FINAL SCRIPT: APPROVED FOR FILMING

Submission ID #: 62499
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Project Page Link: https://www.jove.com/account/file-uploader?src=19062243

Title: Leukodepletion Filter-Derived CD34$^+$ Cells as a Cell Source to Study Megakaryocyte Differentiation and Platelet Formation

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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. **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: 12
Number of Shots: 32

Introduction

1. Introductory Interview Statements

REQUIRED:

1.1. **Anaïs Pongérard:** This protocol proposes a simple method for obtaining human hematopoietic progenitors and for inducing their differentiation into megakaryocytes. It can serve as a basis for understanding megakaryopoiesis **[1]**.

1.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 5.2.2 for "obtain human hematopoietic progenitor".*

1.2. **Anaïs Pongérard:** This technique uses a source that offers the advantage of being abundant and affordable for laboratory research in hematopoiesis **[1]**.

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

OPTIONAL:

1.3. **Catherine Strassel:** This method is used to better understand megakaryopoiesis. For these cells to be used in therapy, they would need to be prepared under GMP conditions, which is currently not the case **[1]**.

1.3.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 4.1.2 for "improve platelet production".*

1.4. **Léa Mallo:** Understanding the steps, including the PBMC collection, can only be fully complete with a visual demonstration **[1]**.

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

Ethics Title Card

1.5. Procedures involving human subjects have been approved by the French Ministry of Higher Education and Research.

Protocol

2. Leukoreduction Filter (LRF) Back-Flushing Modality Preparation

2.1. Before beginning an experiment, connect a leukoreduction filter to an empty 600-milliliter transfer bag **[1-TXT]** and to a tubing set **[2]**. In a biosafety cabinet, inject 25 milliliters of filtered elution buffer per leukoreduction filter into the empty bag **[3]** and use a 30-milliliter syringe to gently aspirate the bag contents through the filter **[4]**.

2.1.1. WIDE: Establishing shot of talent connecting the LRF to transfer bag.
Videographer: This step is important!

2.1.2. Talent connecting LRF to the tubing set.

2.1.3. Talent injecting elution buffer into the bag.

2.1.4. Talent aspirating the bag contents through filter into syringe.

2.2. Transfer the collected red blood cells into a new 50-milliliter tube **[1]** and dilute the cell suspension by half with 2% dextran **[2]**. Mix well to aggregate the red blood cells **[3]** and allow the cells to sediment for 30 minutes at room temperature **[4]**.

2.2.1. Talent transferring the cells into tube.

2.2.2. Talent diluting the red blood cells suspension.

2.2.3. Talent mixing the suspension.

2.2.4. Talent setting timer, with tube on bench visible in frame.

3. PBMC Collection

3.1. When the red blood cells have settled, transfer the supernatant to a 50-milliliter tube **[1]** and fill the tube with 2-millimolar PBS-EDTA solution **[2-TEXT]**.

3.1.1. WIDE: Talent transferring the supernatant into tube.

3.1.2. Talent adding PBS-EDTA to the tube. **TEXT: See text for all solution and medium preparation details**

3.2. Gently overlay half of the supernatant onto 25 milliliters of density gradient medium in each of two 50-milliliter tubes without disturbing the gradient surface **[1]** and separate the cells by density gradient centrifugation **[2-TXT]**.

3.2.1. Supernatant being layered onto density gradient medium. *Videographer: This step is important!*

3.2.2. Talent placing tubes in centrifuge. **TEXT: 30 min, 400 x *g*, RT, no brake**

3.3. Use a disposable pipette to transfer the cells from each interface to a new 50-milliliter tube **[1]**. Wash the cells two times in 50 milliliters of 2 millimolar PBS-EDTA per wash **[2-TXT]**. After the second wash, resuspend the pellets in a single, 50-milliliter volume of fresh PBS-EDTA for counting .Here it is possible to stop the procedure by maintaining the collected cells under agitation at 4°C during the night **[3-TXT]**.

3.3.1. Shot of layers in both tubes, then cells at one interface being collected/transferred to tube. *Videographer: This step is important!*

3.3.2. Talent adding PBS-EDTA to tube. **TEXT: 10 min, 200 x *g*, RT**

3.3.3. Replaced shot: Talent placing the tube with the cells in a cold room in agitation
TEXT: Optional: Store cells at 4 °C O/N

NOTE: Shot number 3.3.3. was modified while filming.

4. CD34$^+$ Cell Selection

4.1. For CD34-positive cell selection, determine the cell number and collect the cells by centrifugation **[1-TXT]** and resuspend the cell pellet in 300 microliters of 2-millimolar PBS-EDTA/1 x 10^8 cells **[2]**. Add the appropriate volume of FcR (F-C-receptor) blocking reagent and 50 microliters of CD34 (C-D-thirty-four) microbeads/1 x 10^8 cells **[3]**.

Added shot: Talent determining the number of cells before centrifugation.

NOTE: A shot was added before 4.1.1. The VO text “For CD34-positive cell selection, determine the cell number” should be used for the added shot.

4.1.1. WIDE: Talent placing tubes in centrifuge. **TEXT: 10 min, 400 x *g*, RT**

4.1.2. Talent resuspending pellet in PBS-EDTA.

4.1.3. Talent adding the reagent, with microbead and reagent containers visible in frame.

4.2. After 30 minutes at 4 degrees Celsius, wash the cells with 2-millimolar PBS-EDTA **[1]** and resuspend in the pellet in the 500 microliters of fresh 2-millimolar PBS-EDTA/1 x 10^8 cells **[2]**.

