Submission ID #: 62108
Scriptwriter Name: Domnic Colvin
Project Page Link: https://www.jove.com/account/file-uploader?src=18942988

Title: Transplantation of Neonatal Mouse Cardiac Macrophages into Adult Mice

Authors and Affiliations:

Yandong Li[1], Jie Feng[1], Yan Li[1], Jianqiu Pei[1], Shengshou Hu[1], Yu Nie[1,2#]

1. Fuwai Hospital & Chinese Academy of Medical Sciences, State Key Laboratory of Cardiovascular Disease, Cardiovascular Institute, National Center for Cardiovascular Diseases, Peking Union Medical College
2. National Health Commission Key Laboratory of Cardiovascular Regenerative Medicine, Fuwai Central-China Hospital, Central-China Subcenter of National Center for Cardiovascular Diseases, Zhengzhou, China

Corresponding Authors:
Yu Nie: nieyu@fuwaihospital.org

Email Addresses for All Authors:
yandong_li1231@hotmail.com
fengjie0420@163.com
lyyy5125@163.com
peijianqiu0620@126.com
huss@fuwaihospital.org
nieyu@fuwaihospital.org

Author Questionnaire

1. **Microscopy**: Does your protocol require the use of a dissecting or stereomicroscope for performing a complex dissection, microinjection technique, or something similar? **Yes**

   If **Yes**, can you record movies/images using your own microscope camera?

   Yes

   Authors: Please use your microscope camera to film the SCOPE shots and upload the video files to your project page: https://www.jove.com/account/file-uploader?src=18942988.

2. **Software:** Does the part of your protocol being filmed include step-by-step descriptions of software usage? **No**

3. **Interview statements:** Considering the COVID-19-imposed mask-wearing and social distancing recommendations, which interview statement filming option is the most appropriate for your group? **Please select one**.

   ☒ Interviewees wear masks until videographer steps away (≥6 ft/2 m) and begins filming, then the interviewee removes the mask for line delivery only. When take is captured, the interviewee puts the mask back on. Statements can be filmed outside if weather permits.

4. **Filming location:** Will the filming need to take place in multiple locations? **No**

Current Protocol Length

Number of Steps: 17
Number of Shots: 43

Introduction

1. Introductory Interview Statements

REQUIRED:

1.1. **Dr. Yandong Li:** This protocol demonstrates neonatal cardiac macrophage separation and transplantation into adult cardiac regeneration, which might be a promising way to promote cardiac repair.

1.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

OPTIONAL:

1.2. **Dr. Yandong Li:** The implications of this technique can be extended toward the Heart regeneration field.

1.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

Ethics Title Card

1.3. All animal protocols were approved by the Institutional Animal Care and Use Committee (IACUC) at Fuwai Hospital, Chinese Academy of Medical Sciences.

Protocol

2. Apical resection operation in neonatal 1-day-old Cx3cr1GFP/+mouse

2.1. Begin by precooling the bronze operating platform overnight at minus 20 degrees Celsius **[1]**. Take the anesthetized mouse out of the ice box **[2]** and put it on the platform **[3]**. Anchor the mouse in a supine position using medical adhesive tape **[4]**.

2.1.1. Talent placing the bronze operating platform at minus 20 degrees overnight.

2.1.2. Talent taking out the anesthetized mouse out of the box.

2.1.3. Talent putting the mouse on the bronze operating platform.

2.1.4. Talent anchoring the mouse using adhesive tape.

2.2. Put the operating platform with the mouse under a stereoscope **[1]** and disinfect the mouse chest using a prep pad soaked with betadine and 70% alcohol **[2]**.

2.2.1. Talent placing the platform under a stereoscope.

2.2.2. Talent disinfecting the mouse chest with pad soaked in betadine and alcohol.

2.3. Incise the skin with a 1-centimeter cut at the fourth intercostal area of the chest cavity **[1]** and separate the intercostal muscles using microsurgical scissors until the heart is accessed **[2]**. *Videographer: This step is important!*

2.3.1. SCOPE: Talent incising the skin in the chest cavity of the mouse. Authors: Please use your microscope camera to film the SCOPE shots and upload the video files to your project page: https://www.jove.com/account/file-uploader?src=18942988.

2.3.2. SCOPE: The intercostal muscles being separated using microsurgical scissors.

2.4. Alternately press the chest and abdomen with the help of two forceps until the heart is exteriorized out of chest without any mechanical damage **[1]**. *Videographer: This step is important!*

2.4.1. SCOPE: Talent pressing the abdomen and chest of the mouse using two forceps.

2.5. Set the ribs below and above the small incision as a natural fixation to immobilize the heart **[1]**. *Videographer: This step is important!*

2.5.1. SCOPE: Talent setting the ribs below and above the small incision.

2.6. Locate the apex of the left ventricle and cut a 1-millimeter diameter of ventricular apex tissue using iridectomy scissors **[1]**. *Videographer: This step is difficult and important!*

2.6.1. SCOPE: Talent cutting ventricular apex tissue using iridectomy scissors.

2.7. Confirm that the left ventricular chamber is exposed and begins oozing **[1]**. Gently press the heart back into chest cavity using a cotton swab **[2]**. Suture the muscles, ribs, and skin using 8 by 0 Prolene sutures **[3]** and clean the mouse thoroughly after the operation **[4]**. *Videographer: This step is difficult and important!*

2.7.1. SCOPE: Talent exposing the left ventricular chamber.

2.7.2. SCOPE: Talent pressing the heart back to the chest cavity using cotton swab.

2.7.3. SCOPE: Talent suturing the muscles, ribs and skin.

2.7.4. Talent cleaning the mouse.

2.8. Transfer the mouse from the operating platform to a 37-degree heating blanket to warm up the body immediately after the operation **[1]**. Confirm anabiosis of the mouse by observing for the signs of spontaneous respiration restoration, skin color change from pale to pink, and movement of limbs **[2]**.

