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Title: Intramuscular Transplantation of Human Pluripotent Stem Cell-derived Pancreatic Endocrine Cells in Mice

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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? **NO**

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

3. Filming location: Will the filming need to take place in multiple locations? **Different rooms in the same building**

Same building (ground floor, 2nd floor)

4. Testimonials (optional): Would you be open to filming two short testimonial statements **live during your JoVE shoot**? These will **not appear in your JoVE video** but may be used in JoVE's promotional materials. **No**

Current Protocol Length

Number of Steps: 18

Number of Shots: 39

Introduction

Videographer: Obtain headshots for all authors available at the filming location.

Authors: Please note that **the questions will not appear on screen**. Please answer in **stand-alone full sentence with sufficient context**

NOTE: The authors have added additional statements as backup. Select only 5 statements

INTRODUCTION:

~~REQUIRED: Summarize your research focus and the main questions it aims to answer.~~

- 1.1. **Mario Hernanz:** We study human endocrine pancreas development using stem cell-derived islets to understand the genetic basis of diabetes.
 - 1.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B roll: 2.3.1*

~~Describe the research gap or limitation in existing methods and how this protocol addresses them.~~

- 1.2. **Mario Hernanz:** Traditional kidney capsule transplantation is highly invasive; intramuscular transplantation protocol offers a simpler, reproducible, and minimally invasive alternative.
 - 1.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

~~Describe the research contexts or systems in which this protocol can be applied.~~

- 1.3. **Mario Hernanz:** This protocol is useful in diabetes research to study the in vivo maturation and function of human stem cell derived islets.
 - 1.3.1. ~~INTERVIEW: Named talent says the statement above in an interview style shot, looking slightly off camera.~~ **NOTE: This is an extra statement, please do not include this in the video**

CONCLUSION:

~~What does this protocol allow researchers to study or measure?~~

- 1.4. **Alberto Bartolomé**: This protocol facilitates *in vivo* functional assays and the evaluation of the survival and maturation of transplanted human grafts.
 - 1.4.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

~~Describe the most important consideration or challenge when performing this protocol.~~

- 1.5. **Alberto Bartolomé**: The most important consideration is ensuring high-quality stem cell differentiation and gentle handling during cluster formation to preserve viability and maximize graft survival.
 - 1.5.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

~~Are there any additional methods or analyses that can be performed following this procedure?~~

- ~~1.6. **Alberto Bartolomé**: Recovered grafts can undergo histological and transcriptomic analyses to assess endocrine cell identity and differential gene expression.~~
 - ~~1.6.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.~~
- NOTE: This is an extra statement, please do not include this in the video**

~~How can future studies build on this work? Describe the questions or research directions that can be investigated next.~~

- 1.7. **Alberto Bartolomé**: These models facilitate the interrogation of genetic mechanisms underlying monogenic diabetes and beta-cell dysfunction.
 - 1.7.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

Videographer: Obtain headshots for all authors available at the filming location.

Ethics Title Card

Human pluripotent stem cell research and animal procedures were conducted under protocols approved by the Ethics Committee of the Consejo Superior de Investigaciones Científicas (CSIC) and the Consejería de Medio Ambiente, Agricultura e Interior de la Comunidad de Madrid.

