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Title: Proplatelet Formation Dynamics of Mouse Fresh Bone Marrow Explants

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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? **Yes**

   If **Yes**, we will need you to record using screen recording software to capture the steps. If you use a Mac, QuickTime X also has the ability to record the steps. Please upload all screen captured video files to your project page as soon as possible.

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

Current Protocol Length

Number of Steps: 13
Number of Shots: 22

Introduction

1. Introductory Interview Statements

REQUIRED:

1.1. **Inès GUINARD:** The overall objective of this procedure is to follow in real time the formation of proplatelets from mouse megakaryocytes which have matured in their native environment.

1.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested b-roll: 4.1.2, 4.1.3*

1.2. **Inès GUINARD**: This method has the advantage of being simple, reproducible and fast. One can analyze many megakaryocytes and visualize the formation of proplatelets in only 6 hours, instead of waiting for the 4th day of culture as for mouse megakaryocytes *in vitro*

1.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested b-roll: 4.2.1*

OPTIONAL:

1.3. **Inès GUINARD:** An interesting application of this model is the possibility to study the effect of pharmaceutical treatments or genetic mutations specifically on the step of proplatelet extension. Here, there are no interferences with the differentiation process as in the case of *in vitro* culture.

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

1.4. **Inès GUINARD:** This video helps in showing the key steps of flushing and cutting the bone marrow. It also explains how to classify the morphology and quantify MKs, which is really useful for the characterization of defects in thrombopoiesis.

1.4.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested b-roll: 3.3.1,3.3.2, 4.3.1 and 4.3.2*

Ethics Title Card

1.5. All animal experiments were performed in accordance with European standards 2010/63/EU and the CREMEAS Committee on the Ethics of Animal Experiments of the University of Strasbourg.

Protocol

2. Preparation of the Experimental Set Up

2.1. To begin, warm Tyrode's buffer at 37 degrees Celsius **[1]** and turn on the heating chamber of the microscope to bring the temperature to 37 degrees Celsius **[2]**.

2.1.1. WIDE: Talent warming solution

2.1.2. Talent turning on the heating chamber of the microscope

2.2. Prepare all necessary tools such as a timer, incubation chambers, 5-milliliter 21-Gauge syringes, forceps, a razor blade, Pasteur pipettes, glass slides, and 15 milliliter centrifuge tube **[1]**.

2.2.1. Timer, incubation chambers, syringes, forceps, razor, Pasteur pipette, slides and centrifuge tube on the work bench

3. Bone Marrow Sectioning and Placement into the Incubation Chamber

3.1. Flush the bone marrow with 2 milliliters of Tyrode's buffer by introducing a 21-gauge needle into the opening of the femur, on the knee side **[1-TXT]**, and slowly press the plunger to retrieve an intact marrow cylinder **[2]**.

3.1.1. Talent flushing the bone marrow **TEXT: Syringe: 5 mL**

3.1.2. Talent pressing the plunger

3.2. Use a 3-milliliter plastic pipette to carefully and gently transfer the intact bone marrow onto a glass slide **[1]**.

3.2.1. Talent transferring intact bone marrow onto the slide using plastic pipette

3.3. Under a stereomicroscope, cut off the ends of the marrow that may have been compressed at the time of the flush. Then, cut thin transversal sections of 0.5-millimeter thickness using a sharp razor blade without damaging the megakaryocytes **[1]**.

3.3.1. Talent cutting the ends and sections of the bone marrow under microscope *Videographer: this step is important!*

3.4. Using a plastic pipette, collect 10 sections into a 1-milliliter tube containing Tyrode's buffer **[1]**.

3.4.1. Talent collecting sections into centrifuge tube

3.5. Carefully transfer the sections to an incubation chamber with a diameter of 13 millimeters **[1]**.

3.5.1. Talent transferring the sections into the incubation chamber

3.6. Aspirate the buffer **[1]** and adjust the volume to 30 microliters of Tyrode's buffer supplemented with 5 percent mouse serum **[2]**.

