UC Berkeley
UC Berkeley Previously Published Works

Title
Small-animal blood exchange is an emerging approach for systemic aging research

Permalink
https://escholarship.org/uc/item/1c52m9gj

Journal
Nature Protocols, 17(11)

ISSN
1754-2189

Authors
Mehdipour, Melod
Amiri, Payam
Liu, Chao
et al.

Publication Date
2022-11-01

DOI
10.1038/s41596-022-00731-5

Peer reviewed

Small-animal blood exchange is an emerging approach for systemic aging research

Melod Mehdipour[1,3], **Payam Amiri**[2,3], **Chao Liu**[1,3], **Jonalyn DeCastro**[2], **Cameron Kato**[1], **Colin M. Skinner**[1], **Michael J. Conboy**[1], **Kiana Aran**[2], **Irina M. Conboy**[1]

[1]Department of Bioengineering and QB3 Institute, University of California, Berkeley, Berkeley, CA, USA.

[2]Keck Graduate Institute, The Claremont Colleges, Claremont, CA, USA.

[3]These authors contributed equally: Melod Mehdipour, Payam Amiri, Chao Liu.

Abstract

We describe a small-animal blood exchange approach developed for aging research as an alternative to heterochronic parabiosis or plasma injections. In parabiosis, animals are surgically coupled, which has several disadvantages, including difficulty controlling experimental procedure, the effects of shared organs, environmental enrichment from jointly exploring the housing enclosure, involuntary exercise and an imprecise onset of blood sharing. Likewise, in plasma injections, the added volumes need to be small, and there is little flexibility in changing the relative contributions of ectopic to endogenous blood components. These factors complicate the conclusions and interpretations, including the identification of key mechanisms and molecular or cellular determinants. Our approach, where blood is exchanged between animals without them being surgically coupled, is less invasive than parabiosis. The percentage of exchanged blood or other exchanged fluids is known and precise. The age of plasma and cells can be mixed and matched at all desired relative contributions to the endogenous systemic milieu, and the onset of the effects can be accurately delineated. In this protocol, we describe the preparatory and animal surgery steps required for small-animal blood exchange in mice and compare this process with parabiosis and plasma injections. We also provide the design, hardware and software for the blood exchange device and compare automated and manual exchange methods. Lastly, we report mathematical modeling of the dilution of blood factors. The fluid exchange takes ~30 min when performed by a well-trained biomedical scientist; the entire process takes ~2 h.

Introduction

Heterochronic parabiosis experiments, wherein animals of different ages are surgically coupled, have demonstrated that mammalian aging is systemic and responsive to the age of circulatory milieu[1-4]. Namely, aged stem cells and the signaling networks that regulate their responses can be rejuvenated upon exposure to a young systemic milieu, while the function and molecular regulation of young stem cells is negatively affected by parabioses to old partners[5,6]. Studies of the mechanisms that underlie these phenomena have focused on the circulating factors that might be responsible for pro-geronic or rejuvenating phenotypes[1,7-16].

Despite the insights that resulted from parabiosis experiments, it is unclear whether the reported effects originated from the blood or from shared organs[5,6]. Young liver and kidneys clear waste metabolites of aged partners, heart and lungs from young animals can improve blood oxygenation and perfusion of old mice, and young gastrointestinal tract and thymus can all improve the health of old parabionts[5,17]. Youthful pheromones and environmental enrichment can also positively impact parameters such as neurogenesis in aged partners[18,19]. Finally, simple behavioral changes, such as regular eating and having to exercise by running next to the younger partner, can also contribute to rejuvenative effects. Conversely, the younger parabiont must maintain the older organ systems with their typical chronic inflammation and fibrosis.

Development of the protocol

This prompted us to develop a small-animal blood exchange system for a better controlled and more precise way to study the effects of blood alone on tissue aging and rejuvenation[20-22]. Unlike in parabiosis, animals that undergo blood exchange are not surgically connected to each other. Catheters are installed into the veins by performing a minimally invasive procedure known as jugular vein cannulation. This allows the mice to be connected or disconnected at will, either to an automated blood exchange device or to a manual Y-coupler and syringe. Consequently, only blood can be exchanged from one partner to the other. Alternatively, blood can be exchanged from one animal to a tube containing a designed blood solution. The amount of blood exchanged can be precisely manipulated, and the onset and duration of any observed effects can be easily known.

Subsequently, we have further developed a modified plasmapheresis system for mice, termed neutral blood exchange (NBE), to determine whether plasma dilution can be rejuvenating[20,21]. During NBE, we estimated that approximately half of mouse plasma is removed and replaced by 5% mouse serum albumin (MSA) in saline after a total of 15 blood exchanges. Importantly, mice will lose blood cells if only the 5% MSA solution is used for blood exchange. It is therefore essential to obtain red blood cells (RBCs) and peripheral blood mononuclear cells (PBMCs) from a donor mouse to ensure that recipient mice do not become depleted of blood cells during the NBE process. The combination of donor mouse RBCs and PBMCs and 5% MSA solution constitute a complete blood solution, although the complete blood solution may contain any other physiological fluids that need not be 5% MSA, but supplementation with RBCs and PBMCs is required.

To perform blood exchanges, we have developed a blood exchange device, which is a computer-controlled peristaltic pump designed for transfusing small volumes of blood between live mice. The pump consists of a flexible silicone tube that is intermittently compressed by a series of rollers that are radially spaced around a rotating pump head[23]. A motor controls the pump, which is used to withdraw blood from the cannulated mouse, i.e., mouse A. As the rollers move along the silicone tube, blood is pulled into the pump and infused into the mouse's exchange partner, i.e., mouse B. After a predetermined duration of time, the roller reverses direction and blood will flow out of the mouse that had just been infused (mouse B), back into the mouse whose blood was initially drawn (mouse A). The roller design minimizes friction and fraying of the silicone tube and ensures that a smooth compression of the tube occurs to avoid ruptures. These characteristics improve the reproducibility and reliability of the system. The transferred volume is a function of the silicone tubing internal diameter, motor step rate and duration of exchange. The flow rate can be modified by adjusting the motor step rate and can be optimized to account for various factors, including, but not limited to, fluid viscosity, blood exchange volume, hemolysis and coagulation[24,25]. These parameters will be discussed in more detail within the protocol to provide a more experimental frame of reference. Components for design and fabrication are also discussed below ('Materials', 'Equipment setup').

Comparison with other methods

Heterochronic parabiosis experiments have been conducted for decades and have shown that young partners suffer pro-geronic changes and old partners become rejuvenated[1-4]. However, unlike blood exchange where only blood factors are shared, in these experiments, organ sharing, pheromones and involuntary exercise may also play roles. Moreover, the parabiosis procedure itself is highly invasive and leaves newly established pairs vulnerable to parabiotic disease, which might be due to epigenetic differences[26,27]. The number of surviving pairs can therefore be reduced at random by up to 50% (refs. [6,17]). This implies that double the number of pairs would be necessary to maintain a sufficient sample size, and that the data and conclusions derive from the selected surviving animals. The animals are also connected for 4–5 weeks, making it difficult to determine the onset of effects.

An alternative to parabiosis has been the injection of a small volume of plasma from one mouse into another. Repeated injections of young mouse plasma or plasma from aged mice that underwent physical exercise were reported to have rejuvenating properties on the brain[2,28,29]. Interestingly, 50/50 blood exchange of old mice with young blood using our approach failed to promote such a rejuvenation[22]. The differences between plasma injections and blood exchange are many, including the relatively small contribution of ectopic factors to endogenous systemic milieu in the injection approach and the fact that the factors injected through the tail vein collect in the liver and the metabolized byproducts is the main differential effect[30]. In contrast, the effect of plasmapheresis through the jugular vein are truly systemic. Additionally, in plasmapheresis, there is no repeated animal handling that is typical for the cycles of plasma injections. Hence, the stress of repeated handling is reduced and the environmental differences between experimental cohorts are minimized in plasmapheresis, as compared with plasma infusions. Table 1 illustrates key methodological differences between parabiosis, blood exchange and plasma infusions.

Applications

Using the blood exchange system, we recapitulated a majority of the findings of heterochronic parabiosis, with important additional conclusions on the dominance of old blood for aging of the brain, yet absence of old brain rejuvenation by the young blood[22]. We were further able to show that aged mice that underwent NBE exhibited significant and robust rejuvenation of muscle, liver and brain[20,21]. Moreover, many pro-regenerative proteins were found to be upregulated in old mouse blood sera 6 d post-NBE and 3 weeks after a similar procedure in humans, which is called therapeutic plasma exchange[20,21]. It was postulated that plasmapheresis rejuvenates the systemic circulation of aged individuals by diluting dominant pro-geronic blood factors.

These studies have shown that an acute and large dilution of age-accumulated plasma factors rapidly and robustly rejuvenates the maintenance and repair of muscle, liver and brain and stably resets aged systemic proteomes to a healthier state, and that young blood factors are not essential for rejuvenation[20,21]. The studies of blood dilution are not feasible with parabiosis or plasma injections (Table 1).

Overview of the protocol

Successful small-animal blood exchange studies require knowledge of the methodology, which includes surgical technique, blood exchange equipment featuring the peristaltic pump and Y-coupler methods, and accurate calculation of exchanged fluids. Here we describe the detailed protocol for small-animal blood exchange, including the following steps:

1. *Design and fabrication of the small-animal blood exchange device.* Detailed instructions for the peristaltic pump that controls the rate and volume of exchange, connecting cannulated animals to the blood exchange device (Procedure 1). We describe the fabrication of a dual pump (with two pump heads; Steps 1–16) and a single pump (with one pump head; Steps 17–29).

2. *Jugular vein cannulation surgery.* Instructions to obtain and maintain the central venous access (Procedure 2, Steps 1–24).

3. *Blood exchange process.* Protocols for blood exchanges with the peristaltic pump (Procedure 2, Steps 32–54) and the alternative of manual syringes and Y-couplers exchanges (Procedure 2, Steps 55–64). We also provide details for producing a complete blood solution (Procedure 2, Steps 25–32).

Through heterochronic blood exchange, it was previously assumed that ~50% of blood was exchanged between a young and old mice after repeated exchanges[20-22]. However, an accurate model for precisely calculating the amounts of blood exchanged between mice is yet to be established. Exchanging blood can be lengthy and stressful for the mice, so it is crucial to calibrate experiments accordingly. Therefore, in Procedure 3 we provide a detailed protocol for blood exchange mathematical modeling. We derive well-correlated empirical and mathematically simulated data on the gradual dilution of blood factors, e.g., complement C3 protein.

Experimental design

A pair of mice can be exchanged simultaneously as in heterochronic blood exchange experiments (back-and-forth blood exchange), or a mouse can be connected to and exchanged with a tube containing a complete blood solution (forward blood exchange). Here we describe a forward blood exchange using our peristaltic pump (Procedure 2; Steps 45–54) and a back-and-forth blood exchange using a Y-coupler (Procedure 2; Steps 55–64); however, both types of blood exchange can be performed by connecting mice to either the peristaltic pump or to a Y-coupler. Ideally, blood exchange experiments should be performed with the automated peristaltic pump to ensure the accuracy of exchanged volumes and the constant rate of exchange. However, the manual syringe and Y-coupler method could be used instead when access to the materials and equipment needed to fabricate a blood exchange device is limited. Automated and manual exchanges attain similar desired outcomes, but there are several methodological differences that are outlined in Table 2. The complete blood solution presented here consists of 5% MSA in saline and synchronic syngeneic blood cells, as previously described for NBE[20,21]. Note: it is imperative to include age-matched syngeneic donor blood cells in the complete blood solution to avoid anemia and leukopenia.

Isochronic blood exchange experiments must be performed to account for physiological alterations that may occur owing to the heparin, anesthesia, etc. parts of the procedure. Such exchanges control for every aspect of heterochronic exchanges and NBE, as each mouse is given the same dose of heparin and anesthesia to ensure consistency. Of note, in our published reports muscle, liver and brain parameters that were measured in animals receiving isochronic blood exchange versus untreated age-matched animals were not significantly different[20-22].

In addition, while one may continue to exchange blood to approach their desired blood ratio, it is important to consider the time required and the stress on the mice. It can be a lengthy process as the exchanges typically take ~5 min each when performed with either the peristaltic pump or manually with the Y-coupler and syringe. Experiments and procedures should therefore be planned accordingly.

