UCLA

UCLA Previously Published Works

Title

Parabiosis in mice: a detailed protocol.

Permalink
https://escholarship.org/uc/item/0wv4w7ts

Authors
Kamran, Paniz
Sereti, Konstantina-Ioanna
Zhao, Peng
et al.

Publication Date
2013

DOI
10.3791/50556

Peer reviewed

eScholarship.org Powered by the California Digital Library
University of California

Video Article

Parabiosis in Mice: A Detailed Protocol

Paniz Kamran[1,2], Konstantina-Ioanna Sereti[1,2], Peng Zhao[1,2], Shah R. Ali[3], Irving L. Weissman[3], Reza Ardehali[1,2]

[1]Department of Medicine-Division of Cardiology, University of California, Los Angeles

[2]Eli and Edythe Broad Center of Regenerative Medicine and Stem Cell Research, University of California, Los Angeles

[3]Institute for Stem Cell Biology and Regenerative Medicine, Stanford University School of Medicine

Correspondence to: Reza Ardehali at RArdehali@mednet.ucla.edu

URL: http://www.jove.com/video/50556
DOI: doi:10.3791/50556

Keywords: Medicine, Issue 80, Biomedical Engineering, Anatomy, Physiology, Surgery, Blood Cells, Hematopoietic Stem Cells (HSC), Blood, Blood Vessels, Cell Biology, Parabiosis, mouse, inflammation, microvasculature, immune reaction, animal model

Date Published: 10/6/2013

Citation: Kamran, P., Sereti, K.I., Zhao, P., Ali, S.R., Weissman, I.L., Ardehali, R. Parabiosis in Mice: A Detailed Protocol. *J. Vis. Exp.* (80), e50556, doi:10.3791/50556 (2013).

Abstract

Parabiosis is a surgical union of two organisms allowing sharing of the blood circulation. Attaching the skin of two animals promotes formation of microvasculature at the site of inflammation. Parabiotic partners share their circulating antigens and thus are free of adverse immune reaction. First described by Paul Bert in 1864[1], the parabiosis surgery was refined by Bunster and Meyer in 1933 to improve animal survival[2]. In the current protocol, two mice are surgically joined following a modification of the Bunster and Meyer technique. Animals are connected through the elbow and knee joints followed by attachment of the skin allowing firm support that prevents strain on the sutured skin. Herein, we describe in detail the parabiotic joining of a ubiquitous GFP expressing mouse to a wild type (WT) mouse. Two weeks after the procedure, the pair is separated and GFP positive cells can be detected by flow cytometric analysis in the blood circulation of the WT mouse. The blood chimerism allows one to examine the contribution of the circulating cells from one animal in the other.

Video Link

The video component of this article can be found at http://www.jove.com/video/50556/

Introduction

Parabiosis, the surgical joining of two organisms, was first described in 1864 by Paul Bert as a way to develop a model to study shared circulatory systems and consisted of the joining of the skin and muscular walls of two rats[1]. Parabiosis promotes formation of microvasculature at the site of inflammation[3] and has had several applications in physiological studies, such as the hormonal communication between the pituitary gland and gonads as well as the role of the kidney in hypertension[4]. It has been further employed to investigate the recruitment and integration of progenitor cells in neovascularization[5], migration of hematopoietic stem cells[6], and lymphocyte trafficking[7], as well as the role and kinetics of circulating inflammatory or stem cells in tumor metastasis[8,9], and neurodegenerative disease[10].

One significant advantage of parabiosis lies in that the partnered animals share common circulating antigens, allowing cell migration and neovascularization without triggering an immunological reaction. Importantly, Weissman *et al.* have shown that parabiosis between male and female mice does not lead to formation of anti H-Y antibodies[11].

In the original protocol described by Paul Bert the two animals were joined together through connection of the skin and muscle walls[1]. This method however, caused significant strain to the animals and resulted in high mortality due to infection of the wound. Since then the parabiosis technique has been revised by several groups with the most predominant being the protocol proposed by Bunster and Meyer in 1933[2]. Their method included joining of the scapula joints, body cavities, and skin, permitting better support and less pain for the animals. At the same time, the new method resulted in minimal post-operative care and significantly decreased mortality rates. The protocol described herein is a modification of the Bunster and Meyer technique that is less invasive and allows firmer joining. Namely, mice are connected through the elbow and knee joints as well as the skin. This joining prevents extension of the skin and therefore causes less pain and complications. Here we describe the joining of a wild type (WT) adult mouse to a constitutive GFP expressing mouse. We show that two weeks following surgery we can achieve 50% of blood chimerism demonstrating the efficacy of this surgical procedure to create a shared circulatory system.

