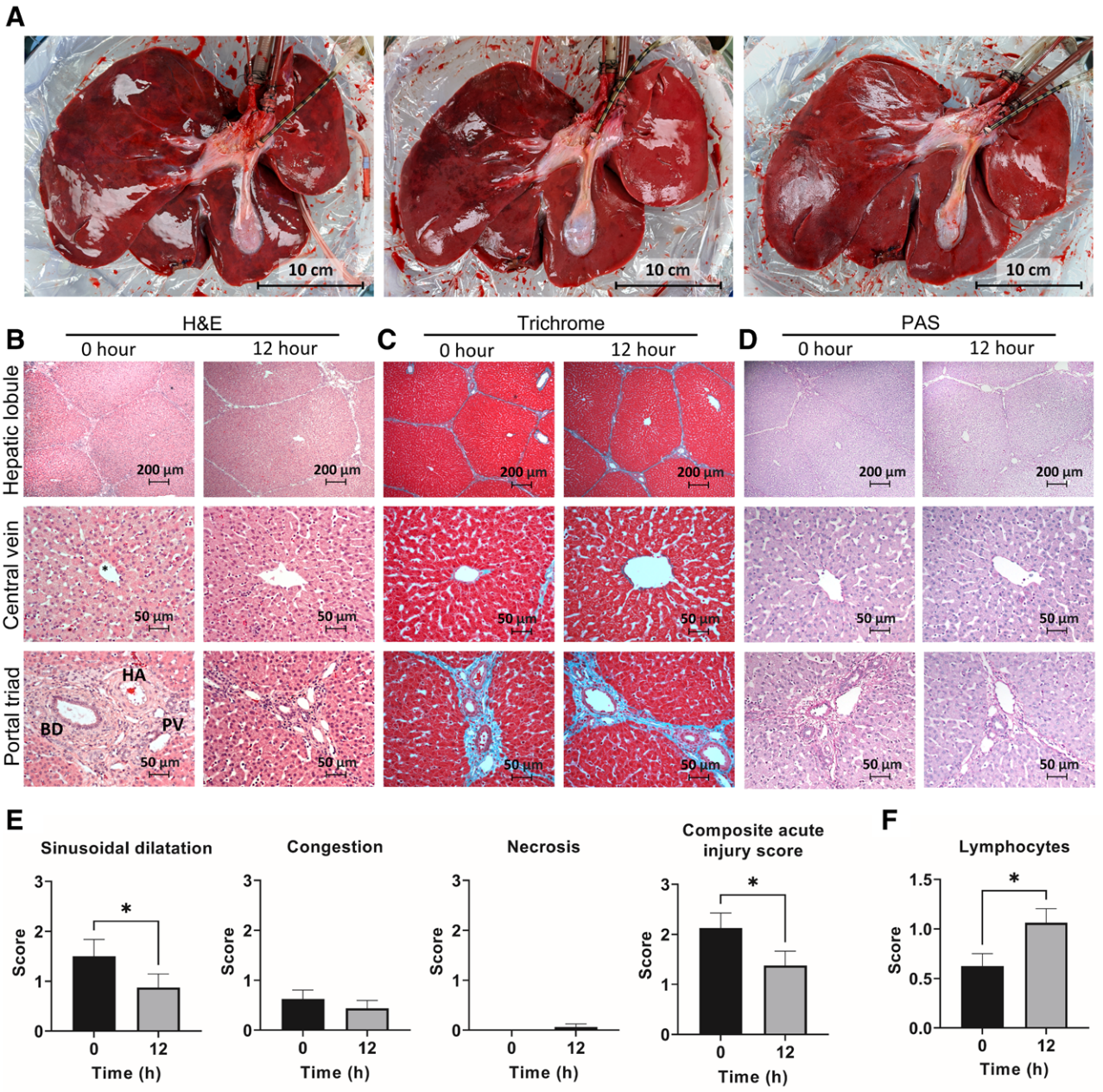

Figure 4. Multiscale analyses of extracorporeal livers. **A**: Photographic global appearance. Micrographs of hepatic lobules, central vein (*), and portal triad: (**B**), H&E stain, (**C**), trichrome stain, (**D**), PAS stain. **E**: Histopathologic evaluation and liver injury scoring. Composite score represents sum of sinusoidal dilatation, hepatocyte congestion, hepatocellular necrosis, and neutrophilic infiltration scores. *$p < 0.05$. **F**: Lymphocytes. *$p < 0.05$. Values are presented as mean ± SEM. BD, bile duct; H&E, hematoxylin and eosin; HA, hepatic artery; PAS, periodic acid-Schiff; PV, portal vein; SEM, standard error of the mean. full color online

hours (Figure 4F, 0.44 points; 95% CI: 0.10 to 0.77 points; $p = 0.014$).

Functional and Metabolic Assessment

Liver weight remained stable over the course of XC (Figure 5A). Oxygen consumption, calculated based on the Fick principle (Supplementary Methods, http://links.lww.com/ASAIO/A697), did not demonstrate a statistically significant change (Figure 5B, 0 hours: 1.7 ± 0.6 ml/min/100 g; 12 hours: 2.4 ± 0.2 ml/min/100 g; $p = 0.22$). Lactate clearance (percent change between inflow and outflow lactate levels) by the extracorporeal liver increased (Figure 5C, 0 hours: 24% ± 13%; 12 hours: 48% ± 7%; 95% CI: 0.02–48.5%; $p = 0.0499$). Blood urea nitrogen (BUN), representing contributions from both extracorporeal liver and host, increased significantly over time (Figure 5D, $p = 0.004$). The extracorporeal liver demonstrated stable bile production with alkaline bile composition throughout XC (Figure 5E, F). Species of the most abundant bile acids were unaltered (Figure 5G).

Markers of Hepatocellular Injury

Aspartate aminotransferase (AST) levels increased at reperfusion, but thereafter remained stable throughout XC (Figure 6A; pre-XC: 15.8 ± 5.5 U/L; 0 hours: 163 ± 54 U/L; $p = 0.13$). ALT levels likewise increased at reperfusion and stabilized (Figure 6B; pre-XC: 24.3 ± 6.4 U/L; 0 hours 37.3 ± 3.2 U/L; $p = 0.17$). Alkaline phosphatase and lactate dehydrogenase levels remained stable (Figure 6C, D).

Discussion