3.6.1. Talent aspirating the buffer

3.6.2. Talent adjusting the volume of Tyrode's buffer

3.7. Position the sections at a distance **[1]**. Seal the self-adhesive chamber with a 22 by 55-millimeter coverslip, inclining the coverslip to avoid the formation of air bubbles **[2]**.

3.7.1. Talent positioning the sections at a distance

3.7.2. Talent sealing the chamber with coverslip

3.8. Place the chamber in the heating chamber at 37 degrees Celsius **[1]**. Start the chronometer and run the experiment for 6 hours at 37 degrees Celsius **[2]**.

3.8.1. Talent placing the chamber in the heating chamber

3.8.2. Talent starting the timer

4. Real-Time Observation of Marrow Explants and Quantification of the Proplatelet-Extending Megakaryocytes

4.1. Make videos to record the transformation of the megakaryocytes **[1]**. After 30 minutes, the marrow cells gradually migrate to the periphery of the explant **[2]**, forming a monolayer **[3]**. After 1 hour of incubation, megakaryocytes can be identified by their large size and polylobulated nuclei **[4]**.

4.1.1. LAB MEDIA: Talent starting the video recording.PNG

4.1.2. LAB MEDIA: migrated marrow cells in a monolayer.PNG

4.1.3. LAB MEDIA: migrated marrow cells in a monolayer 2.PNG

4.1.4. LAB MEDIA: polylobulated and large megakaryocyte.PNG

4.2. The number of megakaryocytes increases **[1],** after 3 hours of incubation **[2]** and some have long extensions **[3]**.

4.2.1. LAB MEDIA: megakaryocyte with extension.PNG

4.2.2. LAB MEDIA: megakaryocyte with extension 2.PNG

4.2.3. LAB MEDIA: megakaryocyte with extension 3.PNG

4.3. Draw a map **[1]** to localize each section **[2]** in the incubation chamber **[3].** After 1 hour, identify the visible megakaryocytes, which are giant polylobulated cells on each section's periphery **[4]**, and plot their positions on the drawing. Repeat this procedure after 3 and 6 hours **[5].**

4.3.1. LAB MEDIA: Talent drawing a map 1.PNG

4.3.2. LAB MEDIA: Talent drawing a map 2.PNG

4.3.3. LAB MEDIA: Talent drawing a map 3.PNG

4.3.4. LAB MEDIA: polylobulated and large megakaryocyte.PNG

4.3.5. LAB MEDIA: Talent plotting the position of MKs.PNG

Results

5. Results: Morphological Assessment of Megakaryocytes in Explants

5.1. Megakaryocytes are counted manually and classified according to their morphology at 3 and 6 hours after sealing of the incubation chamber. They are classified as small, large, with thick extension, or proplatelet-extending **[1]**.

5.1.1. LAB MEDIA: Figure 2A

5.2. With the help of mapping, their evolution can be followed over time. The results are expressed as a percentage of each class at each observation time. Classically, half of the megakaryocytes visible at the periphery extend proplatelets at 6 hours for wild type mouse bone marrow **[1]**.

5.2.1. LAB MEDIA: Figure 2B

5.3. The fate of round megakaryocytes was followed by capturing sequential images over time to image how they form proplatelets **[1].**

5.3.1. LAB MEDIA: video_WT_201210.avi

Conclusion

6. Conclusion Interview Statements

6.1. **Inès GUINARD:** An important thing to remember when attempting this procedure is to keep the explants at 37 degrees Celsius for the duration of the experiment. A different temperature can change the results.

6.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested b-roll: 2.1.1, 2.1.2 and 3.8.1*

6.2. **Inès GUINARD:** Interestingly, the explant protocol can be used after intravital microscopy experiments and hence, the megakaryocytes in the bone marrow explants will be naturally fluorescent and their behavior can be easily investigated. This makes it possible to combine different treatments on the same animal and thus reduce the number of mice.

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

6.3. **Inès GUINARD:** In conclusion, the explant method is simple, fast and provides key information on the capacity of native megakaryocyte to extend proplatelets. The resulting qualitative and quantitative findings are key to our understanding of thrombopoiesis in a physiological or pathological context.

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