Protocol

All animal studies were performed according to the guidelines of UCLA's animal care and use committee and the National Institutes of Health Guide for the Care and Use of Laboratory Animals. The duration of the procedure described below is approximately 45-60 min from beginning to end.

Copyright © 2013 Journal of Visualized Experiments

1. Preparation of Surgical Field

1. Perform procedure in a clean animal surgery room.
2. Equipment: isoflurane Vaporizer, Gaymer T Pump with heating pad.
3. Sterile tools: two curved forceps, fine scissors, needle holder.
4. Sterile gloves must be used during the entire procedure.

2. Preparation of Animals

1. Place two male or female mice, from same genetic background, of similar weight and size in the same cage and monitor for at least two weeks to ensure harmonious cohabitation. Female mice are preferred due to their less aggressive behavior.
2. Anesthetize animals by using an isoflurane vaporizer. Place mice in a Posi-Seal Induction Chamber connected to the isoflurane vaporizer (4-5% v/v). Once anesthesia is induced, transfer the animal to the fur shaving area and maintain the anesthesia throughout the procedure through a nose cone connected to isoflurane (1.5-2% v/v). Apply ophthalmic ointment with a Q-tip to prevent dry eyes.
3. Place the animal on the supine position. Thoroughly shave the left side of the mouse placed on the left and the right side of the mouse placed on the right starting at approximately 1 cm above the elbow to 1 cm below the knee.
4. Aseptically prepare the shaved areas by thoroughly wiping (2-3x) with Betadine-soaked wipes followed by alcohol wipes. Place the mice on a heated pad covered by a sterile pad.
5. For analgesia, administer Carprofen and Buprenorphine intraperitoneally or subcutaneously at a dose of 10 mg/kg and 0.1 mg/kg respectively.
6. Place animals on their side, back to back, with adjacent shaved areas facing up. To avoid any contamination of the surgical area, cover the mice with a sterile drape exposing only the operation area. Create a small drape opening to stay sterile when performing the surgery. We made the drape window large to have a better viewing during videotaping.

3. Parabiosis

1. Using a sharp scissor, perform longitudinal skin incisions to the shaved sides of each animal starting at 0.5 cm above the elbow all the way to 0.5 cm below the knee joint (**Figure 1**). Following the incision, gently detach the skin from the subcutaneous fascia by holding the skin up with a pair of curved forceps and separate the fascia with a second pair to create 0.5 cm of free skin. Perform this separation along the entire incision.
2. Begin the joining by attaching the left olecranon of one animal to the right olecranon of the other. Both olecranons and knee joints are clearly distinguishable following the skin incision. To facilitate the joining, bend the elbow of the first mouse and pass the needle of the non-absorbable 3-0 suture under the olecranon. Similarly, bend the elbow of the second mouse and pass the same suture under it. Attach joints tightly by a double surgical knot.
3. Connect the knee joints following the same procedure.
4. Following the attachment of the joints, connect the skin of the two animals with a continuous absorbable 5-0 Vicryl suture starting ventrally from the elbow towards the knee. To prevent skin rupture and separation perform a tight suture closure of the skin in the area around the elbows and knees. Once the ventral skin attachment has been completed, perform a double surgical knot. Place the mice in the prone position and continue the suture dorsally ending with a double surgical knot. Verify the continuity of the suture and confirm the lack of openings.
5. Administer 0.5 ml of 0.9% NaCl subcutaneously to each mouse to prevent dehydration.