Materials

Biological materials

Male C57/B6 mice of young (2–4 months) or old (18–24 months) of age **! CAUTION** Procedures involving animals and human tissues must conform to relevant institutional and national regulations. Informed consent must be obtained from each patient for human tissue **! CAUTION** All experiments involving live vertebrates were performed in accordance with the regulations and guidelines of the Northwest Animal Facility veterinary personnel at the University of California, Berkeley.

Reagents

- MSA (Innovative Research, cat. no. IMSALB100MG)

- Complement 3 (C3) Mouse ELISA Kit (Abcam, cat. no. ab157711)

- Isoflurane (provided by veterinary staff; Piramal Critical Care, cat. no. DVM-102190)

- Buprenorphine (provided by veterinary staff as a 0.3 mg/mL solution in sterile water)

  ! CAUTION Buprenorphine is a controlled substance and formal approval must be obtained for its use. If approval cannot be obtained, the veterinary staff can provide injections as needed.

- Antibiotic ointment (bacitracin/neomycin/polymyxin, obtained at a local pharmacy)

- Meloxicam (provided by veterinary staff as a 5 mg/mL solution in sterile water)

- Heparin sodium injection (HSI; Sagent Pharmaceuticals)

- Ophthalmic ointment (obtained at a local pharmacy)

- Betadine (antiseptic solution, 10% (wt/vol) povidone-iodine in purified water, obtained at a local pharmacy)

- Nair hair remover (obtained at a local pharmacy)

- Sterile saline (0.9% (wt/vol) NaCl)

- 10% (vol/vol) bleach

- 70% (vol/vol) ethanol

Equipment

- Anesthesia machine (Parkland Scientific, cat. no. V3000PK)

- 10 μL, 50 μL or 100 μL Hamilton syringe (Grainger)

- 70% ethanol wipes

- Heating pad

- Weight scale

- Clock

- Tape

- Cotton swabs

- Paper towels

- 6–0 sutures (provided by veterinary staff)

Surgical tools

- Wound clips (CellPoint Scientific)

- Dissection and microdissection scissors (Bio Corporation)

- Dissecting forceps, sharp and blunt (Millipore Sigma)

- Suture clamps (Global Industrial)

- Cannulation forceps (World Precision Instruments, cat. no. 500377)

- Hemostat clamps (Global Industrial)

- Dermabond (provided by veterinary staff)

- Automatic hair trimmer (obtained at a local pharmacy)

- Dissecting microscope (New York Microscope Company)

Tubing, syringes, needles and related equipment

- 4 cm 2F mouse jugular vein catheters (Instech, cat. no. C20PU-MJV1450)

- 22 G Luer stubs (Instech, cat. no. LS22)

- Catheter plugs (Instech, cat. no. SP22/12)

- Metal tubing couplers (Instech, cat. no. SC22/8)

- Y-coupler (Instech, cat. no. SCY22)

- 23 G needles

- 16 G needles

- 22 G tubing, 30 m in length, nonsterile (must be sterilized; Instech, cat. no. BTPE-50)

- 3 mL hypodermic syringes (Bowers Medical Supply)

3D-printed hard casing

- Ultimaker S5 3D Printer (Ultimaker, cat. no. S5)

- Acrylonitrile butadiene styrene (ABS) 2.85 mm filament (MatterHackers, cat. no. M-Z7W-ZEMW)

- Polyvinyl alcohol (PVA) 2.85 mm filament (MatterHackers, cat. no. M-N9S-LY7R)

- J-B Weld 5-min two-part epoxy adhesive (J-B Weld, cat. no. 50240)

Pump external hardware and tubing

- 22 G hypodermic needle metal tubing (McMaster-Carr, cat. no. 5560K72)

- 18 G hypodermic needle metal tubing (McMaster-Carr, cat. no. 201808232015)

- Silicone tubing (Instech, cat. no. P720/TS-22020S22)

- PE-50 polyethelene tubing (Instech, cat. no. BTPE-50)

- Silicone oil (McMaster-Carr, cat. no. 13805K54)

Electronic circuitry for Arduino

- Arduino Uno (Arduino, cat. no. Uno R3)

- Adafruit motor shield (Adafruit, cat. no. 1438)

- Motor driver integrated circuit (Texas Instruments, cat. no. L293D)

- Adafruit 44 mm × 34 mm stepper motor (Adafruit, cat. no. 324)

- 22 American wire gauge (AWG) stranded wire (McMaster-Carr, cat. no. 9948T25)

- Single-row header pin connectors (Adafruit, cat. no. 3662)

- Toggle switch (McMaster-Carr, cat. no. 7343K184)

- 9 V battery (McMaster-Carr, cat. no. 71455K56)

- 9 V battery clip to barrel connector adapter (McMaster-Carr, cat. no. 7712K181)

- 2 A blade fuse (McMaster-Carr, cat. no. 7460K38)

- Blade fuse holder (McMaster-Carr, cat. no. 8110K3)

- 12 W (12 V) alternating current (AC)–direct current (DC) adaptor (McMaster-Carr, cat. no. 70235K14)

Electrical connections

- Soldering iron (Weller, cat. no. WESD51D)

- Rosin-core solder (McMaster-Carr, cat. no. 765A112)

- Heat-shrink tubing (McMaster-Carr, cat. no. 73095K48)

- Wire stripper (McMaster-Carr, cat. no. 8372K11)

Reagent setup

MSA—Dissolve 1 g MSA in 20 mL saline to make a 5% (mass/vol) solution. This can be stored at 4 °C for up to 3 years without preservatives.

Buprenorphine—Obtain a vial of 0.3 mg/mL buprenorphine from the institution's veterinary staff. This can be stored at room temperature (22–25 °C) until the indicated expiration date (see container). Administer subcutaneously, at a dose of 0.1 mg/kg, 0.3 μL/g mouse of the 0.3 mg/mL solution.

Meloxicam—Obtain a vial of 5 mg/mL meloxicam from the institution's veterinary staff. This can be stored at room temperature (22–25 °C) until the indicated expiration date (see container). Administer subcutaneously, at a dose of 5 mg/kg, 1 μL/g mouse of the 5 mg/mL solution, after recovery from anesthesia and for 2 d post-procedure, one dose per day.

Equipment setup

3D-printed components—The dual-pump small-animal blood exchange device consists of rotating pump heads, pump head rollers, 22 G tubing connectors, and polyethylene and polyurethane tubing (Fig. 1a). The casing for the dual pump is 3D-printed in three separate parts with an Ultimaker S5 using an ABS 2.85 mm filament, owing to its high impact

strength and impact resistance[31-33]. Briefly, each part is designed with computer-aided design (CAD) software (SolidWorks) (Fig. 1b) and printed through additive manufacturing path generated with the accompanying printer software (Cura). The layer height for the structures is set to 0.2 mm, with a wall thickness of 2 mm, and the infill is set to 10%. Moreover, each part is designed to friction fit together as the final housing around the mechanical and electrical structures of the small-animal exchange pumps (Fig. 1c). Similarly, the housing for the single pump is 3D-printed in two separate parts with the same ABS filament and printer settings as described previously using the Ultimaker S5. The single pump (Extended Data Fig. 1a,b) is designed in a way that the two parts also pressure fit together as well (Extended Data Fig. 1c). Two additional parts are printed using the ABS filament and used for constructing the pump head(s). In this case, the printed parts for the pump head are designed to fit together permanently using a two-part epoxy glue (J-B Weld 5-min epoxy adhesive). The pump head(s) are then pressure fit onto the stepper motor and further secured with the two-part epoxy glue. The pump head rollers are fabricated from 18 G and 22 G hypodermic needle metal tubing. The 18 G tubing is used to create bushings that are fixed into the 3D-printed pump head with the two-part epoxy glue. A section of 22 G tubing placed inside the bushings and fixed with the two-part epoxy is used as a shaft. A section of 18 G tubing is set surrounding the 22 G shaft and allowed to freely rotate. The free-rotating section of 18 G tubing is the component of roller that makes contact and compresses the silicone tubing of the peristaltic pump, ultimately allowing for smooth fluid movement when pumping. Finally, an inert silicone oil lubricant is applied to the rollers (Fig. 1a).

Electronic components for the dual-pump small-animal exchange device—
The pump system is controlled by an Arduino Uno microcontroller electronic board that allows for both digital and analog input and output pins that can be integrated with different circuitry and an accompanying programmable computer code through an Arduino software (https://learn.sparkfun.com/tutorials/what-is-an-arduino). This unit is paired with an Adafruit motor shield that enables the ability to control the direction and speed of the motor using the Arduino code to operate a single motor (Supplementary File 1) or both motors simultaneously (Supplementary File 2). The motor shield is connected to the Arduino using soldered single-row header pin connectors (see Fig. 1d for overview and Fig. 2 for labeled diagram). To power the exchange device, the Arduino microcontroller utilized a 9 V battery. The positive line for the battery is interrupted by a toggle switch, used to turn the system on or off. Furthermore, the motor shield is powered by a 12 W (12 V) AC–DC adaptor. An AC–DC adaptor is used to satisfy the power demands of the motor shield. The positive line for the adaptor is interrupted by a two-amplitude (A) blade fuse and serves to protect the circuitry from an unexpected power surge. Finally, two stepper motors are used in this pump. Stepper motor 1 (SM1) is connected to the M1 and M2 terminal block of the Adafruit motor shield and stepper motor 2 (SM2) is connected to the M3 and M4 terminal block of the Adafruit motor shield. The stepper motors allow the conversion of electrical pulses into small discrete mechanical movements. Stepper motors are favorable for the case of small-animal exchange devices in that stepper motors have extremely precise positioning and repeatability of movement within a 3–5% accuracy per step (with this error being noncumulative with each successive step). Furthermore, stepper motors are ideal in

exchange devices given there are no contact brushes in the motor, so the lifetime of the pump relies only on the lifetime of its bearings and is therefore extended compared with other pumps such as DC motors.

Electronic components for the single-pump small-animal exchange device—Like the dual-pump system, the single-pump system is controlled by an Arduino Uno microcontroller, a written Arduino code (Supplementary File 3) and paired with a motor driver integrated circuit (L293D). This motor driver functions in a comparable way as the motor shield for the dual-pump device. The motor driver is connected to the single stepper motor and Arduino following the wiring diagram (Extended Data Fig. 1d). The system is powered by a 12 W (12 V) AC–DC adaptor. The positive line for the adaptor is interrupted by a 2 A blade fuse and a toggle switch, used to turn the system on or off.

Pump mechanics—To reduce mechanical friction and to prevent motor stalling, the pump head is lubricated using a silicone oil lubricant. Silicone tubing is used to wrap around the pump head (Instech; product number P720/TS-22020S22). The silicone tubing was subsequently crosses over to connect into the 3D-printed socket in the pump body (Fig. 1a). Lastly, 22 G connectors cut from the silicone tubing are used to connect the polyethylene 50 tubing (Instech; PE-50 tubing, 0.02-in inner diameter × 0.083-in outer diameter) (Fig. 1a).

Jugular vein cannulation equipment sterilization

- Syringes, heparin solutions, catheters and needles are typically presterilized. There is no need to sterilize these items unless indicated otherwise. See the Checklist document to review heparin standard operating procedures

- Autoclave sterilize cotton swabs, paper towels (wrapped in aluminum foil first) and normal saline. Pass the autoclaved saline through a 0.22 μm filter in a sterile culture hood to remove lingering particulate matter

- Cut the 6–0 silk sutures into 4-cm segments and collect them in a clean Petri dish. While the Petri dish is open, leave the sutures in UV light from a tissue culture hood for 5 min. One can alternatively irradiate using a UV Stratalinker at the standard cross-link setting, with a duration of ~1 min.

- Sterilize Y-couplers and nonsterile tubing in the following fashion: pass 10% bleach through the tubing for 10 s, 70% ethanol for an additional 10 s and then sterile saline five times to rinse

- Sterilize surgical tools with a bead sterilizer on the day of the procedures. Plug in the bead sterilizer and wait for the green light to turn on. It will take ~30–40 min for the bead sterilizer to warm up.

- Wipe table spaces used for animal procedures with cleaning solutions (provided at the animal facility) before and after the procedures

Procedure 1: design and fabrication of the small-animal blood exchange device

▲ **CRITICAL** Steps are given for the fabrication of both a dual pump (Steps 1–16) and a single-pump (Steps 17–29) device.

Dual-pump fabrication ● Timing 1 week

▲ **CRITICAL** The device must be prepared and tested before starting Procedure 2. Refer to detailed schematic Fig. 2

Electrical wiring to Adafruit motor shield

1. Solder the single-row header pins into the top and bottom row of the Adafruit motor shield

   ! CAUTION Solder in an area with good ventilation. Fumes can be hazardous.

