

SUPPLEMENTAL DATA

Construction of Isolated Perfused Heart System

Our isolated perfused heart system was constructed on a general two-layer trolley cart (height \times long \times wide (mm) = 950 \times 900 \times 600) with wheels (Supplemental Fig. 1). All devices were installed on the cart, and the mobility of this system facilitated easy access to the micro PET system. A 10-liter carbogen gas cylinder, a bottle for waste solution and a heating thermostat with a 25-liter water sink (Alpha A 24, Lauda Dr. R. Wobser GmbH & Co. KG) were placed on the lower layer of the cart. The rest of the items including 2-liter double lumen buffer reservoir tanks (Radnoti LLC), a buffer pump (REGLO Digital MS-2/6, Ismatec), a 0.45 μ m cellulose acetate filter (Ciro Manufacturing Corp.) covered by an in-line holder (EMD Millipore Corp.) and a small air bubble trap filter (Speedflow Kids 5.0 μ m, GVS group) were located on the upper layer.

The part from the filter to the heart was fixed using metal stands, rods and connections (Supplemental Fig. 2). While the total length of the buffer tube was approximately 1 m, temperature loss was minimized by wrapping the buffer tube together with the hot water circuit tube using aluminum foil. The end of the buffer tube was connected to a three-way stopcock and connected to an aortic cannula of a metal pipe of 1.7 mm outer diameter. A drip collecting system, created by cutting a 100 ml standard syringe, was held by a flexible stand clamp.

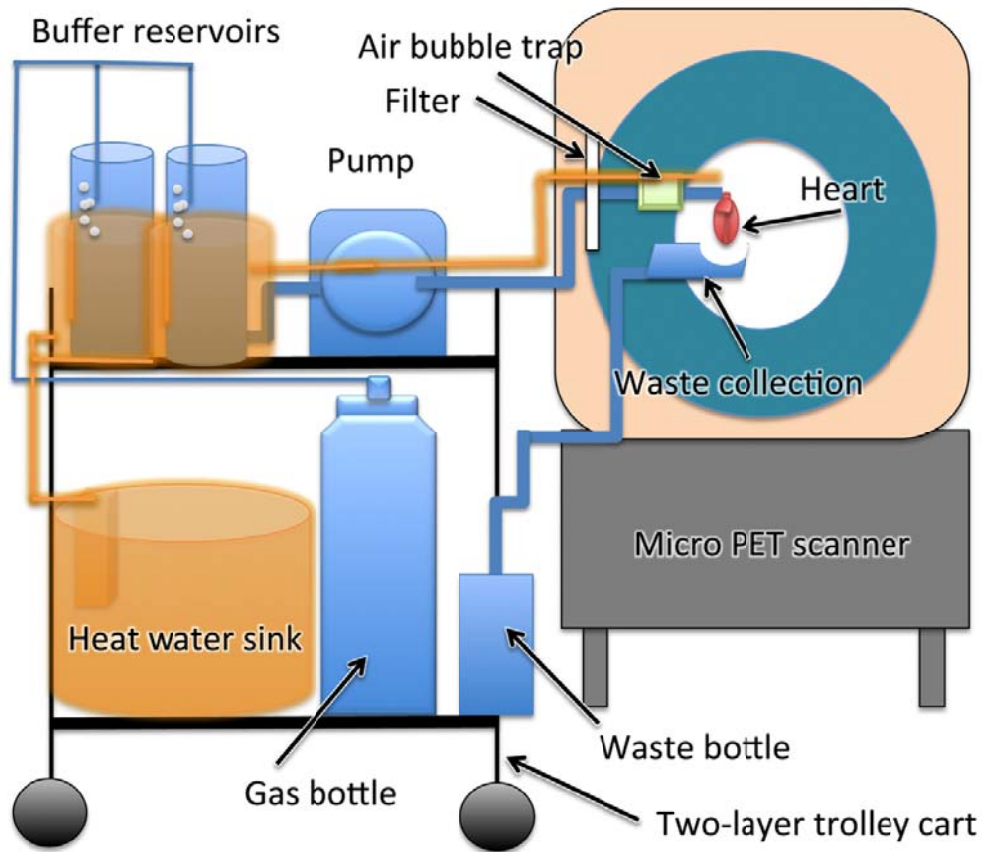
Protocol with Isolated Perfused Heart Study

Krebs-Henseleit buffer (Sigma-Aldrich Co. LLC) was poured into the perfusate circuits and oxygenated by carbogen (95% oxygen and 5% carbon dioxide) for 1 h or more. The pH of the buffer was adjusted to 7.2 ± 0.1 by adding hydrochloric acid, and checked by a pH meter (EL2, Mettler Toledo AG). The temperature of the buffer was monitored at the aortic cannula by a digital thermometer (Testo 925, Testo AG) and kept at $38.0 \pm 1.0^\circ\text{C}$ during all experiments using hot water circuits.

