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Title: Intramyocardial Transplantation of MSC-Loading Injectable Hydrogels after Myocardial Infarction in a Murine Model

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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?

   No

   If your protocol involves microscopy but you are not able to record movies/images with your microscope camera, JoVE will need to use our scope kit (through a camera port or one of the oculars). Please list the make and model of your microscope.

   OLYMPUS SZ61 (WHSZ10X-H/22)

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? **Yes**

   If **Yes**, how far apart are the locations? **Around 100-200 m, different places in the same location.**

Current Protocol Length

Number of Steps: 14
Number of Shots: 35

Introduction

1. Introductory Interview Statements

REQUIRED:

1.1. **Chan Joon Kim:** This method can help address key issues in current cell-based cardiac therapy, such as its low applicability due to low retention and survival of transplanted cells in infarcted hearts.

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

1.2. **Chan Joon Kim:** To address this issue, this method can provide a reliable technique to study intramyocardial transplantation of stem cells using injectable hydrogels in a murine model, which is an excellent platform to use for investigating cardiac tissue repair and regeneration after myocardial infarction.

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

1.3. **Kiyuk Chang (or Chan Woo Kim):** The main advantage of this technique is that it is a feasible method to improve the retention and survival of transplanted stem cells after intramyocardial transplantation using in situ cross-linkable injectable hydrogels.

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

OPTIONAL:

1.4. **Kiyuk Chang (or Chan Woo Kim):** These hydrogels offer diverse opportunities for cardiac tissue engineering, such as delivering not only stem cells, but also growth factors, genetic materials, and drugs to maximize their therapeutic effects in infarcted heart tissues.

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

Introduction of Demonstrator on Camera

1.5. **Kiyuk Chang (or Chan Woo Kim):** Demonstrating the procedure for in vitro experiments will be Eunmi Lee, a research technologist from my laboratory. Eun-Hye Park and Eunhwa Seong, research technologists, will demonstrate the procedure for in vivo experiments.

1.5.1. INTERVIEW: Author saying the above.

1.5.2. The named demonstrator(s) looks up from workbench or desk or microscope and acknowledges the camera.

Ethics Title Card

1.6. Procedures involving animal subjects were performed in accordance with the Institutional Animal Care and Use Committee (IACUC) at the School of Medicine of The Catholic University of Korea.

Protocol

2. Preparation of MSCs and Injectable Gelatin Hydrogels

2.1. Culture mesenchymal stem cells, or MSCs, in a 100-millimeter culture dish at 37 degrees Celsius and 5% carbon dioxide **[1]**. When the cells reach 80% confluence, wash the dish twice with DPBS **[2]** and incubate them with 1 milliliter of trypsin-substitute at 37 degrees Celsius for 3 minutes **[3]**.

2.1.1. WIDE: Establishing shot of talent taking cells out of the incubator.

2.1.2. Talent washing the cells, with the DPBS container in the shot.

2.1.3. Talent adding trypsin-substitute to the cells.

2.2. Then, add 9 milliliters of culture medium to the cells **[1]** and centrifuge them at 500 x *g* for 3 minutes **[2]**. Discard the resulting supernatant **[3]**, resuspend the cells in 1 milliliter of PBS **[4]**, and maintain the cell suspension on ice **[5]**.

2.2.1. Talent adding medium to the cells.

2.2.2. Talent putting the cells in the centrifuge and closing the lid.

2.2.3. Talent removing supernatant.

2.2.4. Talent resuspending the cells, with the PBS container in the shot.

2.2.5. Talent putting the cell suspension on ice.

2.3. Dilute 10 microliters of cell suspension with 10 microliters of Trypan blue and determine the cell concentration using an automated cell counter **[1]**. Resuspend the MSCs to a density of 1 x 10^7 cells per milliliter **[2]** and transfer them to a 1-milliliter tube **[3]**.