2. Using the header pins, connect the Adafruit motor shield onto the Arduino Uno.

3. Connect SM1 to the M1 and M2 terminal block of the Adafruit motor shield and SM2 to the M3 and M4 terminal block.

4. Connect the 12 W AC–DC adapter to the power terminal block of the Adafruit motor shield.

5. Cut the positive line (electrical wire from + terminal of 12 W AC–DC adapter) with the wire strippers and solder a blade fuse holder in-line. Insulate the newly joined electrical connections with heat-shrink tubing.

6. Insert a 2 A blade fuse into the blade fuse holder.

7. Connect the 9 V battery clip to barrel connector adapter to the Arduino Uno.

8. Cut the positive line of the 9 V battery clip to barrel connector adapter with the wire strippers and solder a toggle switch in-line. Insulate the newly joined electrical connections with heat-shrink tubing.

Printing of 3D-printed hardware casing

9 Load the STL files for the different components onto the Cura software connected to the 3D printer (STL files can be found at https://doi.org/10.6084/m9.figshare.19401263.v1). Print the components with a layer height of 0.2 mm, wall thickness of 2 mm and infill of 10%. Select ABS filament for build material and PVA as support material.

10 After print completion, dissolve PVA support material in a bath of room temperature (24–26 °C) water until material has dissolved.

11 Pressure fit the 3D-printed components around the electrical components.

12 Pressure fit the two components of the pump heads together and reinforce the connection with a two-part epoxy.

Final small-animal blood exchange device assembly

13	Use the 18 G hypodermic needle tubing to create bushings that are fixed into the 3D-printed pump head with the two-part epoxy glue.

14	Place 22 G sections of hypodermic needle tubing in the bushings and pass a section of 18 G hypodermic needle tubing around this 22 G tube. Fix the 22 G section with a two-part epoxy and allow the 18 G section to freely rotate.

15	Apply the silicone oil lubricant to the metal components of the rollers and fix the pump head structure to the stepper motor with a two-part epoxy.

16	Wrap the Instech silicone tubings around the pump heads and fit the posts in the sockets on the 3D-printed structure.

Single-pump fabrication ● Timing 1 week

▲ **CRITICAL** The device must be prepared and tested before starting Procedure 2. Refer to detailed schematic Extended Data Fig. 1.

Electrical wiring to Arduino Uno motor driver

17	Solder the Arduino Uno to the motor driver and the stepper motor following the wiring diagram in Extended Data Fig. 1d using 22 AWG stranded wire. Insulate the connections heat-shrink tubing.

! **CAUTION** Solder in an area with good ventilation. Fumes can be hazardous.

18	Connect the 12 W AC–DC adapter to the Arduino Uno and the motor driver following the wiring diagram in Extended Data Fig. 1d using 22 AWG stranded wire. Insulate the newly joined electrical connections with heat-shrink tubing.

19	Cut the positive line of the AC–DC power adapter with the wire strippers and solder a blade fuse holder in-line. Insulate the newly joined electrical connections with heat-shrink tubing.

20	Insert a 2 A blade fuse into the blade fuse holder.

21	Cut the positive line of the AC–DC adapter, downstream of the fuse, with the wire strippers and solder a toggle switch in-line. Insulate the connections with heat-shrink tubing.

Printing of 3D-printed hardware casing

22	Load the STL files for the different components onto the Cura software connected to the 3D printer (STL files can be found at https://doi.org/10.6084/m9.figshare.19401263.v1). Print the components with a layer height of 0.2 mm, wall thickness of 2 mm and infill of 10%. Select ABS filament for build material and PVA as support material.

23	After print completion, dissolve PVA support material in a bath of room temperature water until material has dissolved.

24	Pressure fit the 3D-printed components around the electrical components.

25   Pressure fit the two components of the pump heads together and reinforce the connection with a two-part epoxy.

26   Use the 18 G hypodermic needle tubing to create bushings that are fixed into the 3D-printed pump head with the two-part epoxy glue.

27   Place 22 G sections of hypodermic needle tubing in the bushings and pass a section of 18 G hypodermic tubing around this 22 G tube. Fix the 22 G section with a two-part epoxy and allow the 18 G section to freely rotate.

28   Apply the silicone oil lubricant to the metal components of the rollers. Fix the pump head structure to the stepper motor with a two-part epoxy.

29   Wrap the Instech silicone tubings around the pump heads and fit the posts in the sockets on the 3D-printed structure.

Procedure 2

! CAUTION All experiments involving live vertebrates were performed in accordance with the regulations and guidelines of the University of California, Berkeley, under an institutional animal care and use committee (IACUC)-approved protocol.

! CAUTION Hair net, surgical gown, gloves, face mask and shoe covers must be worn when handling the mice during procedure day.

! CAUTION Mice may be under anesthesia for extended periods of time (2–2.5 h), and it is imperative to administer boluses of saline to each mouse when cannulation and blood exchanges procedures have been completed.

! CAUTION Do not let air enter any of the lines that are directly connected to the right jugular vein. Doing so will be lethal to the animal.

Jugular vein cannulation surgery ● Timing ~1–1.5 h for novice surgeons and half an hour or less for experienced proceduralists.

▲ **CRITICAL** Please refer to Fig. 3 for select illustrations of the procedure.

1.   Administer buprenorphine subcutaneously (0.1 mg/kg from stock) and anesthetize the animals using an anesthesia machine with 1.5–3% isoflurane at 0.5 to 1 L/min until the breathing rate drops to one breath per second and there is a lack of response to toe pinch.

2.   Lay the mouse on the heating pad in dorsal recumbency (laid flat on its back) and treat its eyes with ophthalmic ointment eye drops.

3.   Use Nair and/or the hair trimmer to remove hair from a >1 cm square area around the right central neck and at a point in between the scapulae (back side at the level between the shoulders).

4.   Scrub the neck incision site three times, alternating between Betadine surgical scrub and an alcohol wipe.

5.   Orient the dissecting microscope above the animal and check for a response to toe pinch. Begin the procedure when the mouse does not flinch.

6.   Make a 1–1.5 cm ventral incision to right of the midline terminating at the level of the clavicle.

7.   Isolate the right internal jugular vein (RIJV) by trimming the surrounding fat and fascia while being careful to not nick the jugular vein. Be sure to regularly rehydrate the exposed vein area with saline.

8.   Use fine forceps to create space underneath the RIJV so that two 4-cm pieces of 6–0 silk sutures can be passed directly underneath it.

9.   Separate each suture—one toward the cranial end of the RIJV and the other toward the caudal end of the RIJV.

10.   Tightly ligate the cranial suture to discontinue blood flow, and then loosely ligate the caudal suture. Use hemostat clamps to gently apply tension to the vein.

11.   Bend a 23 G needle so the beveled end is 90° outward, and have ready a catheter preloaded with heparin flush solution. To prepare the preloaded catheter, simply draw enough heparin saline to fill the entire dead space of the catheter (Box 1).

12.   Prepare to perforate the RIJV for cannula insertion. Use the bent 23 G needle to make a venotomy on the ventral aspect of the RIJV (small nick on the exposed portion of the vein) while simultaneously feeding the catheter down the lumen of the vein by using cannula forceps. Tighten the caudal ligature once the catheter is placed. Ensure all bleeding has ceased before continuing.

13.   Check catheter patency by flushing additional heparinized saline through the catheter using a Hamilton syringe. Once patency is confirmed, apply heparin lock and then plug the catheter (Box 1).

      ▲ CRITICAL STEP It is crucial to ensure that the catheter is patent because it is not possible to transfuse blood through occluded cannulas.

      ? TROUBLESHOOTING

14.   Anchor the catheter in place by passing a third 4-cm suture on the cranial end of the cannulated vein.

15.   Lay the mouse in the left lateral decubitus position (lying on its left side).

16.   Insert a pair forceps through the incision site while bluntly dissecting space underneath the skin toward the midpoint between the scapulae. Once this midpoint is reached, spread the forceps open underneath the skin. Wipe the mid scapula skin with alcohol and insert an 18 G needle through the skin mid-scapula and between the tips of the forceps, and feed the needle tip under the skin toward the neck incision site and cannula.

17.   Feed the plug end of the catheter through the lumen of the 18 G needle to guide it to the mouse's back. Withdraw the needle; the catheter should be protruding

mid-scapula. Apply wound clips to close the incision sites, and then apply Dermabond to the exit point of the catheter on the mouse's back.

18. Apply antibiotic ointment (bacitracin/neomycin/polymyxin) directly to the closed incision site(s) topically for 2 d post-procedure.

19. Remove the mouse from anesthesia and allow it to recover in a single-housed cage.

     ▲ **CRITICAL STEP** Ensure that the mice are completely removed from anesthesia. The combination of meloxicam and isoflurane will cause a lethal reaction.

20. If not directly proceeding with blood exchange, administer the first dose of meloxicam subcutaneously as described in the 'Reagent setup' section. Alternatively, proceed with blood exchanges returning mice to anesthesia (as described in Step 1) once they have been allowed to rest for at least 1 h. Analgesia is not required for blood exchange.

Monitoring recovery ● Timing 48 h

▲ **CRITICAL** Not every mouse exhibits the exact same recovery pattern, but the following behaviors listed below are stereotyped across most mice. Mice generally recover in 30–45 min irrespective of their age.

21   Immediately after the procedure, monitor for the following signs of recovery:

     •   Breathing rate; this should increase gradually

     •   Jerking movements while lying still

     •   Bursts of movement

     •   Signs of coordinated movement after 30–45 min from the time mice were removed from anesthesia

22   Pay close attention to the following within the first 48 h post-procedure. If the mice appear healthy during this time period, they will most likely remain in good health throughout the duration of the experiment. The following indicate that the mice have reverted to good health:

     •   Nests are made from the nesting material provided in the cage

     •   The mice appear to be very active

     •   The mice appear to be well groomed

     •   The mice are maintaining their body weights

Clinical effects and changes to animal health and behavior post-procedure monitoring ● Timing 1 week

▲ **CRITICAL** Once the catheters are successfully installed, they will have little effect on the health and behavior of the animal. Closing off flow to the RIJV will not cause adverse effects owing to the presence of redundant bilateral blood flow. Blood exchanges will not

illicit a negative effect on animal health either so long as the experimenter strictly adheres to sterile technique and avoids air bubbles in the lines.

23   After the cannulation and blood apheresis procedures, the following steps must be adhered to:

- House mice individually and feed wet food for the first 2 d

- Apply antibiotic ointment (bacitracin/neomycin/polymyxin) directly to the closed incision site(s), topically for 2 d post-procedure

- Administer topical antibiotic ointments to the incision sites for the first 2 d

- Weigh mice daily for the first 7 d. During this time, mice will be given meloxicam subcutaneously

- Monitor the health of each animal on a daily basis. This must be carried out by trained personnel who will be vigilant of any adverse clinical signs and symptoms that may arise. Indications of ill health include but are not limited to loss of 15% or more of body weight, necrosis, shivering, hunching or sunken eyes, greater than expected loss of mobility, reluctance to respond to stimulus, or disinterest in food/or water

  ▲ **CRITICAL STEP** A veterinarian is to be consulted or the animal euthanized if any of these symptoms arise.

Catheter patency and maintenance ● Timing 1 week

24   Maintain catheters for the duration of the experiment, which is 1 week, as follows:

- Flush catheters daily with a small volume of heparinized saline to prevent clotting (Box 1)

- Use alcohol wipes to clean the catheter area

- If the catheter site is unresponsive to standard care and/or compromised such that the animal is in severe discomfort or pain, remove the animal from the study and immediately euthanize. Note that successfully cannulated mice will appear well (properly groomed and not hunched) and active. Their catheters should be intact and patent for the duration of the desired experiment. The cannulated mice should be healthy enough to survive indefinitely.

Preparation and extraction of donor mouse blood samples for supplementing the NBE fluid with the mouse circulating cells ● Timing 2 h

25   Anesthetize the donor C57/B6 mouse with isoflurane until the behaviors described in Step 1 are observed to ensure the mouse is properly asleep.

26 Once the mouse is anesthetized, draw blood through cardiac puncture[20,21] into a 3 mL hypodermic needle, prefilled with 10 units of heparin. Occasionally invert the syringe to encourage mixing of the heparin with the blood to prevent clotting.

27 Pass the extracted donor blood through a FACS tube to remove blood clots and record the total volume acquired. This is done by running the blood through the filter of the FACS tube's cap. Enough blood needs to be collected to provide the exchange fluid tube with the blood cells (erythrocytes plus leukocytes; the latter are also known as PBMCs).