4. Postoperative Recovery

1. Keep animals on heated pad until recovery.
2. Following recovery, provide analgesics carprofen and buprenorphine. Repeat intraperitoneally or subcutaneously every 24 and 12 hr, respectively, for 48 hr at the same doses described above (step 2.5). Monitor animals for signs of pain and distress such as shaking, lethargy, chewing of tail, arched back, lack of grooming, *etc.* daily for two weeks.
3. Prophylactically, treat mice with Sulfamethoxazole /Trimethoprim oral suspension in their water bottle 2 mg sulfa/ml +0.4 mg trim/ml for 10 days to prevent bacterial infections.
4. House each parabiotic pair in a clean cage with monolithic bedding material (*e.g.* paper towel or absorbent sterile pad) to prevent aspiration of bedding material. Return the animals to a bedding filled cage when they are able to maintain sternal recumbency with their head up. To minimize the strain of reaching for food while adjusting to parabiotic existence, place the moistened food pellets on the cage floor. Provide nesting material. In 1-2 weeks parabiotic mice have the ability to ambulate normally on surgically paired fore-and hind-limbs.
5. Blood chimerism occurs 10-14 days following the surgery.

5. Confirmation and Reverse Procedure

1. After two weeks draw blood from the tail veins of each mouse for flow-cytometric analysis to confirm blood chimerism (**Figure 3**).
2. Blood processing for flow cytometric analysis: dilute 2-3 drops of blood in 500 µl of 10 mM EDTA. Add 500 µl of 2% dextran (diluted in PBS), mix thoroughly and incubate at 37 °C for 30 min. Transfer the supernatant in a new tube, spin at 1,200 rpm for 5 min and resuspend the pelleted cells in PBS (or other buffer) for flow cytometric analysis.
3. Depending on experimental design, the parabiotic pair can be separated at later time points. In our experience, we have maintained parabiosed pairs for up to 9 months without any complications.
4. Perform reverse procedure on a sterile surgical surface. Anesthetize mice by placing them in an induction chamber connected to an isoflurane vaporizer (4-5%) and maintain anesthesia with 1.5-2% isoflurane, as described above (step 2.2).

Copyright © 2013 Journal of Visualized Experiments

5. Remove hair from the area surrounding the initial suture and aseptically prepare the shaved areas as described above (steps 2.3, 2.4).
6. For analgesia, administer Carprofen and Buprenorphine intraperitoneally or subcutaneously at a dose of 10 mg/kg and 0.1 mg/kg, respectively.
7. Cover surgical area with a sterile drape as described above (step 2.6).
8. Using sharp scissors, separate the mice through a longitudinal incision along the lateral suture and gently detach the newly formed fascia between the two mice with a pair of curved forceps. To separate the joints, cut the knots of the suture connecting them. Trim off the skin along the incision to achieve smooth edges and reattach skin with an absorbable continuous 5-0 coated Vicryl suture.
9. To prevent dehydration, administer 0.5 ml of 0.9% NaCl subcutaneously to each mouse. Keep animals on heated pad until recovery.
10. After recovery, repeat analgesic administration, carprofen (10 mg/kg) every 24 hr and buprenorphine (0.1 mg/kg) every 12 hr for 48 hr.
11. To prevent bacterial infections treat mice with Sulfamethoxazole /Trimethoprim oral suspension (2 mg sulfa/ml +0.4 mg trim/ml) in their water bottle for 10 days.

Representative Results

The anticipated outcome of parabiosis of two organisms is the equal contribution of each animal's circulatory system to a common blood circulation (**Figure 2**). One can easily verify the successful equilibration of the blood of the parabiosed WT and GFP positive mice by flow cytometric analysis. Here, venous blood was obtained from the tails of both parabionts at 2 weeks after the surgery and was fractionated for peripheral blood cells (to exclude erythrocytes). The fractionated hematopoietic-derived cell fraction was subsequently analyzed by flow cytometry for the presence of GFP positive and WT cells. Consistent with previous studies, blood from the WT mouse revealed presence of chimerism, as indicated by approximately half GFP positive and half WT blood cells (53% WT cells and 47% GFP cells) (**Figure 3**).