In this feasibility study, we established a novel physiologic *ex vivo* organ support system capable of maintaining the structure, viability, and function of extracorporeal livers for 12 hours while providing circulatory support to the host. Throughout V-AV XC support, extracorporeal donor livers demonstrated preserved global and microscopic hepatic architecture, improved injury scoring, robust metabolic activity, and intact synthetic function. Hosts maintained normal hemodynamic and physiologic profiles after initial reperfusion.

Despite initial transaminitis upon reperfusion, plateau of all markers of liver injury during XC indicates that injury was transient, within expectations for organ reperfusion, and analogous to initial hepatic biomarker elevations observed clinically after liver transplantation.[22,23] Although plateau ALT levels remained at the upper limit of normal and plateau AST levels remained above normal ranges reported for swine,[24] these changes are likely explained by contributions from both the *in situ* host liver and the *ex vivo* donor liver, as well as from other tissues, for example, AST also arises from muscle, heart, brain, red blood cells.[25] The maintenance of hepatic arterial pressure and flow within physiologic ranges reflects intact autoregulatory functions of the myogenic response and the hepatic arterial buffer response.[26] Robust oxygen consumption and lactate clearance by the extracorporeal liver, with rates comparable to those reported for NMP, indicate preserved metabolic activity.[27] Improvements in histopathologic injury score demonstrate the ability to rehabilitate acute hepatocellular injury incurred during procurement. Although hepatocellular viability is indisputably necessary for the preservation of global liver function, bile duct health and physiologic biliary metabolism have emerged as critical determinants of donor organ viability.[28] Livers maintained on the V-AV XC system demonstrated stable bile production, alkaline bile composition, and preserved species of bile acids produced (similar to those previously reported in swine), which reflect physiologic biliary metabolism and robust bile duct health.[28,29]