28 Place the FACS tube in a conical vial so that it fits in a swinging bucket centrifuge.

29 Centrifuge the sample at $400g$ for 5 min at room temperature.

30 Post-centrifugation, collect donor plasma to a separately labeled microcentrifuge tube, leaving a blood cell pellet. Donor plasma can be stored on ice and used later on for separate studies, for example, proteomics analysis, or discarded.

▲ **CRITICAL STEP** Careful technique is required to ensure that the cell pellets are not disturbed.

31 Resuspend the blood cells with normal sterile saline (0.9% NaCl) to two times the original volume of the blood collected to rinse the blood cells.

32 Repeat the centrifugation in Step 29 and decant the saline. Replace the saline fraction with a solution of 5% MSA in sterile normal saline at a volume that is equal to the volume of decanted plasma (from Step 30). This makes us the complete exchange solution for its use in Step 40. Use this solution for exchange immediately after blood exchange device calibration is ensured (see Step 33).

Calibration of the small-animal blood exchange device ● Timing 70 min

▲ **CRITICAL** Calibration of the small-animal blood exchange device (single or dual pump) is essential before use.

33 For adequate calibration, reload the silicone tubing for the pump with 100 U/mL of heparin in saline and incubate at room temperature for at least 1 h.

▲ **CRITICAL STEP** Failure to calibrate will result in inaccurate exchange volumes.

34 Prepare two 5-cm lengths of PE-50 tubing. Join one 22 G coupler each to both tubings.

▲ **CRITICAL STEP** The couplers allow easier insertion and joining of tubing to microcentrifuge collection tubes or the mouse catheter.

35 Loop the silicone tubing closed with two 5-cm lengths of PE-50 tubing.

▲ **CRITICAL STEP** This ensures sterility and helps avoid evaporation.

36 Calibrate the pump to an exchange volume of 300 μL in each direction (Supplementary Files 1 and 2 for a dual pump and Supplementary File 3 for a single pump; change from '*const int half_cycle = xxxx*' to '*const int half_cycle = 0300*').

▲ **CRITICAL STEP** While the exchange volume is set to 300 μL, the dead space in the tubing will be 50 μL. This results in an effective exchange volume of 250 μL.

37 Ensure that the small-animal exchange device has no occlusions to flow throughout the tubing. To confirm that the metal contact points are free of occlusions, turn the pump on and allow the system to flow from Port A to Port B with 300 μL of exchange volume (Fig. 4a). If swelling is observed in the silicone tubing downstream of a metal connector, it is likely that an occlusion is present within the connector.

▲ **CRITICAL STEP** Empirically, it has been observed that metal encourages blood aggregation. Heparin coating can limit this phenomenon; however, there are still situations when aggregation can occur.

? TROUBLESHOOTING

38 After programming, perform a test run with the pump. Take note of the length of time for one full cycle, and take note of when the polarity of the pump changes direction.

39 Program into the Arduino microcontroller code the total number of exchanges, Supplementary Files 1-3 '*const int exchange_number*'.

40 Finally, unwrap the silicone tubing around the pump head until ready for the experiment. This avoids excessive prolonged tension on the tubing.

Small-animal exchange device preparation ● Timing 45 min

▲ **CRITICAL** Before performing a blood exchange procedure with the small-animal exchange device, the system should be flushed with blood to ensure no air is present within the tubing line.

41 Feed the PE-50 tubing of the exchange device into the young or old donor microcentrifuge tube, for example, the complete blood solution prepared in Steps 25–32 and turn on the pump. We will denote this initial side as Port A in the described experiment. The second line of PE-50 tubing designated for mouse jugular vein catheter attachment will be denoted as Port B.

▲ **CRITICAL STEP** For ease of tubing insertion and keeping Port A tubing from falling out of the microcentrifuge tube, we advise piercing a hole into the side of the microcentrifuge tube. This can be done using the tip of sharp dissecting forceps. Pierce the forceps through the plastic of the microcentrifuge tube downwards at a 45° angle to ensure tubing will remain in place and not draw in air at any point of the experiments.

42 Attach the PE-50 tubing for Port B (port pulling from the mouse, Fig. 4b) to the jugular vein catheter of the anesthetized recipient mouse (see Step 20). Figure 4a illustrates a cannulated mouse that is connected to the pump and the tube containing the complete blood solution.

▲ **CRITICAL STEP** For this step, it is crucial to ensure that no air bubbles are present in any of the tubing. The mouse continues to be under the general anesthesia during this process

43 Flip the toggle switch on the pump to turn it on. Note that the pump's Arduino code is designed in a way that, if the pump is switched off, it will reset to the initial position once turned back on. This initial position is programmed to withdraw fluid from Port B, in a clockwise direction.

44 Perform an initial withdrawal from the mouse to allow the blood to be withdrawn into the tubing. Turn off the pump when the tubing is completely filled with blood and a droplet is observed at the very tip of the Port A outlet, and then stop pump (Fig. 4b). Ensure that no air bubbles have formed.

- Once the pump power switch is toggled to the OFF position, the two tubing lines for Port A and Port B are pinched by the pump rollers on the pump head. This ensures that no air will infiltrate the tubing

- As initially described, once the pump is turned on again, it restarts at initial position to start pulling toward Port B again for the start of experiment

- Figure 4b illustrates the preparation of the device for exchange. Take note that the pump head has the shape of a hexagon with rollers on every other corner. Ensure that the pump head is positioned such that the front most corner does not have a roller at the start of the exchange. If needed, the pump head can be rotated by hand to the correct start position

Forward blood exchanges with the small-animal blood exchange device ● Timing 5 min for a single exchange, 30–45 min to exchange 50% blood

▲ **CRITICAL** It is possible to perform repeated exchanges across the span of multiple days so long as the cannula remains patent. To accomplish this, flush the catheter with 'B' solution and then add 20 μL of 'A' as a catheter locking solution (as described in Box 1). Repeat flushing and locking daily. For the exchange procedure, confirm that the catheter of the recipient mouse is connected to the pump via the PE-50 tubing (Port B in the previously described section).

45 Prepare x microcentrifuge tubes, labeled with $e = 0$ to $e = x$ number of exchanges to serve as the blood samples of different timepoints of different exchanges throughout the experiment and have ready.

▲ **CRITICAL STEP** As previously described, it is helpful to pierce a hole in the side of the microcentrifuge collection tube to ensure tubing will remain inside the tube for the duration of the exchange cycle.

46   Release the hemostat that was previously occluding the tubing.

47   Turn on the pump and allow blood to be withdrawn from the mouse (Port B) for collection into a sample collection tube, exchange (e) = 0, i.e., e_0 (Port A) (Fig. 4c).

▲ **CRITICAL STEP** This step generates an additional reserve of blood cells, in case more saline + MSA + blood cells exchange fluid is needed for an ongoing procedure.

? TROUBLESHOOTING

48   Just as the pump is about to change polarity (~5 s before switch), feed the PE-50 tubing of Port A into the complete blood solution tube. Once the polarity changes, fluid from Port A donor tube will be withdrawn and pumped into the recipient mouse. Note each time that the pump changes polarity.

▲ **CRITICAL STEP** *One full exchange is denoted as the arrow symbol '→'.* This is the point when all the fluid from the complete blood solution tube is pumped into the mouse and there is another polarity change where blood from the mouse would return to the complete blood solution tube (Fig. 4c).

? TROUBLESHOOTING

49   About 5 s before the polarity change from when blood is withdrawn from the mouse back to Port A, feed the PE-50 tubing of Port A into a new 'sample exchange, e_x tube'. These samples serve as blood samples of the exchanged points for n exchanges. If not interested in sampling blood drawn from the mouse to sample at different exchange points, only one sample collection tube is needed.

50   About 5 s before the next polarity change from when the complete blood solution is pumped back to the mouse, move the PE-50 tubing of Port A into the complete blood solution tube.

51   Continue transfusing the blood into the mouse using this technique until all the volume is infused into the mouse. We have observed that approximately five to seven transfusions are needed to reach 50% exchange; more precise calculations and equations are provided below.

? TROUBLESHOOTING

52   To address gravity-induced blood cell sedimentation, occasionally disturb the complete blood solution microcentrifuge tube by either by pipetting the suspension up and down or lightly flicking the side of tube. Periodically perform this tube disturbance for the duration of the exchange.

53 To infuse the last of the complete blood solution into the mouse, allow the pump to collect all the blood in the tube and then shut off just before any air reaching the mouse.

54 Trim the mouse catheter, then plug and glue to the back of the mouse to seal the catheter and prevent it from being pulled out.

Back-and-forth blood exchanges with Y-coupler ● Timing 5 min for a single exchange, ~35 min to exchange 50% blood

▲ **CRITICAL** Schematics of this procedure are illustrated in Fig. 5.

55 Heparinize the Y-couplers; draw heparin saline into each line (Box 1), then lariat the lines to close them against each other. Let the heparin incubate inside the lumen of the tubing for at least 1 h at room temperature. Minimize bubble formation to maximize the available volume of tubing that will be in contact with the heparin. Heparinizing the Y-couplers in this fashion will minimize occlusions within the tubing.

56 Anesthetize the mice and apply ophthalmic ointment as previously stated in the 'Jugular vein cannulation surgery' section (Steps 1–24).

57 Lay the mice on a heating pad in ventral recumbency (lying on their front).

58 (Optional) If exchanges are to be performed the day after cannulation, secure the catheter with a hemostat clamp. Remove the catheter plugs, remove the lock solution from the catheters and then flush the catheters with heparin saline; this step is performed with a Hamilton syringe. Otherwise, the mouse is still heparinized from the cannulation procedure that was completed hours prior on the same day.

59 Connect the mice to a Y-coupler; each mouse is connected to a line that passes through the Y-coupler; be sure to draw blood up from both lines to ensure that they do not contain air.

? TROUBLESHOOTING

60 Using a hemostat clamp, occlude one of the lines (Line 1) and begin drawing 150 μL of blood from the non-occluded line (Line 2); once the desired volume is reached, occlude Line 2 and then inject the blood into the next mouse; these mark one exchange.

61 Repeat each exchange cycle 14 more times to attain ~50% blood exchange in each mouse. In other words, roughly half of each mouse's total blood volume has been replaced with half of its partner's blood.

62 Disconnect the mice from the Y-coupler using hemostat clamps, and then add heparin lock solution to the catheters (Box 1). Plug the catheters once locking solution has been added.

63 Remove the mice from anesthesia and allow them to recover and administer meloxicam.

64   Perform post-procedural care (see Steps 21–24).

Procedure 3: blood exchange mathematical modeling

▲ **CRITICAL** According to the National Centre for the Replacement, Refinement, and Reduction of Animals in Research, mice are estimated to have ~58.5 mL of blood per kilogram of body weight (www.nc3rd.org.uk/mouse-decision-tree-blood-sampling).

Back-and-forth-exchange function derivation ● Timing Variable (once the equations are derived, it is not necessary to perform this section again)

▲ **CRITICAL** First, we present the derivation of the 'back-and-forth exchange' mathematical model where blood is being exchanged between two mice in a back-and-forth fashion.

1.   Set the definition for an exchange as: mouse 1 has its blood removed and exchanged into mouse 2, followed by removing the same volume of blood from mouse 2 and exchanging it back to mouse 1. We will use the following variables:

Variable	Description
$r(x)$	Residual blood fraction
$i(x)$	Infused blood fraction
x	Number of exchanges
l_1 and l_2	Total blood volume of mice 1 and 2
v	Blood volume moved for each exchange

2   Begin with a residual blood fraction of 1 for mouse 1 because no blood has left mouse 1, and 0 for mouse 2 infused blood because none of mouse 1's blood has mixed with mouse 2's blood. We also denote midpoints in the exchange, when mouse 2 has received blood but not exchanged it back to mouse 1, as $X.5$ steps, where X is the previous exchange number. This assumes that sufficient time has been given for the blood to mix in each mouse between steps. See the following table:

Exchange	Mouse 1 residual blood $r(x)$	Mouse 2 infused blood $i(x)$
0	1	0
0.5	1	$\dfrac{v}{v + l_2} = a_1$
1	$\dfrac{l_1 - v + v * a_1}{l_1} = a_2$	a_1
1.5	a_2	$\dfrac{a_1 * l_2 + a_2 * v}{v + l_2} = a_3$
2	$\dfrac{a_2(l_1 - v) + v * a_3}{l_1} = a_4$	a_3
2.5	a_4	$\dfrac{a_3 * l_2 + a_4 * v}{v + l_2} = a_5$

Exchange	Mouse 1 residual blood $r(x)$	Mouse 2 infused blood $i(x)$
$\lim x \to \infty$	$\dfrac{l_1}{l_1 + l_2}$	$r(x) = Ae^{-B \times x} + C$

We substituted certain fractions as a_i to prevent the equations from becoming too complex. This recursive formula behaves like an exponential decay function in the form of $r(x) = Ae^{-B \times x} + C$. Solve for constants A, B and C to obtain the final equation:

$$r(x) = \frac{l_2}{l_1 + l_2} \times e^{-Bx} + \frac{l_1}{l_1 + l_2}$$

where B is equal to:

$$\ln\left(\frac{(l_1 \times (v + l_2))}{l_1 \times l_2 - l_2 \times v}\right)$$

Forward exchange function derivation ● Timing The time required is variable. Once the equations are derived, it is not necessary to perform this section again.

