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Title: Preparation of Mesh-Shaped Engineered Cardiac Tissues Derived from Human iPS Cells for In Vivo Myocardial Repair

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Author Questionnaire

1. Microscopy: Does your protocol involve video microscopy, such as filming a complex dissection or microinjection technique? **N**

2. Software: Does the part of your protocol being filmed demonstrate software usage? **N**

3. Filming location: Will the filming need to take place in multiple locations (greater than walking distance)? **N**

Introduction

1. Introductory Interview Statements

REQUIRED:

1.1. **Takeichiro Nakane**: This protocol describes a novel and easy method for creating mesh-shaped engineered cardiac tissues derived from human induced pluripotent stem cell-derived cardiac cells **[1]**.

1.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera Videographer NOTE: Mr. Nakane wore a name tag in the first take of his first interview shot (filename: A007_08241632_C126.mov) that he later took off. His interview shots without the name tag start from A007_08241633_C128.mov.

REQUIRED:

1.2. **Hidetoshi Masumoto**: The flexibility of the tissue geometry and the scalability of the preserved tissue function are the main advantages of this technique **[1]**.

1.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera Videographer NOTE: Focus is soft at start of this shot. Focus is correct from :30 onwards. (filename A007_08241616_C123.mov)

OPTIONAL:

1.3. **Takeichiro Nakane**: The implanted mesh shaped ECTs can restore cardiac structure and function in a rat myocardial infarction model, confirming its feasibility for use in cardiac regenerative therapy **[1]**.

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

OPTIONAL:

1.4. **Hidetoshi Masumoto:** These techniques require reproducible human stem cell processing and tissue culture skills for consistent results **[1]**.

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

Protocol

2. Polydimethylsiloxane (PDMS) Tissue Mold Fabrication

2.1. Begin by cutting a cured PDMS (P-D-M-S) sheet to the appropriate size **[1-TXT]** to allow the sheet to be bonded with silicone adhesive to fabricate a 21- x 20.5-millimeter rectangular tray with 7-millimeter-long, 0.5-millimeter-wide, and 2.5-millimeter-high rectangular posts at staggered positions **[2]**.

2.1.1. WIDE: Talent cutting sheet **TEXT: See text for PDMS preparation details**
2.1.2. Sheet being bonded to tray *Videographer: Important step; Video Editor: please emphasize posts when mentioned*

2.2. The horizontal spacing between two lines of posts should be 2.5 millimeters **[1]**.

2.2.1. Shot of tray *Video Editor: please emphasize spacing between posts*

2.3. Autoclave the tray at 120 degrees Celsius for 20 minutes **[1]** before coating the mold with 1% poloxamer 407 (four-oh-seven) in PBS for 1 hour **[2]**.

2.3.1. Talent placing tray into autoclave
2.3.2. Talent coating tray, with poloxamer container visible in frame *Videographer: Important step*

2.4. Then thoroughly rinse the mold with PBS and place the mold into one well of a 6-well plate **[1]**.

2.4.1. Mold being rinsed, with PBS container visible in frame
~~2.4.2. Talent placing mold into well~~

3. Engineered Cardiac Tissue (ECT) Construction

3.1. After cell lineage analysis, combine the cells from the cardiomyocyte plus endothelial and vascular mural cell protocols **[1-TXT]** so that the final concentration of mural cells is 10-20% in a total cell number of 6 x 10^6 cells per construct **[2-TXT]**.

3.1.1. WIDE: Talent adding cells to container, with both cell culture setups visible in frame *Videographer: Important step* **TEXT: See text for cell preparation and lineage analysis details**
3.1.2. Shot of cells in container *Videographer: Important step* **TEXT: See text for all medium and solution preparation details**

3.2. Mix 133 microliters of acid-soluble rat tail collagen type one solution **[1]** and 17 microliters of alkali buffer to 17 microliters of 10x minimum essential medium on ice **[2]**.

3.2.1. Talent adding collagen to MEM, with MEM container visible in frame
3.2.2. Talent adding buffer to solution on ice and solution turning on pink **TEXT: If solution does not become pinkish, add additional buffer**

3.3. Then add 67 microliters basement membrane matrix to the collagen solution **[1]**.

3.3.1. Talent adding basement membrane matrix to container

3.4. Next, centrifuge the cells **[1-TXT]** and resuspend the pellet in 167 microliters high-glucose Dulbecco's minimal essential medium supplemented with fetal bovine serum and antibiotics **[2]**.