Protocol

***Videographer:** Please always record an audio slate while filming each shot mentioned in the shotlist.*

2. Generation of 3D Clusters and Differentiation to Insulin-Producing Cells

Demonstrator: Mario Hernanz

- 2.1. To begin, add 0.5 milliliters of 5 percent anti-adherence solution to cover the bottom of a 24-well microwell plate containing inverted pyramidal microwells [1]. Centrifuge the plate at 1300 *g* for 10 minutes at room temperature [2].
 - 2.1.1. WIDE: Talent adding an anti-adherence solution into the wells of the 24-well microwell plate. NOTE: Videographer took a WIDE (2.1.1.1) and a CLOSE (2.1.1.2) take of this.
 - 2.1.2. Talent placing the microwell plate into the centrifuge.
- 2.2. Then, examine the microwells under a microscope for air bubbles [1]. After re-centrifuging the plate to remove any trapped air, aspirate the anti-adherence solution [2]. Then, wash each well with 0.5 milliliters of PBS and repeat the washing step once more [3-TXT].
 - 2.2.1. SCOPE: Shot of microwells.
 - 2.2.2. Talent aspirating the anti-adherence solution from the wells. NOTE: 2.2.2 and 2.2.3 Recorded continuously in the same take and file.
 - 2.2.3. Talent washing the wells with PBS. TXT: Leave the wells covered in PBS until ready for use
- 2.3. Next, wash the cultured pancreatic progenitors developed from hPSCs (*H-P-S-Ceez*) with 1 milliliter of PBS with 0.5 millimolar EDTA [1]. Aspirate the PBS with 0.5 millimolar EDTA [2] and cover the wells with trypsin [3]. Then, incubate at 37 degrees Celsius for 8 to 12 minutes [4].
 - 2.3.1. Talent adding PBS-0.5 millimolar EDTA to the cells. NOTE: 2.3.1-2.3.3 Recorded continuously in the same take and file.
 - 2.3.2. Talent aspirating the PBS-0.5 millimolar EDTA from the wells.
 - 2.3.3. Talent adding trypsin to cover the wells.
 - 2.3.4. Talent placing the plate for incubation.
- 2.4. Dilute the trypsin with 2 volumes of DMEM-F12 (*D-M-E-M-F-Twelve*) or PBS [1] and gently resuspend the cells by pipetting [2]. Transfer the suspension to a conical tube and immediately proceed to the next step [3].

- 2.4.1. Talent adding DMEM-F12 to dilute the trypsin. **NOTE: 2.4.1, 2.4.2. 2.4.3 Recorded continuously in the same take and file.**
- 2.4.2. Talent gently pipetting the cell suspension to resuspend the cells.
- 2.4.3. Talent transferring the suspension into a conical tube.
- 2.5. Centrifuge the suspension at 200 *g* for 3 minutes at room temperature [1]. Carefully aspirate the supernatant [2] and gently resuspend the pellet in prewarmed S4 medium [3].
 - 2.5.1. Talent placing the conical tube into the centrifuge.
 - 2.5.2. Talent carefully aspirating the supernatant from the tube. **NOTE: 2.5.2 and 2.5.3 Recorded continuously in the same take and file.**
 - 2.5.3. Talent gently resuspending the pellet in prewarmed S4 medium.
- 2.6. Now, aspirate the PBS from the microwell dishes [1]. Gently add 1.25 milliliters of the suspended cells to each well of the microwell plate [2].
 - 2.6.1. Talent aspirating PBS from the microwell dishes. **NOTE: 2.6.1 and 2.6.2 Recorded continuously in the same take and file**
 - 2.6.2. Talent gently adding the suspended cells into each well of the microwell plate.
- 2.7. Centrifuge the plate at 100 *g* for 3 minutes at room temperature to distribute the cells evenly in the microwells [1]. Carefully transfer the microwell plate to a humidified incubator [2].
 - 2.7.1. Talent placing the microwell plate into the centrifuge.
 - 2.7.2. Talent transferring the microwell plate into a humidified incubator.
- 2.8. To perform media changes with differentiation media, gently aspirate and dispense the media along the well walls to prevent turbulence and displacing cell clusters from microwells [1-TXT].
 - 2.8.1. Talent replacing media in the microwell plate with differentiation media. **TXT: Compact spherical clusters form within 24–48 h**

3. Transplantation of SC-islets into the Tight Muscle of Immunodeficient Mice

Demonstrator: Gema García

- 3.1. Transfer the appropriate quantity of generated clusters to a 1.5-milliliter microcentrifuge tube [1].
 - 3.1.1. Talent transferring clusters into a 1.5 milliliter microcentrifuge tube.
- 3.2. Connect the syringe to the needle-tubing assembly [1] and fill it with S7 medium [2].
 - 3.2.1. Talent connecting the syringe to the needle-tubing assembly. **NOTE: Recorded**

continuously in the same take and file. **NOTE: 3.2.1 and 3.2.2 Recorded continuously in the same take and file.**