▲ **CRITICAL** Forward exchanges are best suited for exchanging blood from a mouse with a complete blood solution. NBEs with 5% MSA, saline and synchronic blood cells may be defined as a complete blood solution[20,21].

3   Forward exchanges are defined as: a volume of blood, v, is withdrawn from a mouse with total blood volume l. v is transferred to an empty collection tube. The volume v is then drawn from the tube containing the complete blood solution and then injected back into the mouse.

4   Set l_2 of the back-and-forth function to infinity because, if we assumed that the total volume of mouse 2 blood is infinite, it would never be diluted by mouse 1 blood in an exchange. Thus, the residual fraction of blood in mouse 1 will be identical to described scenario in which its blood was exchanged with a complete blood solution. By doing this, we arrive at:

$$r(x) = e^{-\ln\left(\frac{l}{l-v}\right) \times x}$$

Which can be simplified to:

$$r(x) = \left(\frac{l-v}{l}\right)^x$$

Please note that there are caveats that must be addressed when using the equations.

•   The actual fraction of blood in mice during exchanges can be affected by the time between exchanges because blood needs time to be mixed by the heart

- One may desire to exchange half a volume of blood before starting blood exchanges, so that neither mouse has a full volume more or less than its original blood volume. This will result in blood mixing progressing slightly faster than described in our equations

- Researchers must also consider the dead space in the tubes before exchanging blood. This can simply be accounted for by exchanging more blood than intended, and with our exchange pump, we found that adding 50 μL to each exchange accounted for the difference

The parameters can be set to values that the experimenter deems most reasonable. This system also allows the experimenter to precisely determine the dosage of exchanges. We recommend a v that does not exceed 10% of an animal's total blood volume[22].

Model validation strategies ● Timing 20 min for in vitro validation; 45–60 min min for in vivo validation

▲ **CRITICAL** Validation strategies include in vitro and in vivo components. In vitro experiments (Steps 5–15) involve exchanging a red-dye-containing solution with deionized water.

▲ **CRITICAL** Exchanges here were performed with the peristaltic pump.

5   Set parameters represent exchanges between a young and old mouse or by NBEs. Based on our experience, typical mouse weights and blood volumes, exchange volumes, dead-space volume and effective exchange volumes are as detailed below:

Typical old mouse weight	34 g
Typical young mouse weight	28 g
Old mouse blood volume	1,989 μL
Young mouse blood volume	1,638 μL
Dead space of the system	50 μL
Exchange volume	300 μL
Effective exchange volume	250 μL
Number of back-and-forth exchanges	30
Number of forward exchanges	10

6   Adjust the pump head and tubing as described in Step 41 of Procedure 2.

7   To obtain a useable stock solution of red dye, add one drop of red food dye to a microcentrifuge tube and add enough deionized water to fill the volume to 1,989 μL. Dilute the dye solution serially by twofold and measure absorbances with a spectrophotometer. Obtain measurements until the corresponding reduction of absorbances falls within a linear range. Use the dye solution whose absorbance was obtained at the range's maximum for the in vitro experiments. Connect the tube containing this solution to the right side of the peristaltic pump head.

8 Fill another microcentrifuge tube with 1,638 µL of deionized water and connect it to the left side of the pump head.

9 Switch the pump on to begin exchanging the fluids.

10 Mix the receiving tube by pipetting its contents several times before the pump changing polarity.

11 Measure absorbances of each tube after every five back-and-forth exchanges or after every forward exchange.

12 Repeat these steps until the pump ceases motion.

13 Remove the lines and clear them with water.

14 Compute relative absorbances as absorbance the values obtained at x exchanges (A_x) divided by the absorbance of the stock dye solution (A_o). Note that all absorbance values must be normalized to the deionized water sample blank.

15 Plot relative absorbances as a function of x number of exchanges. Figure 6 illustrates the overlaid plots of our relative residual absorbance results and the curve from our derived functions.

▲ **CRITICAL** In vivo experiments (Steps 16–19) involve exchange of blood with desired physiologic exchange fluid.

! CAUTION All experiments involving live vertebrates were performed in accordance with the regulations and guidelines of the Northwest Animal Facility veterinary personnel at the University of California, Berkeley.

▲ **CRITICAL** The broad biomedical significance of complement factors inspired us to probe concentrations of plasma C3 as a function of the number of exchanges during NBE. C3 is a versatile innate immune sensor that was recently recognized as a multitasking protein that engages in crosstalk with a variety of immunoregulatory pathways. These pathways change with aging and influence both disease-promoting and homeostatic processes[34-37]. Excess complement activation can lead to the deregulated release of proinflammatory/chemotactic mediators[34]. C3 is also elevated in the cerebrospinal fluid of patients with Alzheimer's or Parkinson's disease[38] and in age-related diseases such as in patients with type 2 diabetes or atherosclerosis[38]. Cholesterol crystals may lead to the activation of C3 in atherosclerotic lesions, and this induces the release of potent inflammatory mediators[38,39].

▲ **CRITICAL** We used the forward exchange method to dilute the plasma of young (2- to 4-month-old) mice and performed exchanges using the Y-coupler method. Additionally, two forward NBEs (+/− cells, as described below) were performed using the peristaltic pump under the parameters indicated below.

16 Set the parameters for exchange as follows:

Average weight young mice   24.25 g

Corresponding total blood volume	1,418.3 μL
Dead space of the system	50 μL
Exchange volume	200 μL
Effective exchange volume	150 μL
Number of forward exchanges	10

17 Perform NBEs using forward exchange on four young mice with either 5% MSA in saline or 5% MSA in saline with synchronic blood cells (Fig. 7a,b). The exchange procedure is delineated as follows:

- Occlude Line 2 with a hemostat clamp and draw 150 μL of blood from the mouse through Line 1

- Occlude Line 1 and transfer the 150 μL of blood to a collection tube through Line 2

- While Line 1 is still occluded, submerge Line 2 in a tube that contains a mixture of 5% MSA, saline and synchronic blood cells, and draw 150 μL of blood from this tube

- Open Line 1, occlude Line 2 and inject back into the mouse

- These steps result in one full exchange; repeat ten times

18 Using the 150 μL of blood in the collection tubes, detect C3 levels by ELISA according to the manufacturer's instructions.

19 Divide the concentrations of C3 at exchange x ($C3_{Tx}$) by the concentration of C3 at exchange 0 ($C3_{T0}$) to obtain the relative residual concentration of C3 ($C3_{Tx}/C3_{T0}$). Plot $C3_{Tx}/C3_{T0}$ values as a function of the number of exchanges.

Troubleshooting

Troubleshooting advice can be found in Table 3.

Timing

Procedure 1

Steps 1–16, dual-pump fabrication: 1 week

Steps 17–29, single-pump fabrication: 1 week

Procedure 2

Steps 1–20, jugular vein cannulation surgery: 30–90 min

Steps 21–22, monitoring recovery: 48 h

Step 23, clinical effects and changes to animal health and behavior post-procedure monitoring: 1 week

Step 24, catheter patency and maintenance: 1 week

Steps 25–32, preparation and extraction of donor mouse blood samples for supplementing the NBE fluid with the mouse circulating cells: 2 h

Steps 33–40, calibration of the small-animal blood exchange device: 70 min

Steps 41–44, small-animal exchange device preparation: 45 min

Steps 45–54, forward blood exchanges with the small-animal blood exchange device: 30–45 min

Steps 55–64, back-and-forth blood exchanges with the Y-coupler: 35 min

Procedure 3

Steps 1–2, back-forth-exchange function derivation: variable

Steps 3–4, forward function derivation: variable

Steps 5–19, model validation strategies: 20 min for in vitro validation; 45–60 min for in vivo validation

Anticipated results

The detailed protocols will enable successful experiments with blood exchange between mice or exchange of mouse plasma with any physiologic fluid; the key steps can be accurately performed, including the jugular vein cannulation, the design and fabrication of the exchange device, comparative automated and manual procedures, mathematical modeling of blood factors' dilution and troubleshooting. In comparison with parabiosis and plasma infusions, it is expected that there will be greater experimental flexibility and precision of the small-animal blood exchange over the alternatives. The small-animal blood exchange will bestow more control in the studies focused on exchanging or replacing systemic milieu, making the conclusions on subsequent effects on molecular, cellular, tissue and organismal youth health, aging and disease to be more accurate. The exact process of blood or fluid exchange is likely to be relevant for the intensity and duration of improvements in tissue health and regeneration. It is therefore essential to precisely calculate the exchange or dilution rates and dosages to maximize the resulting effects while minimizing the number of exchanges needed. This protocol enables such important biomedical processes.

As an example of specific anticipated results, Fig. 7c demonstrates the overlay of empirical ELISA data on C3 with the corresponding theoretical curve from the forward exchange function. The empirical results of ten in vivo NBE procedures with subsequent C3 ELISA assays (duplicate ELISAs for each NBE, $N = 6$) agrees very well with the theoretical calculations. It is important to note that the experimentally derived $C3_{TX}/C3_{T0}$ values are consistently lower than the theoretical values. This suggests that less C3 is available in the plasma than expected and that it might be sequestered by to other blood factors, such as

albumin, blood cells, etc. Nonetheless, dilution ratios as a function of NBE can be obtained in a predictable fashion.

Data availability

The main data discussed in this protocol were generated as part of the studies published in the supporting primary research papers[20,21]. Blueprints for the fabrication of the dual- and single-pump devices can be found at https://doi.org/10.6084/m9.figshare.19401263.v1. Source data for Fig. 6 can be found at https://doi.org/10.6084/m9.figshare.19401275.v1, and for Fig. 7 at https://doi.org/10.6084/m9.figshare.19401278.v1.

Code availability

The code used in this protocol can be found in Supplementary Information.