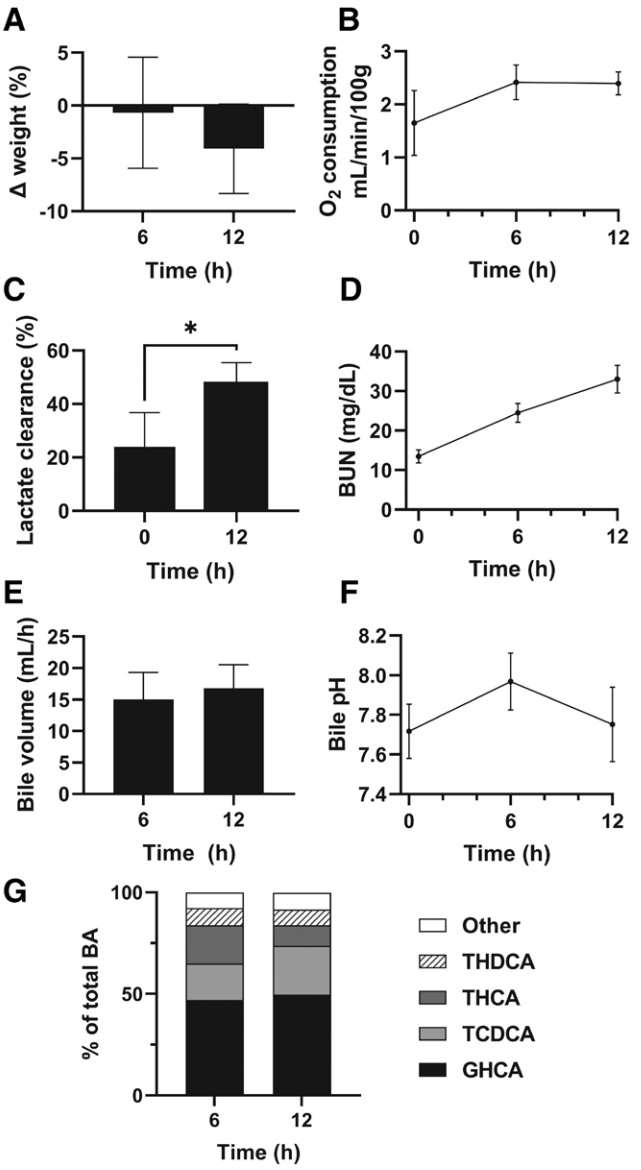

Figure 5. Maintenance of extracorporeal liver function. **A**: Change in liver weight. **B**: Liver oxygen consumption, as computed by Fick's principle, normalized per 100 g of organ weight. **C**: Lactate clearance. *$p < 0.05$. **D**: BUN. **E**: Bile volume. **F**: Bile pH. **G**: Proportions of most abundant bile acid species. Values are presented as mean ± SEM. BA, bile acid; BUN, blood urea nitrogen; GHCA, glycohyocholic acid; SEM, standard error of the mean; TCDCA, taurochenodeoxycholic acid; THCA, taurohyocholic acid; THDCA, taurohyodeoxycholic acid.