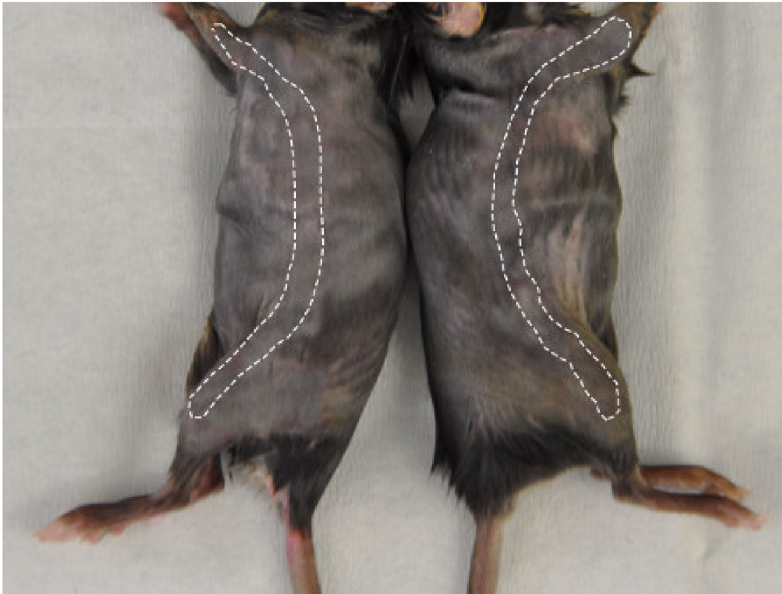

Figure 1. Parabiosis surgery: Incision site. The lateral sides of anesthetized animals are shaved and longitudinal skin incisions are performed (doted area) from 0.5 cm above the elbow to 0.5 cm below the knee joint.

Copyright © 2013 Journal of Visualized Experiments

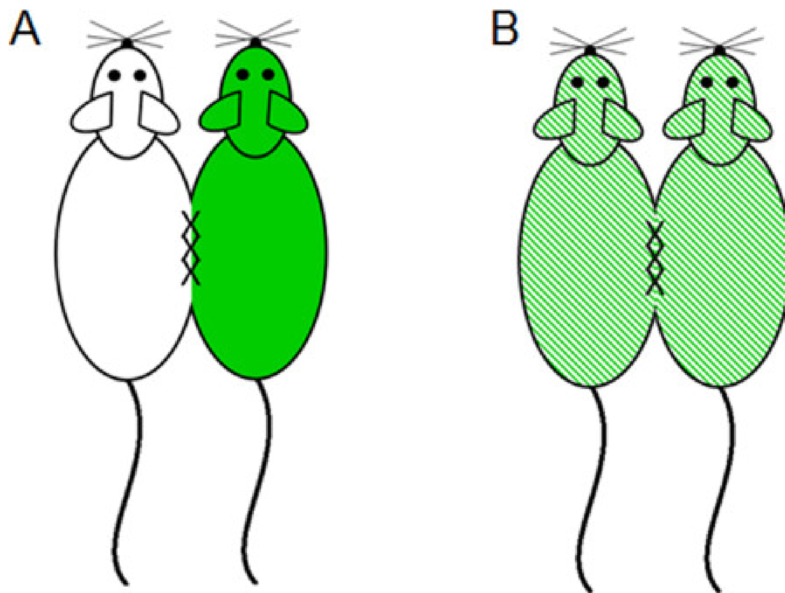

Figure 2. Schematic representation of the parabiosis method. A) Joining of a WT mouse (left) to a GFP positive mouse (right). **B)** Approximately two weeks following surgical attachment, new microvasculature is formed at the location where the two mice are connected allowing joining of the blood circulation.

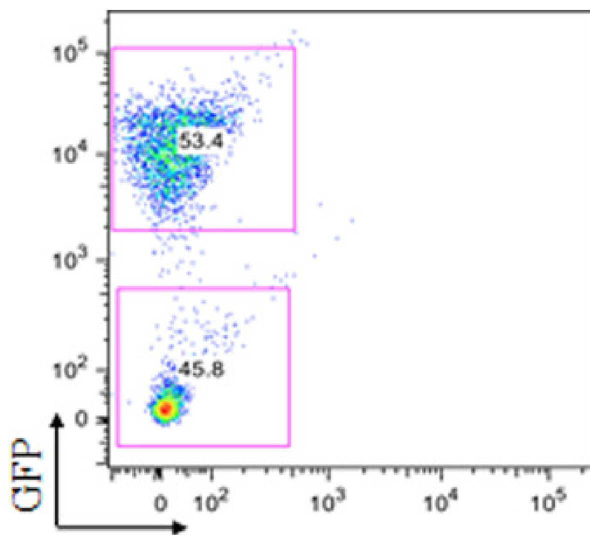

Figure 3. Blood chimerism of parabiotic partners. Flow cytometric analysis of blood sample collected from the WT parabiotic partner two weeks following surgical joining.