Extended Data
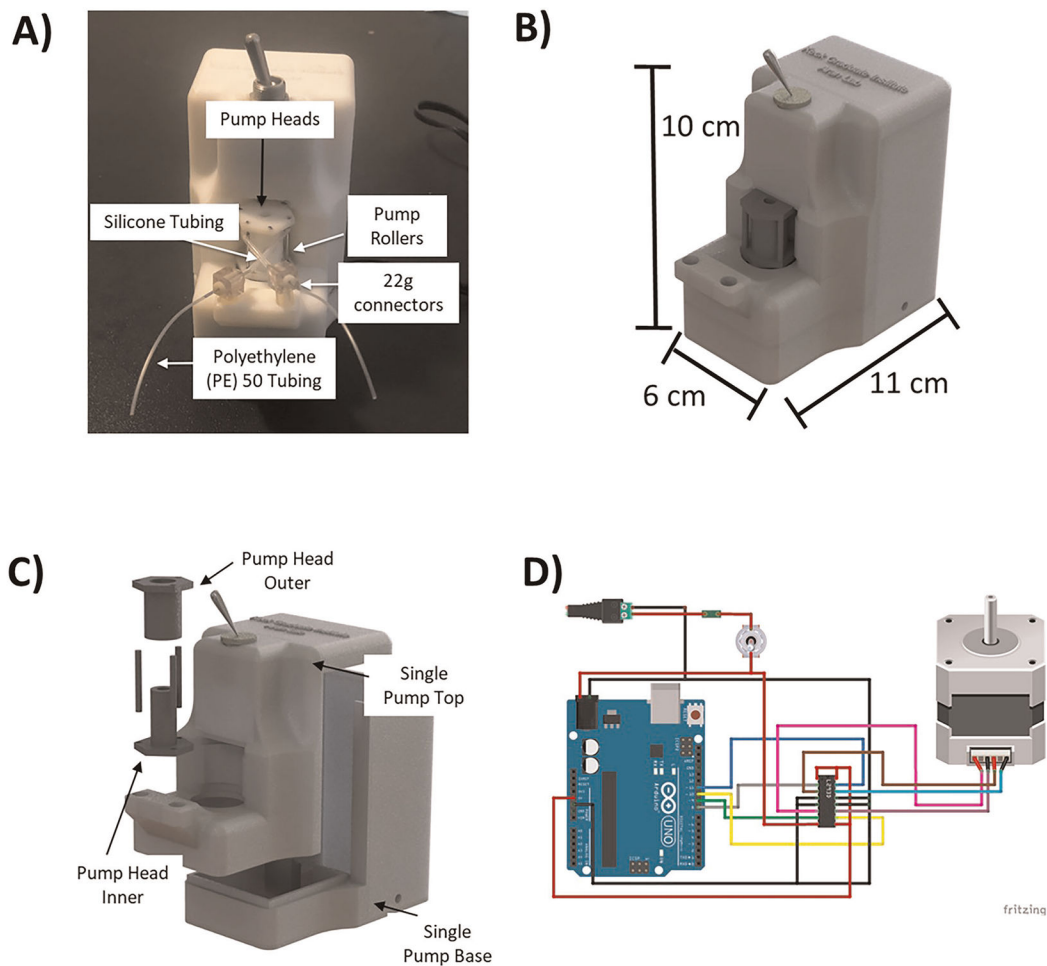
Extended Data Fig. 1 |. Overview and detailed parts and schematics of single-pump small-animal blood exchange device.

a, Overview of single-pump small-animal blood exchange device and detailed description of the parts involved in constructing the external hardware of the pump including appropriate tubing. **b**, CAD image for 3D-printed hardware housing of the pump with dimensions. **c**, Exploded view of the single pump illustrating the intersection of the incorporated components. **d**, Single-pump electrical wiring diagram.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

Related links

Key references using this protocol

References

Rebo J et al. Nat. Commun 7, 13363 (2016): 10.1038/ncomms13363 [PubMed: 27874859]

Mehdipour M et al. Aging 12, 8790–8819 (2020): 10.18632/aging.103418 [PubMed: 32474458]

Mehdipour M et al. GeroScience 43, 1–18 (2021): 10.1007/s11357-020-00297-8 [PubMed: 33191466]

References

1. Villeda SA et al. The ageing systemic milieu negatively regulates neurogenesis and cognitive function. Nature 477, 90–96 (2011). [PubMed: 21886162]

2. Villeda SA et al. Young blood reverses age-related impairments in cognitive function and synaptic plasticity in mice. Nat. Med 20, 659–663 (2014). [PubMed: 24793238]

3. Loffredo FS et al. Growth differentiation factor 11 is a circulating factor that reverses age-related cardiac hypertrophy. Cell 153, 828–839 (2013). [PubMed: 23663781]

4. Conboy IM et al. Rejuvenation of aged progenitor cells by exposure to a young systemic environment. Nature 433, 760–764 (2005). [PubMed: 15716955]

5. Conboy IM & Rando TA Heterochronic parabiosis for the study of the effects of aging on stem cells and their niches. Cell Cycle 11, 2260–2267 (2012). [PubMed: 22617385]

6. Conboy MJ, Conboy IM & Rando TA Heterochronic parabiosis: historical perspective and methodological considerations for studies of aging and longevity. Aging Cell. 12, 525–530 (2013). [PubMed: 23489470]

7. Elabd C et al. Oxytocin is an age-specific circulating hormone that is necessary for muscle maintenance and regeneration. Nat. Commun 5, 4082 (2014). [PubMed: 24915299]

8. Yousef H et al. Aged blood impairs hippocampal neural precursor activity and activates microglia via brain endothelial cell VCAM1. Nat. Med 25, 988–1000 (2019). [PubMed: 31086348]

9. Yousef H et al. Systemic attenuation of the TGF-β pathway by a single drug simultaneously rejuvenates hippocampal neurogenesis and myogenesis in the same old mammal. Oncotarget 6, 11959–11978 (2015). [PubMed: 26003168]

10. Castellano JM et al. Human umbilical cord plasma proteins revitalize hippocampal function in aged mice. Nature 544, 488–492 (2017). [PubMed: 28424512]

11. Yousef H et al. Age-associated increase in BMP signaling inhibits hippocampal neurogenesis. Stem Cells 33, 1577–1588 (2015). [PubMed: 25538007]

12. Yousef H et al. hESC-secreted proteins can be enriched for multiple regenerative therapies by heparin-binding. Aging 5, 357–372 (2013). [PubMed: 23793469]

13. Carlson ME et al. Relative roles of TGF- β1 and Wnt in the systemic regulation and aging of satellite cell responses. Aging Cell 8, 676–689 (2009). [PubMed: 19732043]

14. Carlson ME, Silva HS & Conboy IM Aging of signal transduction pathways, and pathology. Exp. Cell Res 314, 1951–1961 (2008). [PubMed: 18474281]

15. Egerman MA et al. GDF11 Increases with age and inhibits skeletal muscle regeneration. Cell Metab. 22, 164–174 (2015). [PubMed: 26001423]

16. Smith LK et al. β2-Microglobulin is a systemic pro-aging factor that impairs cognitive function and neurogenesis. Nat. Med 21, 932–937 (2015). [PubMed: 26147761]

17. Conboy IM, Conboy MJ & Rebo J Systemic problems: a perspective on stem cell aging and rejuvenation. Aging 7, 754–765 (2015). [PubMed: 26540176]

18. Schaffer DV & Gage FH Neurogenesis and neuroadaptation. Neuromol. Med 5, 1–9 (2004).

19. Bruel-Jungerman E, Laroche S & Rampon C New neurons in the dentate gyrus are involved in the expression of enhanced long-term memory following environmental enrichment. Eur. J. Neurosci 21, 513–521 (2005). [PubMed: 15673450]

20. Mehdipour M et al. Rejuvenation of three germ layers tissues by exchanging old blood plasma with saline-albumin. Aging 12, 8790–8819 (2020). [PubMed: 32474458]

21. Mehdipour M et al. Plasma dilution improves cognition and attenuates neuroinflammation in old mice. Geroscience 10.1007/s11357-020-00297-8 (2020).

22. Rebo J et al. A single heterochronic blood exchange reveals rapid inhibition of multiple tissues by old blood. Nat. Commun 7, 13363 (2016). [PubMed: 27874859]

23. Esser F, Masselter T & Speck T Silent pumpers: a comparative topical overview of the peristaltic pumping principle in living nature, engineering, and biomimetics. Adv. Intell. Syst 1, 1900009 (2019).

24. Horobin JT, Sabapathy S & Simmonds MJ Repetitive supra-physiological shear stress impairs red blood cell deformability and induces hemolysis. Artif. Organs 41, 1017–1025 (2017). [PubMed: 28543744]

25. Zhang T et al. Study of flow-induced hemolysis using novel couette-type blood-shearing devices. Artif. Organs 35, 1180–1186 (2011). [PubMed: 21810113]

26. Finerty JC & Panos TC Parabiosis intoxication. Proc. Soc. Exp. Biol. Med 76, 833–835 (1951). [PubMed: 14844364]

27. Finerty JC Parabiosis in physiological studies. Physiol. Rev 32, 277–302 (1952). [PubMed: 12983225]

28. Horowitz AM et al. Blood factors transfer beneficial effects of exercise on neurogenesis and cognition to the aged brain. Science 369, 167–173 (2020). [PubMed: 32646997]

29. De Miguel Z et al. Exercise plasma boosts memory and dampens brain inflammation via clusterin. Nature (2021) 10.1038/s41586-021-04183-x

30. Zhang G, Budker V & Wolff JA High levels of foreign gene expression in hepatocytes after tail vein injections of naked plasmid DNA. Hum. Gene Ther 10, 1735–1737 (1999). [PubMed: 10428218]

31. McKeen LW Introduction to fatigue of plastics and elastomers. Fatigue and Tribological Properties of Plastics and Elastomers 3rd edn (Elsevier, 2016); 10.1016/B978-0-323-44201-5.00001-0

32. McKeen LW Introduction to the tribology of plastics and elastomers. Fatigue and Tribological Properties of Plastics and Elastomers 3rd edn (Elsevier, 2016); 10.1016/B978-0-323-44201-5.00002-2

33. Ventola CL Medical applications for 3D printing: current and projected uses. P&T 39, 704–711 (2014). [PubMed: 25336867]

34. Mastellos DC, Reis ES, Ricklin D, Smith RJ & Lambris JD Complement C3-targeted therapy: replacing long-held assertions with evidence-based discovery. Trends Immunol. 38, 383–394 (2017). [PubMed: 28416449]

35. Ricklin D, Hajishengallis G, Yang K & Lambris JD Complement: a key system for immune surveillance and homeostasis. Nat. Immunol 11, 785–797 (2010). [PubMed: 20720586]

36. Kolev M, Le Friec G & Kemper C Complement—tapping into new sites and effector systems. Nat. Rev. Immunol 14, 811–820 (2014). [PubMed: 25394942]

37. Hajishengallis G & Lambris JD Microbial manipulation of receptor crosstalk in innate immunity. Nat. Rev. Immunol 11, 187–200 (2011). [PubMed: 21350579]

38. Cardoso AL et al. Towards frailty biomarkers: candidates from genes and pathways regulated in aging and age-related diseases. Ageing Res. Rev 47, 214–277 (2018). [PubMed: 30071357]

39. Niyonzima N et al. Complement activation by cholesterol crystals triggers a subsequent cytokine response. Mol. Immunol 84, 43–50 (2017). [PubMed: 27692470]

40. Björk I & Lindahl U Mechanism of the anticoagulant action of heparin. Mol. Cell. Biochem 48, 161–182 (1982). [PubMed: 6757715]

41. Chuang Y-J, Swanson R, Raja SM & Olson ST Heparin enhances the specificity of antithrombin for thrombin and factor Xa independent of the reactive center loop sequence: evidence for an exosite determinant of factor Xa specificity in heparin-activated antithrombin. J. Biol. Chem 276, 14961–14971 (2001). [PubMed: 11278930]

42. Weitz JI New anticoagulants for treatment of venous thromboembolism. Circulation 110, I-19–I-26 (2004). [PubMed: 15339877]

Box 1 |

Heparin handling and ensuring cannula patency

Proper anticoagulation of each in-dwelling line marks the make-or-break point of these experiments. Heparin is a naturally occurring glycosaminoglycan (a polysaccharide, not a protein) that has potent anticoagulant properties. Heparin binds to and thereby activates antithrombin III (AT)[40,41]. Activated AT in turn inactivates thrombin, factor Xa and other proteases involved in the clotting cascade[34,35]. Heparin has a fairly short half-life in vivo due to degradation and/or binding onto macrophages, endothelial cells or proteins[42]. As such, proper dosing in mice is of the essence.

- Standard dose in mice: 100 U/kg per 12 h at minimum

- Tolerated maximum: 20 U within the indicated 12-h period, though this may vary mouse to mouse. We add 9–11 U; mice do not appear to be negatively affected by this dose and it keeps the catheters patent for several hours (at the very least)

Heparin handling

Before preparation, we recommend ordering pharmaceutical-grade HSI solutions from Sagent Pharmaceuticals. 1,000 U/mL of HSI will suffice for these experiments. Store HSI solutions at 4 °C, and seal the lids for each HSI tube tightly with Parafilm before removal from the sterile hood.

1. Clean and prepare a workspace in a sterile tissue culture hood following sterile technique

2. Use a syringe needle to draw ~2 mL of HSI, then transfer this quantity into a sterile and labeled 2.0 mL microcentrifuge tube

3. Obtain two additional 0.5 mL microcentrifuge tubes

4. Aliquot 175 μL of HSI into one of the 0.5 mL microcentrifuge tubes + 325 μL of sterile normal saline (labeled 'A')

5. Aliquot 50 μL of HSI into the next 0.5 mL microcentrifuge tube + 450 μL of sterile saline (labeled 'B')

6. Aliquot 'A' into four more tubes at 100 μL each (350 U/mL heparin saline)

7. Aliquot 'B' into four more tubes at 100 μL each (100 U/mL heparin saline)

▲ **CRITICAL STEP** Prepare 'A' and 'B' aliquots daily to ensure freshness and optimal activity.