4.2.1. Talent adding PBS-EDTA to tube.

4.2.2. Shot of pellet, then cells being resuspended.

4.3. Humidify an appropriately sized magnetic column with PBS-EDTA and then add the sample on the column **[1]**. Wash the column two times with 3 milliliters of 2-millimolar PBS-EDTA per wash **[2]** before eluting the CD34-positive cells two with 5 milliliter volumes of 2 millimolar PBS-EDTA per elution **[3-TXT]**.

Added shot: Talent humidifying the column before adding the sample on column.

NOTE: A shot was added before 4.3.1. The VO text " Humidify an appropriately sized magnetic column with PBS-EDTA" should be used for the added shot.

4.3.1. Talent adding the sample on column.

4.3.2. Talent washing the column.

4.3.3. Talent eluting cells with PBS-EDTA. **TEXT: Approximately 6.1 x 10^5 $CD34^+$ cells/LRF**

5. Evaluation of $CD34^+$ Cell Purity

5.1. To assess the purity of the magnetic bead-selected cells, add 2 microliters of an appropriate human anti-CD34 antibody to a 100-microliter aliquot of the isolated cells **[1]** and mix well before incubating for 15 minutes at 4 degrees Celsius **[2]**.

5.1.1. WIDE: Talent adding antibody to cell suspension.

5.1.2. Talent mixing cells.

5.2. Following the incubation, wash the cells in PBS **[1]** and resuspend in the pellet in 200 microliters of PBS **[2]** for analysis of the purity of the cell sample by flow cytometry **[3]**.

5.2.1. Talent washing the cells/placing cell suspension in centrifuge.

5.2.2. Talent adding cell pellet to PBS.

5.2.3. LAB MEDIA: Figure 2B. *Video Editor: please emphasize rectangular gate in second plot/cells in rectangular gate of second plot*

6. $CD34^+$ Cell Freezing

6.1. After counting, collect the CD34-positive cells by centrifugation **[1-TXT]** and immediately resuspend the pellet in cold solution 1 to a density of 1 x 10^6 cells/milliliter **[2]** before quickly adding the cell suspension to cold solution 2 **[3]**.

6.1.1. WIDE: Talent adding tube to centrifuge. **TEXT: 5 min, 400 x *g*, RT**

6.1.2. Shot of pellet, then cold solution 1 being added to cells. *Videographer: This step is important!*

6.1.3. Talent adding cells to cold solution 2. *Videographer: This step is important!*

6.2. Then immediately place the cryotube in a minus 80 degree-Celsius freezer for 24 hours **[1]** before transferring the cryotube to liquid nitrogen storage **[2]**.

6.2.1. Talent placing the cryotube in freezer.

6.2.2. Talent placing cryotube in liquid nitrogen tank.

Results

7. Results: Mature Megakaryocyte Differentiation and Platelet Formation

7.1. In this representative study **[1]**, the cells were recovered by leukoreduction filtration as demonstrated **[2]** with an approximate 95% viability **[3]**. After magnetic bead selection, a greater than 90% pure CD34-positive cell population was obtained **[4]**.

7.1.1. LAB MEDIA: Figure 2A i.

7.1.2. LAB MEDIA: Figure 2A i. *Video Editor: Emphasize Cell number graph.*

7.1.3. LAB MEDIA: Figure 2A i. *Video Editor: Emphasize viability graph.*

7.1.4. LAB MEDIA: Figure 2B. *Video Editor: Emphasize cells in rectangular gate in right graph and/or Purity data bar*

7.2. Cell proliferation typically decreases after a week of culture **[1]** but with no significant changes in cell viability **[2]**.

7.2.1. LAB MEDIA: Figure 3B. *Video Editor: Sequentially emphasize "proliferation rate" graphs for D7 and D10.*

7.2.2. LAB MEDIA: Figure 3B i, ii. *Video Editor: Sequentially emphasize "viability" graphs for D7 and D10.*

7.3. By day 7 **[1]**, the CD34-positive cells should begin expressing CD41, a specific and early marker for megakaryocyte and platelet development **[2]**. By day 10, the majority of the cells in the culture typically develop into mature, CD41-expressing megakaryocytes **[3]**.

7.3.1. LAB MEDIA: Figure 3 C.

7.3.2. LAB MEDIA: Figure 3 C.

7.3.3. LAB MEDIA: Figure 3 C. *Video Editor: Emphasize cells in top right quadrant of D7 plot*

7.3.4. LAB MEDIA: Figure 3 C. *Video Editor: Emphasize cells in top right quadrant of D10 plot.*

7.4. By day 13 **[1]**, megakaryocyte proplatelet extension and platelet release can be observed by light microscopy **[2]**. The total number of CD41-CD42-a-positive platelets released into the culture **[3]** can then be quantified using a calibrated number of fluorescent beads **[4]**.

7.4.1. LAB MEDIA: Figure 4 A.

7.4.2. LAB MEDIA: Figure 4 A. *Video Editor: Emphasize Round cell(s) (text and/or image indicated by line and text)*

7.4.3. LAB MEDIA: Figure 4B and C.

7.4.4. LAB MEDIA: Figure 4B and C. *Video Editor: Emphasize cells in gate in Figure 4B iii plot and/or data bar in Figure 4C.*

Conclusion

8. Conclusion Interview Statements

8.1. **Anaïs Pongérard:** This method paves the way for enhancing the underlying mechanisms of megakaryopoiesis and for increasing platelet yields in vivo **[1]**.

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

8.2. **Léa Mallo:** Even if this method seems tedious at first glance, it is very simple. You just need to be patient and to prepare all of the reagents in advance **[1]**.

8.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 2.1.3 for all the reagents".*

8.3. **Catherine Strassel:** Although our protocol concerns megakaryopoiesis, the obtained CD34+ hematopoietic progenitors can be used in other hematopoietic pathways **[1]**.

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