3.2.2. Talent filling the syringe with S7 medium.

3.3. Aspirate the clusters into the tubing directly from the microcentrifuge tube [1]. Fold the tubing in half and insert the folded section into a 200-microliter pipette tip [2]. Then, insert the tip-tubing-needle assembly into a 15-milliliter conical tube and secure the ends to the tube rim using tape [3].

3.3.1. Talent aspirating clusters into the tubing from the microcentrifuge tube. **NOTE: 3.3.1-3.3.3 Recorded continuously in the same take and file.**

3.3.2. Talent folding the tubing and inserting it into a 200 microliter pipette tip.

3.3.3. Talent placing the assembly into a 15-milliliter conical tube and securing it with tape.

3.4. Now, centrifuge the tube at 100 *g* for 2 minutes at 4 degrees Celsius to compact the clusters [1] and immediately place the tube on ice [2].

3.4.1. Talent placing the conical tube into the centrifuge.

3.4.2. Talent placing the tube on ice.

3.5. To transplant the clusters into anesthetized NOD-scid-gamma (*Nod-Scid-Gamma*) mice, shave the posterior side of the right leg below the knee [1-TXT] and disinfect with 70 percent ethanol [2].

3.5.1. Talent shaving the posterior side of the mouse's right leg below the knee. **TXT: Anesthesia: 3-5% Isoflurane** **NOTE: 3.5.1 and 3.5.2 Recorded continuously in the same take and file.**

3.5.2. Talent disinfecting the shaved area with ethanol.

~~3.6. Fill the syringe and needle with 0.9 percent sodium chloride [1].~~ **NOTE: Not filmed**

~~3.6.1. Talent filling the syringe and needle with sodium chloride solution.~~

3.7. Then, detach the tubing from the pipette tip and connect it to the syringe [1]. Cut the tubing close to the compacted clusters and attach it to the blunt end of the needle [2]. Push the saline until the first cluster reaches the tip of the needle [3].

3.7.1. Talent detaching the tubing from the pipette tip and connecting it to the syringe. **NOTE: 3.7.1-3.7.3 Recorded continuously in the same take and file.**

3.7.2. Talent cutting the tubing and attaching it to the blunt end of the needle.

3.7.3. Talent pushing saline through the tubing until the cluster reaches the needle tip.

3.8. Now, position the mouse prone, leaning to the right side, and extend the right leg [1]. Insert the needle 4 to 5 millimeters deep into the medial compartment of the right thigh between the adductor and gracilis muscles [2].

- 3.8.1. Talent positioning the mouse and extending the right leg. **NOTE: 3.8.1-3.9.2 Recorded continuously in the same take and file.**
- 3.8.2. Talent inserting the needle into the specified region of the thigh.
- 3.9. Then, inject the clusters while retracting the needle by approximately 3 millimeters [1]. Rotate the syringe, withdraw the needle, and allow the mouse to recover [2-TXT].
 - 3.9.1. Talent injecting clusters while retracting the needle.
 - 3.9.2. Talent rotating the syringe and withdrawing the needle. **TXT: Recover the graft, 6 weeks post-transplantation**
- 3.10. Following tissue sectioning, perform immunostaining to enable subsequent imaging analyses [1].
 - 3.10.1. Talent adding antibody over the tissue section.