Ensuring cannula patency

1. Before the act of cannulation, perform a catheter patency test: by preloading a sterile catheter with aliquot 'A'. We used a 10.5-cm 1 Fr to 3 Fr catheter that fits a maximum of 20 μL. This means that one requires 20 μL of 'A' to completely fill the lumen. 20 –L of 'A' translates to ~9 U of heparin. Plug the catheter once preloaded.

2. Once the cannula is inserted into the blood vessel, clamp the plugged end of the catheter.

3. Draw 20–30 μL of aliquot 'B' into a Hamilton syringe

4. Unplug the catheter and then connect the Hamilton syringe to the catheter (push a drop of fluid out of the syringe and from the catheter upon clamping to ensure no bubble formation)

5. Draw a small amount of blood from the vein. A properly cannulated vessel will draw blood without complication.

6. At this point, A and B should be well infused in the line. Inject the heparin solutions into the vein once the blood draw has been completed. Be careful to not introduce air from the end of the syringe once the fluid runs out.

7. Plug and unclamp the catheter. The mouse will have ~11–12 U in its system (this appears to be well tolerated by the mice; if any unusual bleeding occurs, limit the heparin).

▴ **CRITICAL STEP** Ensure that oozing has ceased in the surgical site before adding heparin.

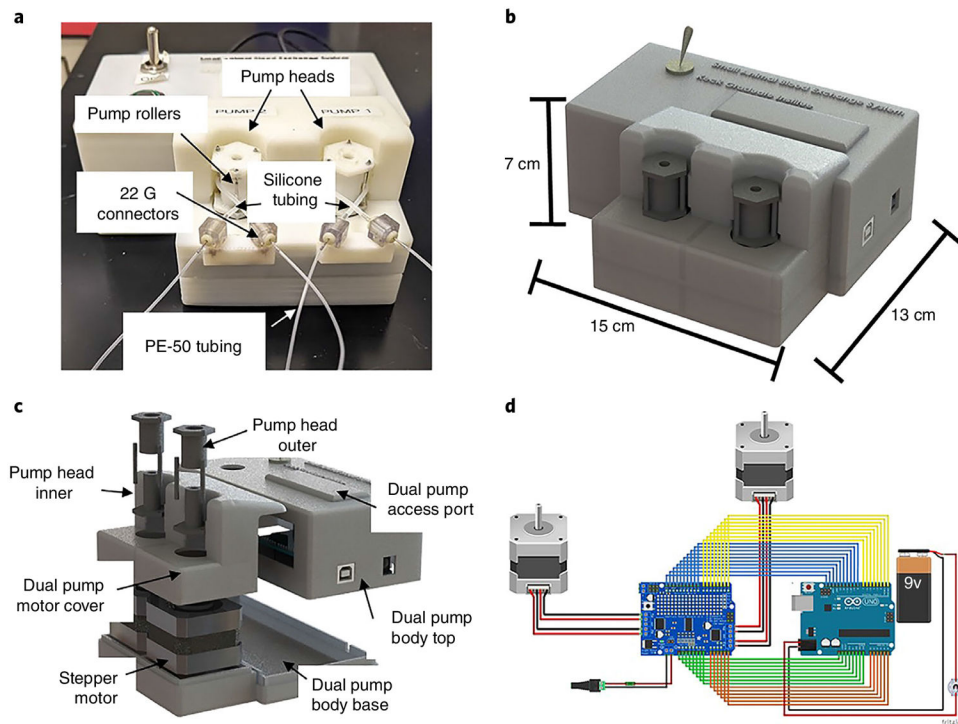**8.** Resume with the surgical protocol as described.

Fig. 1 |. Overview and detailed parts and schematics of dual-pump small-animal blood exchange device.

a, Image of the dual-pump small-animal blood exchange device with denotations of the parts involved in constructing the external hardware of the pump including appropriate tubing. **b**, CAD image for 3D-printed hardware housing of the pump with dimensions. **c**, Exploded view of the dual pump illustrating the intersection of the incorporated components. **d**, Dual-pump electrical wiring diagram.
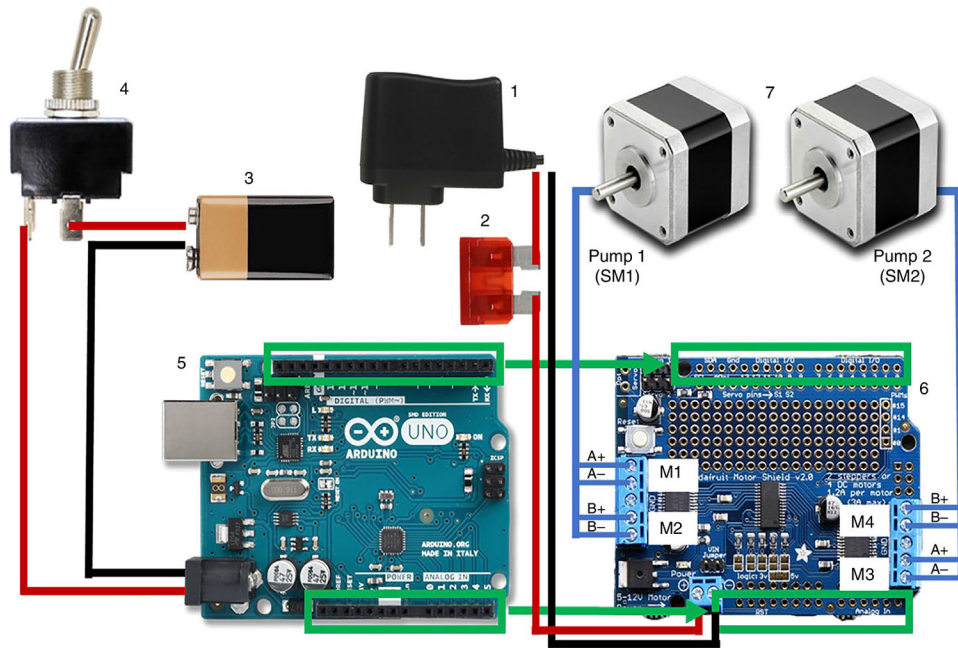
Fig. 2 |. Detailed electrical schematic of dual-pump small-animal blood exchange device.
(1) AC-DC adapter; (2) blade fuse and connector; (3) 9-V battery; (4) toggle switch; (5) Arduino Uno; (6) Adafruit stepper shield for Arduino v2; (7) stepper motor. Red lines denote the positive line extended from the positive battery terminal, while black denotes the negative line. Green boxes and arrows denote header pin connections and blue A+/− and B+/− represent the phases of the stepper motor connected to the Adafruit stepper shield.
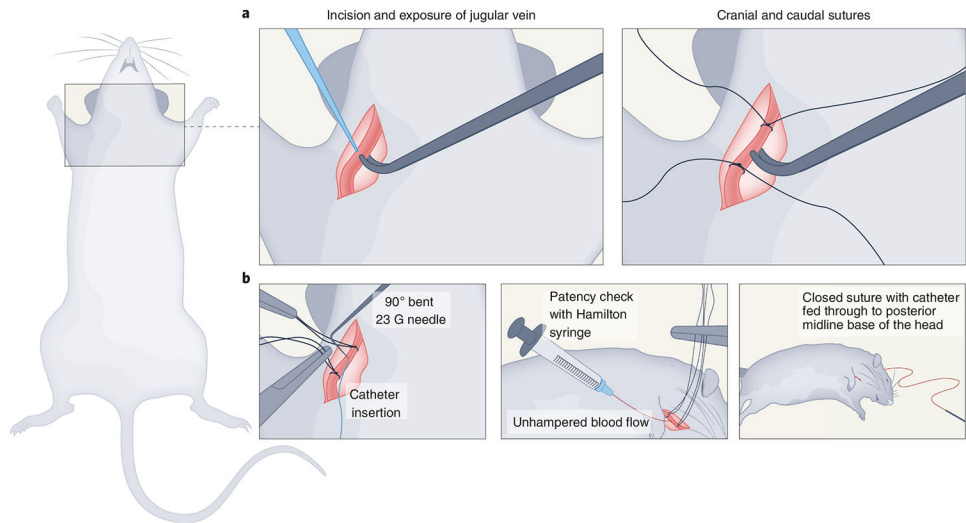
Fig. 3 |. Major stages of jugular vein cannulation procedure (tracing of the photographs).
a, Left: initial incision site for right jugular vein cannulation and exposure of jugular vein, ensuring posterior side of the vein is well exposed and free of fatty or connective tissues. Right: placement of initial two suture for top and bottom knots to be tied around the jugular vein; top knot is tied tightly to occlude blood flow and bottom knot is loosely tied until post catheter insertion. **b**, Left: puncture of the jugular vein was created with a 23 G needle with its tip bent to a 90° angle. Catheter was inserted through puncture and into vessel towards right atrium. Middle: once the catheter is placed, a patency check is performed using a heparin-loaded Hamilton syringe. The syringe is pushed and pulled for forward and backward flow to ensure no occlusions in the catheter. Right: blunt dissection from the incision to the posterior midline of the base of the skull allows for feeding the catheter through the back. All experiments involving live vertebrates were performed in accordance with the regulations and guidelines of the University of California, Berkeley under IACUC-approved protocol.
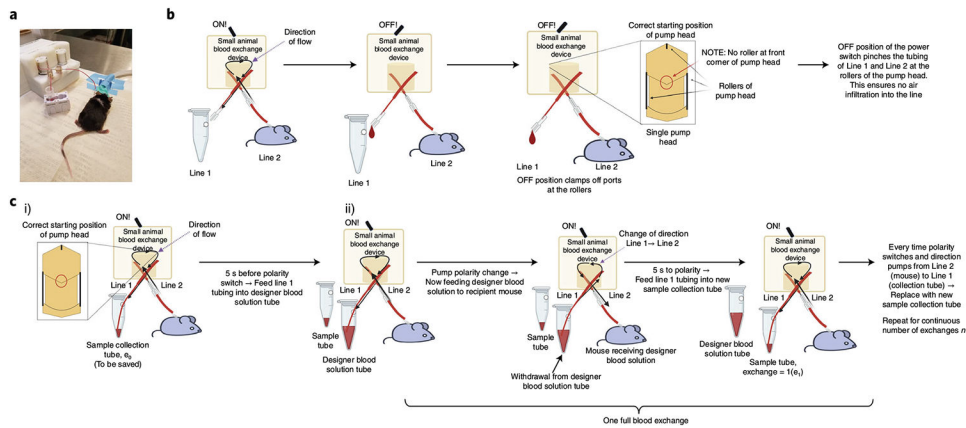
Fig. 4 |. Automation of the forward blood exchange method with dual-pump small-animal blood exchange device.