2.3.1. Talent using the cell counter.

2.3.2. Talent resuspending the cells.

2.3.3. Talent transferring the cells to a 1mL tube.

2.4. Prepare a 6.25% GH conjugate solution in PBS and separate it into 2 vials **[1]**. Next, mix the GH solutions with either 6 micrograms per milliliters of HRP **[2-TXT]** or 0.07% hydrogen peroxide **[3-TXT]**.

2.4.1. Talent separating the GH conjugate solution into 2 vials.

2.4.2. Talent adding HRP to the GH solution, with the HRP container in the shot. **TEXT: GH solution A**

2.4.3. Talent adding hydrogen peroxide to the GH solution, with the hydrogen peroxide solution in the shot. **TEXT: GH solution B**

2.5. Briefly centrifuge the cell suspension at 1,000 x *g* **[1]** and carefully aspirate the resulting supernatant **[2]**, then mix the cell pellet with GH solution A **[3]**.

2.5.1. Talent centrifuging the cell suspension.

2.5.2. Talent aspirating the supernatant.

2.5.3. Talent mixing the pellet with solution A.

3. In Situ MSC-loading and Three-dimensional in Vitro Culture

3.1. Load the MSCs in GH solution A and GH solution B into either side of a dual syringe **[1]**. Plate 300 microliters of the combined GH solutions with MSCs at a final density of 5 million cells per milliliter onto an eight-well chamber slide **[2]**.

3.1.1. Talent loading the solutions into the syringe.

3.1.2. Talent plating the cells on the slide.

3.2. After in situ hydrogel formation and subsequent MSC encapsulation via enzymatic cross-linking, add 700 microliters of DMEM containing 10% FBS and 1% antibiotic–antimycotic solution **[1]**.

3.2.1. Talent adding the DMEM to the slide.

3.3. Incubate the slide at 37 degrees Celsius and 5% carbon dioxide and replace the culture medium every 2 to 3 days **[1]**.

3.3.1. Talent putting the slide in the incubator and closing the door.

4. Induction of Myocardial Infarction in Mice

4.1. Prior to surgery, depilate the mouse chest using hair removal cream **[1]** and sterilize the skin with iodine **[2]**. Place the mouse on an operating table **[3]** and intubate it by inserting a catheter into the trachea to provide supplemental oxygen via mechanical ventilation **[4]**. *Videographer: This step is important!*

4.1.1. Talent depilating the mouse.

4.1.2. Talent sterilizing the skin.

4.1.3. Talent placing the mouse on the operating table.

4.1.4. Talent intubating the mouse.

4.2. Gently cut through the skin using surgical scissors **[1]**, then penetrate the intercostal muscles with micro scissors **[2]**. Separate the second and third left ribs using a 5-0 silk suture to maintain an open chest cavity **[3]**. *Videographer: This step is important!*

NOTE: Authors uploaded additional scope shots for 4.2 – 5.1, please use whatever looks best.

4.2.1. SCOPE: Additional Shot_4.2.1.MOV. Talent cutting the skin.

4.2.2. SCOPE: Additional Shot_4.2.2.MOV. Talent cutting the muscle.

4.2.3. SCOPE: Additional Shot_4.2.3(19s-58s).MOV or Additional Shot_4.2.3(1m38s-2m12s)_X.MOV. Talent opening the chest cavity.

4.3. Carefully ligate the left anterior descending coronary artery using a needle holder with an 8-0 polypropylene suture and cut the suture using electrocautery **[1]**. Observe an immediate color change in the anterior left ventricular wall **[2]**. *Videographer: This step is difficult and important!*

4.3.1. SCOPE: Additional Shot_4.3.1(1m05s-1m45s)+4.3.2(3m50s-4m18s).MOV. Talent ligating the artery and cutting the suture.