Results

4. Results

- 4.1. Single-cell suspensions of Stage 4 pancreatic progenitors were seeded into microwell plates to generate size-controlled 3-dimensional aggregates [1]. Within 24 to 48 hours, compact, spherical clusters formed [2].
 - 4.1.1. LAB MEDIA: Figure 2A. *Video editor: Show the grid of small square wells used for cell seeding*
 - 4.1.2. LAB MEDIA: Figure 2B. *Video editor: Highlight the round clusters visible inside the wells*
- 4.2. Fluorescence microscopy confirmed the presence of PDX1 (*P-D-X-One*) and insulin-expressing cells in reporter hPSC lines [1].
 - 4.2.1. LAB MEDIA: *Figure 2C and D. Video editor: Highlight the bright green clustered regions in the image*
- 4.3. After 6 weeks, the graft site was visualized macroscopically with islet-like masses identifiable within the muscle [1]. Following excision, explants were identifiable under a stereomicroscope as pale, spherical tissue structures [2].
 - 4.3.1. LAB MEDIA: Figure 3A. *Video editor: Highlight the boxed region showing the tissue mass in the muscle*
 - 4.3.2. LAB MEDIA: Figure 3B. *Video editor: Zoom in on the pale, rounded structure in the center of the image*
- 4.4. Robust detection of C-peptide-positive cells was observed in transplanted animals [1]. Insulin staining confirmed the presence of human beta-like cells within the graft [2].
 - 4.4.1. LAB MEDIA: Figure 4A–C. *Video editor: Highlight the green-stained regions labeled “C-peptide”*
 - 4.4.2. LAB MEDIA: Figure 4C. *Video editor: Highlight the red-stained regions labeled “INS”*
- 4.5. Glucagon- and somatostatin-positive cells were detected, indicating preservation of alpha- and delta-cell populations [1].
 - 4.5.1. LAB MEDIA: Figure 4A and B. *Video editor: Highlight the red-stained regions labeled “GCG” and “SST”*

1. Anti-adherence
Pronunciation link: <https://www.merriam-webster.com/dictionary/adherence>
IPA: /,ænti əd'hɪərəns/
Phonetic Spelling: an·tie ad·heer·uhns
2. Microwell
Pronunciation link: No confirmed link found
IPA: /'maɪkroʊ,wəl/
Phonetic Spelling: my·kroh·wel
3. Pyramidal
Pronunciation link: <https://www.merriam-webster.com/dictionary/pyramidal>
IPA: /pə'rɪmɪdəl/
Phonetic Spelling: puh·rim·uh·duhl
4. Centrifuge
Pronunciation link: <https://www.merriam-webster.com/dictionary/centrifuge>
IPA: /'sentrə,fju:dʒ/
Phonetic Spelling: sen·truh·fyooj
5. Aspirate
Pronunciation link: <https://www.merriam-webster.com/dictionary/aspirate>
IPA: /'æspə'reɪt/
Phonetic Spelling: as·puh·rayt
6. Progenitors
Pronunciation link: <https://www.merriam-webster.com/dictionary/progenitor>
IPA: /proʊ'dʒenətər/
Phonetic Spelling: ʒə·h·jen·uh·ter
7. EDTA
Pronunciation link: No confirmed link found
IPA: /,i:di:ti:'eɪ/
Phonetic Spelling: ee·dee·tee·ay
8. Trypsin
Pronunciation link: <https://www.merriam-webster.com/dictionary/trypsin>
IPA: /'trɪpsɪn/
Phonetic Spelling: trip·sin
9. Incubate
Pronunciation link: <https://www.merriam-webster.com/dictionary/incubate>
IPA: /'ɪŋkjə,bet/
Phonetic Spelling: ing·kyuh·bayt
10. DMEM-F12
Pronunciation link: No confirmed link found
IPA: /,di:emem ef twelv/
Phonetic Spelling: dee·em·em ef·twelv
11. Conical
Pronunciation link: <https://www.merriam-webster.com/dictionary/conical>
IPA: /'kɑ:nɪkəl/
Phonetic Spelling: kah·ni·kuhl