a, Typical experimental setup of small-animal blood exchange with dual pump. **b**, Side-view schematic of small-animal blood exchange device preparation for day of experiment. Blood from the recipient mouse is drawn into the silicone tubing to flush the system of any potential air in a clockwise direction. Once line is full of blood and droplet appears at end of Line 1 outlet, shutting the pump off clamps the tubing at the rollers of the pump head to ensure no air infiltration. Zoom-in of the pump head depicting its correct starting position. The hexagonal shape of the pump head should be oriented in a manner such that there is no roller (dark gray bars shown on each side) at the front-most corner (circled in red). **c**, (i) Schematic of initial blood exchange workflow. Once turned back on from the previously described device preparation step, blood from the recipient mouse is withdrawn into an initial sample collection tube. Within 5 s of the polarity change of the pump, the Line 1 tubing is fed into the complete blood solution tube. (ii) Once polarity changes, the directionality is pumping in a counterclockwise fashion, withdrawing from the complete blood solution tube to the recipient mouse. Again, 5 s before the polarity switch, the Line 1 tubing is fed into a new sample collection tube. This signifies one full blood exchange. Line 2 tubing delivers the fluids to the recipient mouse. Adapted from Biorender.com under a paid academic subscription. All experiments involving live vertebrates were performed in accordance with the regulations and guidelines of the University of California, Berkeley, under an IACUC-approved protocol.
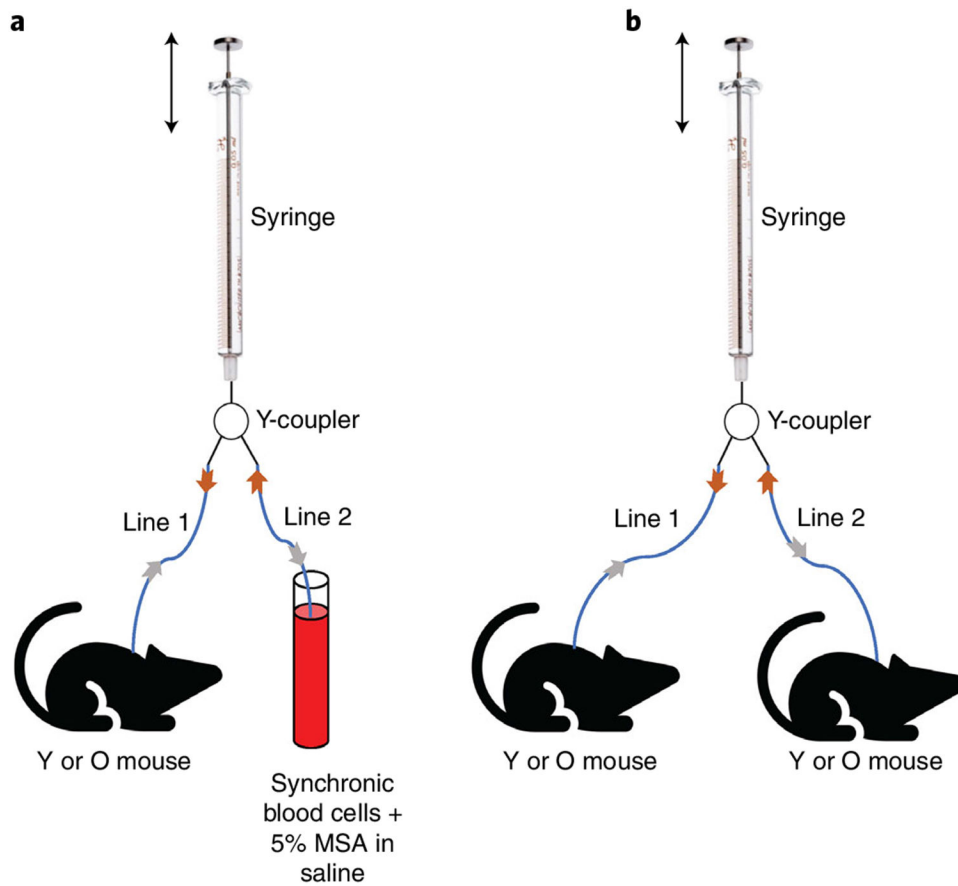
Fig. 5 |. Back-and-forth blood exchanges using the Y-coupler and syringe method.
Young (2–4 months of age) or old (18–24 months of age) mice or a tube containing
a complete blood solution are connected to a Y-coupler. The experimenter draws blood
using the syringe. Lines 1 and 2 are occluded in an alternating fashion to draw or inject
blood volumes between exchange partners. **a**, Blood exchanges between a young (Y) or
old (O) mouse and a tube consisting of syngeneic donor mouse blood cells, 5% MSA
in 0.9% sodium chloride (saline). **b**, Blood exchanges between mice of the indicated
ages. All experiments involving live vertebrates were performed in accordance with the
regulations and guidelines of the University of California, Berkeley, under an IACUC-
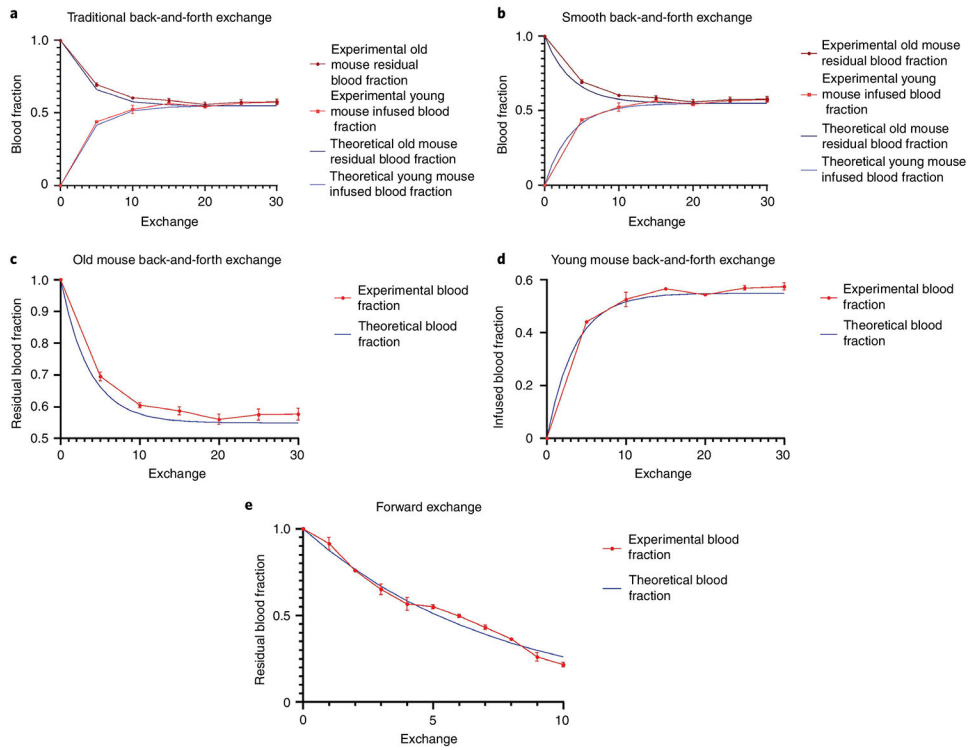approved protocol.

Fig. 6 |. Empirical results of automated mouse blood exchange parameters.
Each plot shows theoretical curves with experimentally derived curves overlaid.
Experimental results represent A_x/A_o versus x curves. **a**, Absorbance values were collected
for every five exchanges. Theoretical plot values are shown accordingly. **b**, The theoretical
plot is shown as a smooth and continuous curve. **c**, Residual blood fractions for the old
mouse tube. **d**, Infused blood fractions for the young mouse tube. **e**, Forward exchanges
performed with old mouse tubes. Empirical exchanges experiments were performed in
triplicates. Error bars represent standard deviations from three samples per timepoint.
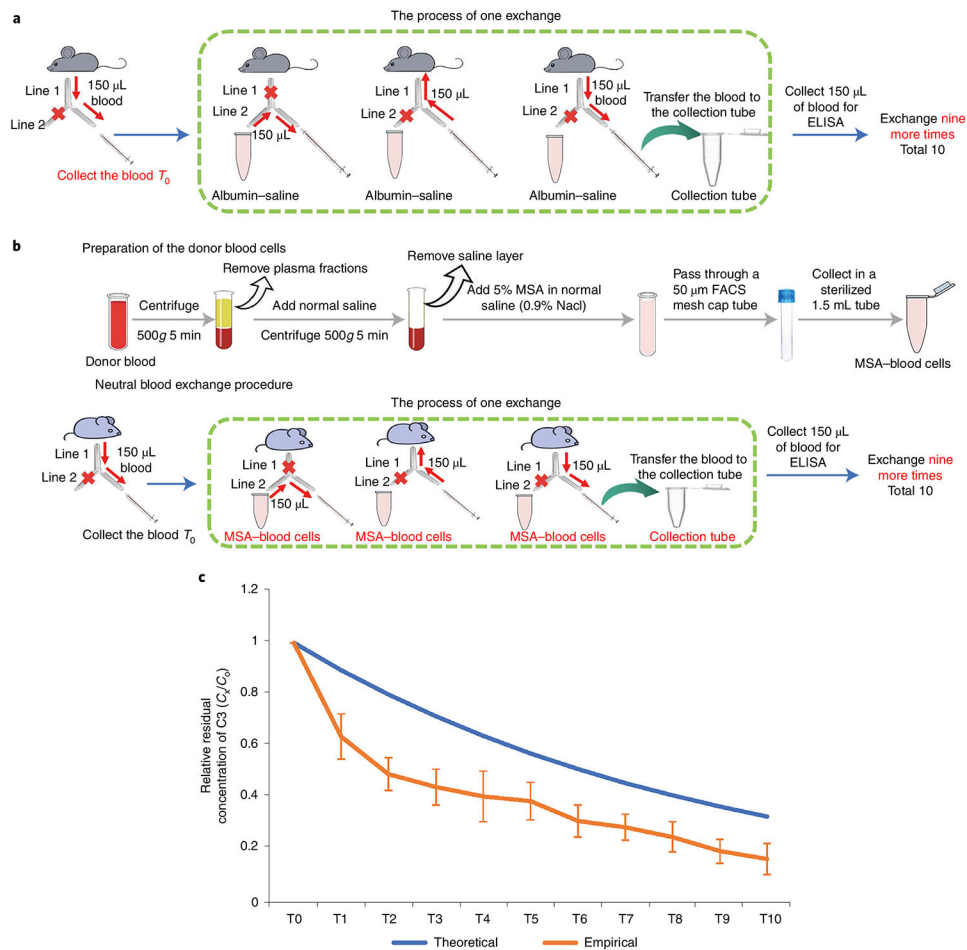
Fig. 7 |. Empirical results of Y-coupler forward exchanges with mouse blood exchange using the indicated parameters in vivo.

a, Experimental schematic of Y-coupler forward exchanges with 5% MSA and saline without syngeneic blood cells. **b**, Experimental schematic of Y-coupler forward exchanges with 5% MSA in saline with syngeneic blood cells. **c**, Data were obtained by performing eight Y-coupler exchanges ($N = 4$ MSA with cells and $N = 4$ MSA without cells) and two pump exchanges ($N = 1$ MSA with cells and $N = 1$ MSA without cells). Experimentally derived $C3_{TX}/C3_{T0}$ versus x curves overlain with the theoretical curve obtained from the function. Error bars represent standard errors of the means. All experiments involving live vertebrates were performed in accordance with the regulations and guidelines of the University of California, Berkeley, under an IACUC-approved protocol.

Table 1 |

Comparison of parabiosis and plasma injections with blood exchange

Experimental parameter	Parabiosis	Plasma injections	Blood exchange
Stress, environmental enrichment and exercise	+	+/− (repeated animal handling)	−
Shared organs	+	−	−
Blood cell sharing	+	−	+/− (as desired)
Maintaining precise control over how much blood is exchanged	−	−	+
Exchange amounts of blood exceeding 50%	−	−	+
Ability to mix complete blood solutions for exchange	−	−	+
Risk of parabiotic disease (epigenetic mismatching)	+	+/− (might manifest with repeated infusions)	−
Concentrated in the liver	−	+	−

(+) indicates that the respective approach exhibits these characteristics and (−) indicates the opposite.

Table 2 |

Comparison between blood exchange using a peristaltic pump and a Y-coupler and syringe

Peristaltic pump exchange	Y-coupler and syringe exchange
More materials and resources are needed for operation and maintenance	Less complex, fewer materials are required
Hands-off: exchanges are performed automatically	Hands-on: exchanges are performed manually
Optimized to exchange precise volumes of fluid at a precise rate with minimal error	Volumes and rates of exchange might be imprecise owing to researchers' errors, syringe handling, air bubbles, etc

Table 3 |

Troubleshooting table

Step	Problem	Possible reason	Solution
Procedure 2, Step 13	Catheter is not patent when initially checked	The catheter may not have entered the lumen of the vein properly and may have contacted fascia or entered the mediastinum	Loosen the caudal ligature and reposition the catheter. To ensure that the catheter is properly inserted in the vein, the surgeon should not feel any resistance upon insertion. If the catheter is properly inserted but it still is not patent, remove the catheter and carefully inspect for occlusions
Procedure 2, Steps 37, 48, 59	Blood does not draw into tubing	Occlusions within the tubing that require dislodging	Perform a sternal rub: gently massage the sternum using the index finger in an undulating fashion
			Turn off the pump and manually rotate the pump head to dislodge any occlusions
			Check the metal points of contact for obstructions
Procedure 2, Step 47	The original saline + MSA + donor-mouse blood cells exchange fluid becomes accidentally depleted before the completion of the procedure	Miscalculation of the exchange volumes	Resuspend the host reserve blood cells that were collected during the procedure in saline + MSA, and use this exchange fluid to complete the procedure
Procedure 2, Step 51	Exchange volumes appear to vary/drift over time	Stretch or wearing out of the silicone tubing	Check the elasticity of the silicone tubing. If elasticity of silicone tubing is stretched passed original length (of when initially assembled), replace the tubing
			If tubing is not the issue, the pump may be recalibrated through the Arduino code by modifying the variable 'const int half_cycle = xxx.' Increasing the value of this variable will increase the volume exchanged; decreasing the value of this variable will decrease the volume exchanged. The variable value should be divisible by 66.6 (Supplementary Files 1–3). Upload the updated code onto the Arduino and continue to calibrate until the desired parameters have been achieved