Discussion

The parabiosis method discussed here presents minimal technical difficulties and results in low mortality rates. The attachment of knee and elbow joints is a significant improvement of the Bunster and Meyer technique. However, the procedure remains invasive thus maintenance of sterile conditions throughout the surgery is imperative. To further prevent infection of the surgical site, it is important that the parabiosed animals receive a combination of antibiotics and be monitored regularly. To ensure firm support and prevent pain, the suture connecting the elbows and knees should surround the joints rather than passing through the tissue.

As an alternative to isoflurane inhalation, anesthesia may be induced with the use of intraperitoneal injection of ketamine/xylazine or other anesthetic drugs approved by institutional committees. The advantage of using isoflurane is that it can rapidly induce anesthesia and significantly reduce the recovery time. Furthermore, the level of anesthesia can be precisely controlled.

Parabiosis, which allows circulatory systems from two animals to commingle and equilibrate, is a powerful experimental procedure for physiological studies. It presents several advantages to other commonly used techniques such as bone marrow transplantation or cell injection. Cell delivery procedures, at times, provide a short window to examine the effect of transplanted cells. In addition, immunosuppression increases the risk of infection and subsequent morbidity and mortality. However, with parabiosis one can maintain a chimeric circulation for long periods of

Copyright © 2013 Journal of Visualized Experiments

time and study circulating factors (cells, cytokines, *etc.*) independent of their origin. This system can be used to determine the role of circulating cells in wound healing, tumor formation, aging, regeneration, and inflammatory response, among many others.

Disclosures

The authors have no competing financial interests.

Acknowledgements

The authors would like to thank Adriane Mosley and Libuse Jerabek (Stanford) for assistance with the surgical technique.

References

1. Bert, P. Expériences et considérations sur la greffe animale. *Journal de l'Anatomie et de la Physiologie.* **1**, 69-87 (1864).
2. Bunster, E. & Meyer, R.K. Improved methods of parabiosis. *Anat. Rec.* **57**, 339-380 (1933).
3. Waskow, C. Generation of parabiotic mice for the study of DC and DC precursor circulation. *Methods Mol. Biol.* **595**, 413-428, doi:10.1007/978-1-60761-421-0_27 (2010).
4. Finerty, J.C. Parabiosis in physiological studies. *Physiol. Rev.* **32**, 277-302 (1952).
5. Aicher, A. & Heeschen, C. Nonbone marrow-derived endothelial progenitor cells: what is their exact location? *Circ. Res.* **101**, e102, doi:10.1161/CIRCRESAHA.107.162438 (2007).
6. Abe, S., Boyer, C., *et al.* Cells derived from the circulation contribute to the repair of lung injury. *Am. J. Respir. Crit. Care Med.* **170**, 1158-1163, doi:10.1164/rccm.200307-908OC (2004).
7. Donskoy, E. & Goldschneider, I. Thymocytopoiesis is maintained by blood-borne precursors throughout postnatal life. A study in parabiotic mice. *J. Immunol.* **148**, 1604-1612 (1992).
8. Powell, A.E., Anderson, E.C., *et al.* Fusion between Intestinal epithelial cells and macrophages in a cancer context results in nuclear reprogramming. *Cancer Res.* **71**, 1497-1505, doi:10.1158/0008-5472.CAN-10-3223 (2011).
9. Duyverman, A.M., Kohno, M., *et al.* A transient parabiosis skin transplantation model in mice. *Nat. Protoc.* **7**, 763-770, doi:10.1038/nprot.2012.032 (2012).
10. Ajami, B., Bennett, J.L., *et al.* Local self-renewal can sustain CNS microglia maintenance and function throughout adult life. *Nat. Neurosci.* **10**, 1538-1543, doi:10.1038/nn2014 (2007).
11. Weissman, I.L., Jerabek, L., *et al.* Tolerance and the H-Y antigen: Requirement for male T cells, but not B cells, to induce tolerance in neonatal female mice. *Transplantation.* **37**, 3-6 (1984).

Copyright © 2013 Journal of Visualized Experiments