3.4.1. Talent adding tube(s) to centrifuge **TEXT: 5 min, 240 x g, RT**
3.4.2. Shot of pellet if visible, then medium being added to tube, with medium container visible in frame

3.5. Mix the cells and the matrix solution on ice **[1]** and carefully pour 400 microliters of the cell-matrix suspension onto the poloxamer 407 coated PDMS tissue mold **[2-TXT]**.

3.5.1. Matrix being added to cells
3.5.2. Solution being added to mold *Videographer: Important/difficult step* **TEXT: Caution: Avoid bubbles**

3.6. Incubate the cell-matrix mixture in a standard cell culture incubator at 37 degrees Celsius and 5% carbon dioxide for 60 minutes **[1]**.

3.6.1. Talent placing plate into incubator

3.7. When the tissue has formed, carefully soak the mold with 4 milliliters of ECT culture medium **[1]** and return the plate to the cell culture incubator for 14 days **[2-TXT]**.

3.7.1. Medium being added to mold, with medium container visible in frame *Videographer: Important step*

3.7.2. Talent placing plate into incubator **TEXT: Change medium every day**

3.8. Before ECT implantation, use sterilized fine forceps to carefully remove the ECT from the loading posts **[1]**.

3.8.1. Construct being removed OR LAB MEDIA: Figure 3E

Protocol Script Questions

A. Which steps from the protocol are the most important for viewers to see?
2.1., 2.3., 3.1., 3.5., 3.7.

B. What is the single most difficult aspect of this procedure and what do you do to ensure success?
3.5. Pour the mixture quickly, but carefully to avoid generating bubbles in order to prevent filling defects in the poured gel.

Results

4. Results: Representative ECT Design and Generation

4.1. Tissue molds can be fabricated with various patterns, post lengths, and post spacing **[1]** to generate different final ECT geometries **[2]**.

4.1.1. LAB MEDIA: Figure 3 *Video Editor: please emphasize blue and white patterns*
4.1.2. LAB MEDIA: Figure 3 *Video Editor: please emphasize light microscope images of cell patterns*

4.2. For this analysis, a mold with 7-millimeter-long posts with 2.5-millimeter wide intervals was used **[1]**.

4.2.1. LAB MEDIA: Figure 3 *Video Editor: please emphasize/zoom into Figure 3E*

4.3. Poloxamer 407 prevents adhesion of the cells to the mold **[1]** and enables the formation of a characteristic mesh structure following rapid gel compaction in 14 days **[2]**.

4.3.1. LAB MEDIA: Figure 4 Day 0-0 h image
4.3.2. LAB MEDIA: Figure 4 Day 0-0 h image Video Editor: please add Day 0-1h to Day 14 images

4.4. The structure is maintained even after the ECT is released from the mold **[1]**.

4.4.1. LAB MEDIA: Figure 4 Day 14-unloaded image

4.5. The fabricated tissue construct is approximately 1.5 centimeters wide and 0.5 millimeters thick **[1]** and the width of each bundle in the mesh is approximately 0.5-millimeters in average **[2]**.

4.5.1. LAB MEDIA: Figure 4 Day 14-unloaded image *Video Editor: please emphasize width of entire pattern*
4.5.2. LAB MEDIA: Figure 4 Day 14-unloaded image *Video Editor: please emphasize width of one bundle within pattern*

4.6. It is possible to generate a 3-centimeter-final width mesh ECT **[1]** containing twenty-four million cells from a four times larger mold with the same staggered post design **[2]**.

4.6.1. LAB MEDIA: Figures 5A and 5B *Video Editor: please emphasize pattern in Figure 5A*

4.6.2. LAB MEDIA: Figures 5A and 5B *Video Editor: please emphasize mold in Figure 5B*

4.7. This larger mesh ECT can also be easily removed from the mold and generates a local active force equivalent to the smaller mesh ECT **[1]**.

4.7.1. LAB MEDIA: Figure 5C

Conclusion

5. Conclusion Interview Statements

5.1. **Takeichiro Nakane**: It is important to pour the cell-matrix mixture evenly into the tissue mold without generating bubbles to prevent filling defects in the final gel **[1]**.

5.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera (3.5.)

5.2. **Hidetoshi Masumoto**: Functional characterization of the contractile maturation and force-frequency relations can be performed using in vitro muscle bath systems **[1]**.

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

5.3. **Takeichiro Nakane**: Our ultimate goal is the translation of ECT paradigms into clinical therapies that utilize large animal pre-clinical studies **[1]**.

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