Liver NMP is a rapidly evolving technology developed for prolonging organ preservation and facilitating organ recovery. A platform recently published by Eshmuminov *et al*.[27] demonstrated preservation of metabolically active, functionally intact human livers for 7 days. Despite impressive automation

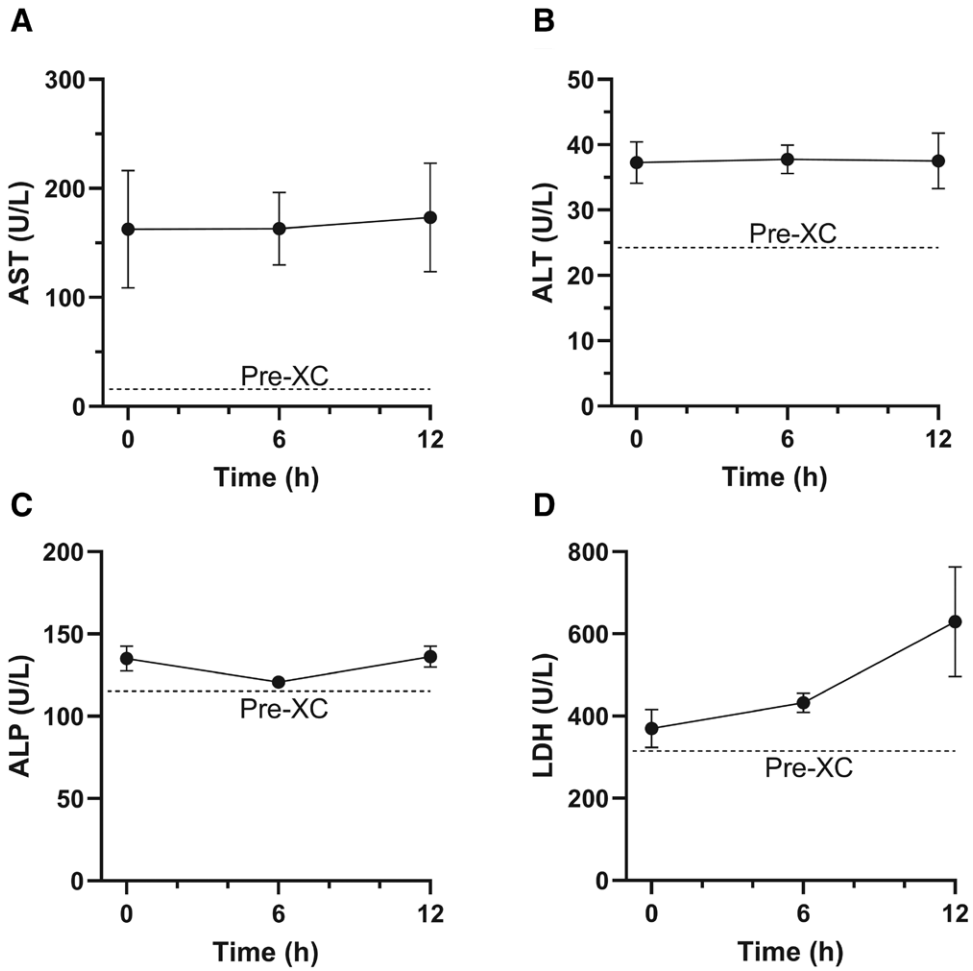

Figure 6. Serum markers of liver injury. **A**: AST. **B**: ALT. **C**: ALP. **D**: LDH. Dotted line represents baseline host assessment before heparin administration and cross-circulation. Values are presented as mean ± SEM. ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; LDH, lactate dehydrogenase; SEM, standard error of the mean; XC, cross-circulation.

of components that provide multiday, multisystem support, the ability of a machine perfusion system to mimic physiologic homeostatic mechanisms and inter-organ signaling pathways is limited—and the prospects of truly duplicating complete homeostatic physiology is unlikely to be achieved in the near future. A XC platform that leverages the intrinsic physiologic milieu provided by a host organism can bridge these gaps and ensure durable physiologic maintenance.[10,12,13] In our study, the bioreactor host served as the source and regulator of metabolic substrates, vasoactive mediators, waste clearance, homeostatic signaling, and all other physiologic processes not yet provided on isolated single-organ machine perfusion platforms.