4.3.2. Color change in the LV wall.

5. Intramyocardial Transplantation of MSC-loading GH Hydrogels

5.1. After inducing the myocardial infarction, inject 10 microliters of the MSC-loading GH solutions into two different points at the infarct border zone **[1-TXT]**. Restore the opened chest cavity and close the muscles and skin using 5-0 sutures **[2]**. *Videographer: This step is difficult and important!*

5.1.1. SCOPE: Additional Shot_5.1.1(1m50s-2m20s).MOV. Talent injecting the MSC-loading GH solutions into the infarct border zone. **TEXT: Total: 2 x 10^5 MSCs in 20 microliters**

5.1.2. SCOPE: Additional Shot_5.1.2.MOV. Talent restoring the chest cavity and closing the muscles and skin.

5.2. Remove the tracheal tube **[1]** and place the mouse in a cage under an infrared lamp during recovery **[2]**.

5.2.1. Talent removing the tracheal tube.

5.2.2. Talent placing the mouse in the cage under the lamp.

5.3. Perform an echocardiography and measure corresponding lines for LV anterior wall, LV internal, and LV posterior wall to obtain cardiac wall thickness, chamber dimension, and fractional shortening **[1]**.

5.3.1. Talent performing an echocardiography on the mouse.

Results

6. Results: Improving Stem Cell Retention and Engraftment with Injectable Hydrogels

6.1. Prior to in vivo transplantation, the proliferation and survival of MSCs in GH hydrogels were confirmed by a 3D in vitro live-dead cell staining assay. Representative images exhibited sufficient MSC proliferation, showing branched networks within GH hydrogels **[1]**.

6.1.1. LAB MEDIA: Figure 2, just days 3 – 7. *Video Editor: Indicate somewhere that the live cells are green and dead cells are red.*

6.2. An extensive multicellular 3D structure of MSCs was clearly observed at day 14, indicating that GH hydrogels could provide a proper microenvironment for the encapsulated cells **[1]**.

6.2.1. LAB MEDIA: Fig S2 Video.mp4.

6.3. After the induction of myocardial infarction, MSC-loading GH hydrogels were intramyocardially transplanted into the peri-infarct areas **[1]**. The MSCs and gel were appropriately sustained within the infarcted region. MSCs **[2]** were well integrated into GH hydrogels **[3]**.

6.3.1. LAB MEDIA: Figure 3 B.

6.3.2. LAB MEDIA: Figure 3 B. *Video Editor: Emphasize the red in the images.*

6.3.3. LAB MEDIA: Figure 3 B. *Video Editor: Emphasize the green in the images.*

6.4. To verify the therapeutic effects of the MSC-loading GH hydrogels, the changes in cardiac function and structure were evaluated by echocardiography and histological analysis at day 28 post-transplantation and compared among the different treatment groups **[1]**.

6.4.1. LAB MEDIA: Figure 4 B.

6.5. The representative echocardiography showed improved cardiac functions, including fractional shortening, ejection fraction, and end-systolic volume, in the MSC-gel treated group **[1]**.

6.5.1. LAB MEDIA: Figure 4 C – E. *Video Editor: Emphasize the MSC/gel bars in all graphs.*

6.6. In addition, histological analysis **[1]** exhibited less fibrosis, thicker infarcted walls, and a smaller infarct size in the MSC-gel treated group than in the other groups, indicating that this protocol significantly attenuated LV remodeling **[2]**.

6.6.1. LAB MEDIA: Figure 5 A.

6.6.2. LAB MEDIA: Figure 5 B – D.

Conclusion

7. Conclusion Interview Statements

7.1. **Kiyuk Chang (or Chan Woo Kim):** Using this technique, we achieved long-term stem cell engraftment and proliferation and led to significant improvements in the cardiac structure and function following myocardial infarction in a murine model.

7.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 5.3.1.*

7.2. **Chan Joon Kim:** This technique can be extended for use in large animals and to clinical translation as a new strategy for prevention of postinfarct hear failure by providing cardiac tissue repair and regeneration.

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