2.8.1. Talent transferring the mouse to heating blanket.

2.8.2. Signs of anabiosis.

2.9. Take the operated mouse back to its mother once it has recovered **[1]**. Mingle the operated mouse and its mother's nesting materials if necessary **[2]**.

2.9.1. Talent returning the operated mouse to its mother.

2.9.2. Talent mingling the operated mouse.

3. Preparation of the neonatal cardiac macrophage suspension

3.1. Harvest the heart of a euthanized neonatal *Cx3cr1*$^{GFP/+}$ *(C-X-three-C-R-one-GFP)* mouse 1-day after apical resection **[1]** and immerse it in a 10-centimeter dish with PBS **[2]**.

3.1.1. Talent harvesting the chest out of the chest.

3.1.2. Talent immersing the heart in PBS containing dish.

3.2. Cut the vessels and remaining connective tissue away from the ventricles **[1]**. Cut the auricular appendix and outflow tract away from the heart **[2]**.

3.2.1. Talent cutting the vessels and connective tissue away from the ventricles.

3.2.2. Talent cutting auricular appendix and outflow tract.

3.3. After the heart stops beating, cut the heart into 1 to 2-cubic millimeter pieces in phosphate saline buffer using microsurgical scissors **[1]**. Transfer them to a tube containing 2.5 milliliters of preheated enzyme mix **[2]** and tightly close the tube **[3]**.

3.3.1. Talent cutting the heart tissue in Phosphate saline buffer

3.3.2. Transfer of tissue to tube containing preheated enzyme mix.

3.3.3. Talent closing the tube.

3.4. Invert the tube and place it with the cap down **[1]**, then run the neonatal heart dissociation program **[2]**. Detach the tube from the dissociator after the program completes **[3]**.

3.4.1. Talent inverting the tube and placing with cap down.

3.4.2. Talent running the neonatal dissociation program.

3.4.3. Talent detaching the tube from the dissociator.

3.5. Add 7.5 milliliters of 1x DMEM with 10% FBS to the tube **[1]**. Filter and transfer the suspension to a 15-milliters centrifuge tube **[2]**. Centrifuge the cell suspension at 300 times *g* for 5 minutes **[3]**.

3.5.1. Talent adding DMEM to the tube.

3.5.2. Talent filtering the suspension into a centrifuge tube.

3.5.3. Talent centrifuging the suspension.

3.6. Resuspend the cell pellet in 1 milliliter of blood cell lysis solution **[1]** and incubate for 2 minutes at room temperature **[2]**. Add 5 to 10 milliliters of phosphate saline buffer to the suspension **[3]** and centrifuge at 300 times *g* for 5 minutes **[4]**.

3.6.1. Talent adding blood cell lysis solution.

3.6.2. Talent incubating the tube at room temperature.

3.6.3. Talent adding Phosphate buffer saline to the suspension.

3.6.4. Talent centrifuging the tube.

3.7. Resuspend the cell pellet in 1 milliliter of DMEM with 10% FBS **[1]**, then sort GFP positive neonatal cardiac macrophages by FACS **[2]**.

3.7.1. Talent adding DMEM to the cell pellet.

3.7.2. Talent sorting the GFP positive neonatal cardiac macrophages.

3.8. Sort macrophages into a sterile tube containing 500 milliliters of DMEM with 10% FBS **[1]**. Count the GFP positive macrophages **[2]** and resuspend them in DMEM with 10% FBS at the concentration of a million macrophages per 200 milliliters of DMEM for later injection **[3]**.

3.8.1. Talent receiving sorted macrophages in sterile tube.

3.8.2. Talent counting the GFP positive macrophages.

3.8.3. Talent resuspending the positive GFP in DMEM with FBS.

Results

4. Immunofluorescence, echocardiogram, masons staining, and statistical analysis of neonatal cardiac macrophage transplantation

4.1. Neonatal cardiac macrophages were injected into the myocardial infarcted adult mouse heart and efficiency of the proliferative cardiomyocytes was confirmed by immunofluorescence staining **[1]**.

4.1.1. LAB MEDIA: Figure 3A. *Video editor emphasize on the cell proliferation shown with a white arrow.*

4.2. Statistical analysis shows that cardiomyocyte proliferation increases after macrophage transplantation **[1]**.

4.2.1. LAB MEDIA: Figure 3A. *Video editor emphasize on the grey bar graph on the right.*

4.3. Echocardiogram images show the cardiac function in an adult mouse 1-month after myocardial infarction **[1]**. Cardiac function is enhanced after macrophage transplantation **[2]**.

4.3.1. LAB MEDIA: Figure 3B.

4.3.2. LAB MEDIA: Figure 3B. *Video editor emphasize on the gradual increase in the grey bar graphs on the right.*

4.4. Masson's staining shows that the infarcted area in an adult mouse 1-month after myocardial infarction was remarkably reduced after the neonatal cardiac macrophage transplantation **[1]**.

4.4.1. LAB MEDIA: Figure 3C.

Conclusion

5. Conclusion Interview Statements

5.1. **Dr. Yandong Li:** Neonatal cardiac macrophage transplantation might be a promising strategy to promote adult mouse heart regeneration. This protocol helps researchers pursue regenerative application development and explore the heart regeneration mechanism.

5.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.