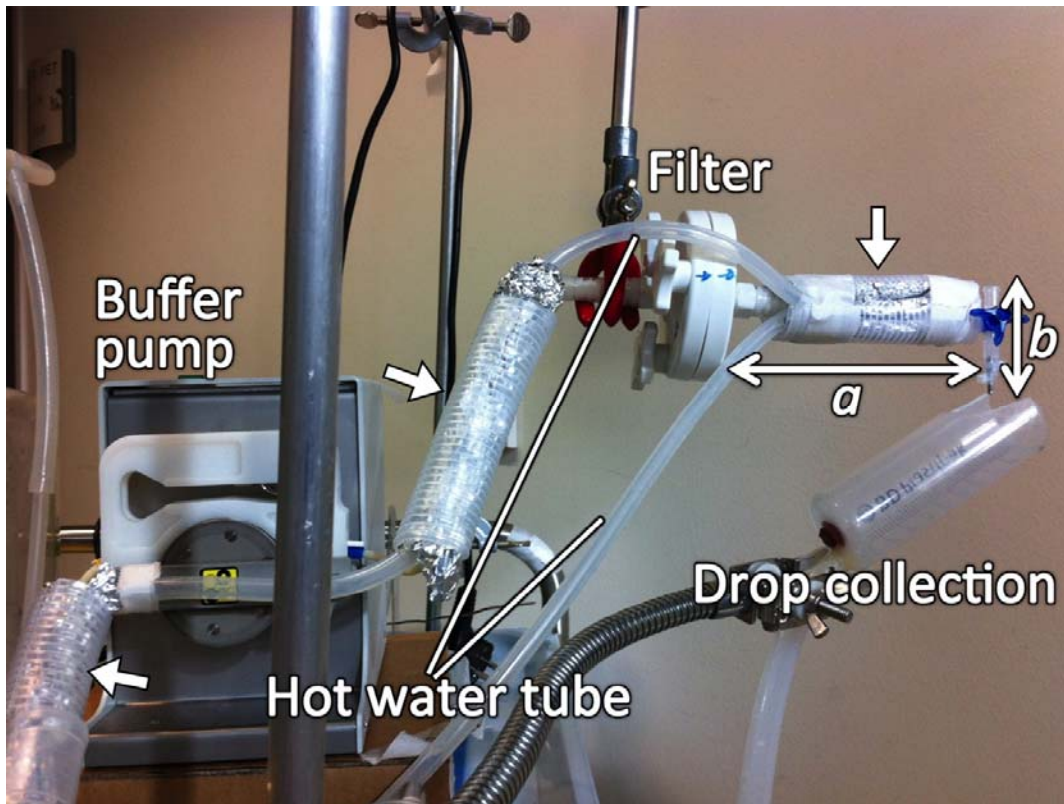
After heparinization of the rats, the hearts were extirpated under deep anesthesia with 2% isoflurane. The heart was clipped to the aortic cannula and quickly mounted by a silk

thread. Plastic paraffin films (Parafilm, Pechiney Plastic Packaging, Inc.) were used to avoid drying and fluttering of the hearts. The drip correcting system was set just under the heart to drain waste solution from the hearts.

To prevent radioactive contamination plastic sheets were used to cover both PET gantry and floor. During the PET measurements, the wheels of the trolley cart were locked after confirmation of the optimal position of the isolated perfused heart (center of the field of view).



SUPPLEMENTAL FIGURE 1. A design of the isolated perfused heart device for micro PET systems. All devices were installed on two-layer trolley cart.



SUPPLEMENTAL FIGURE 2. A photograph of the isolated perfused heart device for micro PET systems. To reduce temperature loss, both the buffer tube and the hot water tubes are wrapped together and covered using aluminum foil (arrows). The part from the filter to the heart ($a = 13.5$ cm and $b = 5.5$ cm) is placed in the micro PET gantry.