In addition to *ex vivo* liver support, this novel configuration of V-AV XC concurrently provided approximately 1 L/min of V-A circulatory support to the host throughout the experiment. Outside of brief periods of hypotension at the onset of XC in two of four hosts, there were no episodes of host instability. This initial hypotension is likely secondary to flushing of metabolites and cold perfusate from the extracorporeal liver, combined with rapid intravascular volume shifts as the extracorporeal liver is perfused. This observation parallels the physiologic response seen clinically in liver transplant recipients experiencing postreperfusion syndrome.[14–16] Reperfusion-associated instability is observed in up to one third of liver transplant recipients and is more often encountered with older donor age, increased cold ischemia time, and increased graft steatosis.[16,30] In designing a XC configuration for future human donor liver support, we considered the heterogeneity in graft quality and preservation conditions and included the V-A component as an adjunct to veno-venous XC to address the clinical challenge of reperfusion-associated instability. In this study, the use of the V-A component minimizes the impact of reperfusion-associated instability and improves host hemodynamic support during XC.

This V-AV XC platform may address challenges seen in combined organ transplant, especially heart-liver, where the heart is typically transplanted first and requires transient pharmacologic and mechanical support as the graft function stabilizes. XC support of the extracorporeal liver graft in addition to mechanical circulatory support of the recipient could improve the physiologic milieu in which the subsequent liver implant is completed by maintaining normothermic perfusion of the liver, minimizing cold ischemic time, and optimizing the recipient before liver implant.

There are several limitations in this study. Our investigation included a small number of experiments for a limited duration, imposing some limits on the opportunity for complex statistical analyses. Nevertheless, our findings demonstrate feasibility

of our original experimental intent of XC for normothermic support of extracorporeal livers. Future studies will include a greater number of donor-host pairs, longer duration of organ support, and a wider variety of experimental conditions including donor quality, circuit parameters, and therapeutic interventions. The mild increase in parenchymal lymphocytes was not further characterized in this study. Although lymphocytic infiltration has been previously described in postreperfusion and post-transplant protocol biopsies without significant clinical consequence,[31,32] further evaluation of this mononuclear population would clarify its immunologic significance. Furthermore, because immunosuppression was used, immunological markers of injury may be different than in the absence of immunosuppression. Additional studies using an extended extracorporeal organ support system could help elucidate the roles of cytokines and host immune system in the bioreactor environment. This study also did not investigate deposition of host cells, platelets, or serum components in extracorporeal livers. Investigating hematologic and immunologic interactions will be critical to assess the safety for clinical translation. All donor livers included in this study were healthy and experienced minimal warm or cold ischemia before XC. The capability of the system to support, recover, or regenerate livers with a greater burden of ischemic insult, or other injury are yet to be investigated. Lastly, transplantation of XC livers was not performed in this series of experiments. Further studies are needed to assess outcomes following transplantation of livers that were maintained on XC.

Despite these limitations, this study demonstrated that the swine V-AV XC platform enables both extracorporeal donor liver preservation and host circulatory support. Parallel to reported success in using XC for the support and recovery of donor lungs, we hypothesize that XC for *ex vivo* liver support can be extended in duration to several days or longer,[11] can be applied in a xenogeneic setting to unallocated human donor livers,[12] and can offer new opportunities to assess and recover donor organs. Beyond clinical applications, liver XC creates novel opportunities for extracorporeal liver manipulation and optimization within a homeostatic setting. The physiologic milieu of XC may be preferable to isolated single-organ support systems for research and development of techniques and therapeutics that rely upon, or are affected by, interactions only present in a complete biosystem. Future investigations using extended organ support could enable advanced interventions through chemical conditioning, immunomodulation, viral transfection, cell replacement, or other bioengineering approaches to improve organ function. We envision broad applications for this system as a translational research and basic science tool to develop technology that enables organ recovery, rehabilitation, and regeneration.

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