12. Supernatant
 Pronunciation link: <https://www.merriam-webster.com/dictionary/supernatant>
 IPA: /,suːpərˈneɪtənt/
 Phonetic Spelling: soo·per·nay·tuhnt
13. Resuspend
 Pronunciation link: No confirmed link found
 IPA: /,riːsəˈspend/
 Phonetic Spelling: ree·suh·spend
14. Humidified
 Pronunciation link: <https://www.merriam-webster.com/dictionary/humidify>
 IPA: /hjuːˈmɪdəˌfaɪd/
 Phonetic Spelling: hyoo·mi·duh·fyd
15. Transplantation
 Pronunciation link: <https://www.merriam-webster.com/dictionary/transplantation>
 IPA: /,trænsplænˈteɪʃən/
 Phonetic Spelling: trans·plan·tay·shuhn
16. Immunodeficient
 Pronunciation link: <https://www.merriam-webster.com/dictionary/immunodeficient>
 IPA: /ɪˌmjuːnoʊdɪˈfɪʃənt/
 Phonetic Spelling: ih·myoo·noh·duh·fi·shuhnt
17. Microcentrifuge
 Pronunciation link: No confirmed link found
 IPA: /,maɪkroʊˈsentərəˌʃjuːdʒ/
 Phonetic Spelling: my·kroh·sen·truh·fyooj
18. Microliter
 Pronunciation link: <https://www.merriam-webster.com/dictionary/microliter>
 IPA: /ˈmaɪkroʊˌliːtər/
 Phonetic Spelling: my·kroh·lee·ter
19. Anesthetized
 Pronunciation link: <https://www.merriam-webster.com/dictionary/anesthetize>
 IPA: /əˈnesθəˌtaɪzd/
 Phonetic Spelling: uh·nes·thuh·tyzd
20. Isoflurane
 Pronunciation link: <https://www.merriam-webster.com/medical/isoflurane>
 IPA: /ˌaɪsoʊˈflorem/
 Phonetic Spelling: eye·soh·floor·ayn
21. Sodium chloride
 Pronunciation link: <https://www.merriam-webster.com/dictionary/sodium>
 IPA: /ˈsoʊdiəm ˈklɔːraɪd/
 Phonetic Spelling: soh·dee·uhm klor·ide
22. Adductor
 Pronunciation link: <https://www.merriam-webster.com/dictionary/adductor>
 IPA: /əˈdʌktər/
 Phonetic Spelling: uh·duk·ter

23. Gracilis
Pronunciation link: <https://www.merriam-webster.com/medical/gracilis>
IPA: /'græsɪlɪs/
Phonetic Spelling: grass·ih·liss
24. Immunostaining
Pronunciation link: No confirmed link found
IPA: /ɪˌmjuːnoʊ'steɪnɪŋ/
Phonetic Spelling: ih·myoo·noh·stay·ning
25. Fluorescence
Pronunciation link: <https://www.merriam-webster.com/dictionary/fluorescence>
IPA: /flʊ'reɪsəns/
Phonetic Spelling: floor·es·uhns
26. Microscopy
Pronunciation link: <https://www.merriam-webster.com/dictionary/microscopy>
IPA: /maɪ'kraːskəpi/
Phonetic Spelling: my·krah·skuh·pee
27. Macroscopically
Pronunciation link: <https://www.merriam-webster.com/dictionary/macroscopic>
IPA: /ˌmækroʊ'skɑːpɪkli/
Phonetic Spelling: mak·roh·skah·pik·lee
28. Stereomicroscope
Pronunciation link: No confirmed link found
IPA: /ˌsteriəʊ'maɪkrə'skoʊp/
Phonetic Spelling: ster·ee·oh·my·kruh·skohp
29. Explants
Pronunciation link: <https://www.merriam-webster.com/medical/explant>
IPA: /'ɛksplænt/
Phonetic Spelling: eks·plant
30. Glucagon
Pronunciation link: <https://www.merriam-webster.com/dictionary/glucagon>
IPA: /'gluːkəˌɡɑːn/
Phonetic Spelling: gloo·kuh·gon
31. Somatostatin
Pronunciation link: <https://www.merriam-webster.com/medical/somatostatin>
IPA: /soʊˌmætə'stætɪn/
Phonetic Spelling: soh·mat·oh·stat·in