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Title: Using Magnetometry to Monitor Cellular Incorporation and Subsequent Biodegradation of Chemically Synthetized Iron Oxide Nanoparticles

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Author Questionnaire

1. **Microscopy**: Does your protocol involve video microscopy, such as filming a complex dissection or microinjection technique? **no**

2. **Software:** Does the part of your protocol being filmed include step-by-step descriptions of software usage? **Possible screen capture from camera equipment**

   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.

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

Introduction

1. Introductory Interview Statements

REQUIRED:

1.1. **Yoann Lalatonne:** This video introduces a fast and efficient method for the synthesis of magnetic nanoparticles and the use of magnetometry to follow their transformations in the biological environment.

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

1.2. **Aurore Van de Walle:** Magnetic nanoparticles have attracted increased attention for biomedical applications and it is important to understand their fate once internalized in cells. This is achieved here by measuring the cellular magnetism.

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

OPTIONAL:

1.3. **Alexandre Fromain:** For both magnetic nanoparticles synthesis and measurement, results could be affected by contamination. Use well-cleaned chemical vials and make sure that the magnetometer equipment is free of contamination.

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

1.4. **Alexandre Fromain:** The magnetometry sample holder is usually used for powder samples. We show how to adapt the use of the holder to liquid or biological samples.

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

Protocol

2. Synthesis of Magnetic Nanoparticles

2.1. In a 30-milliliter monowave glass vial, dissolve 400 milligrams of iron(III) *(pronounce iron three)* acetylacetonate in 10 milliliters of benzyl alcohol **[1]**.

2.1.1. Talent dissolves iron(III) acetylacetonate in benzyl alcohol in a glass vial.

2.2. Using a microwave reactor, heat the suspension from 25 degrees Celsius to 250 degrees Celsius, at a rate of 11.25 degrees Celsius per minute, and maintain it at 250 degrees Celsius **[1]**. *Videographer: This step is important!*

2.2.1. Talent places vial in microwave reactor and programs settings.

2.3. ~~**[1].**~~ To separate the nanoparticles, apply a neodymium magnet for 30 minutes **[2]**.

~~2.3.1. Talent transfers suspension to a glass vial.~~
2.3.2. Talent applies a magnet. NOTE: CU at the end

2.4. To pellet the nanoparticles, perform a series of washes, using 10 milliliters of the designated wash fluid and applying a neodymium magnet for 5 minutes **[1-TXT]**.

2.4.1. Talent washes nanoparticles with 10 milliliters of wash fluid and applies magnet. **TEXT: dichloromethane; 1 M sodium hydroxide; ethanol; pH 7 water (3x)** NOTE: Magnet at the end , take 2 : magnet only , take 3 : water added up

2.5. Remove the filtrate and add 12 milliliters of 10^{-2} molar hydrochloric acid **[1]**. ~~Again~~, centrifuge the suspension in a 100-kilodalton filter at 2,200 times *g* for 10 minutes **[2-TXT]**. Then, resuspend the nanoparticles in 10 milliliters of 10^{-2} molar hydrochloric acid **[3]**.

2.5.1. Talent removes the filtrate and adds hydrochloric acid.
2.5.2. Talent centrifuges the suspension in a filter. **TEXT: Perform 3 X** NOTE: pour solution onto the filter

2.5.3. Talent resuspends nanoparticles in hydrochloric acid.

2.6. Prepare the coating molecule solution as described in the manuscript **[1]**. Add the 10-milliliter nanoparticle aqueous dispersion to the coating molecule solution, at a mass ratio of 5 to 1 between the coating molecules and the nanoparticles **[2]**. Then, agitate the mixture for 2 hours at room temperature, using a magnetic stirrer **[3]**.

2.6.1. Talent prepares the coating molecule solution. NOTE: take 2
2.6.2. Talent adds the nanoparticle suspension to the coating molecule solution.
2.6.3. Talent places the mixture on a magnetic stirrer. NOTE: CU at the end

2.7. After the reaction is complete, adjust the pH of the mixture to 7, using 1 molar sodium hydroxide **[1]**.

2.7.1. Talent adds sodium hydroxide to mixture while measuring pH. NOTE: take 2

3. Culture and Magnetic Labeling of Stem Cells

3.1. When the cultured human mesenchymal stem cells are at passage 5 and 90 percent confluent, remove the medium, and rinse the cells with serum free RPMI without glutamine **[1-TXT]**. To each 150-square-centimeter culture flask, add 10 milliliters of the nanoparticle suspension **[2]**. *Videographer: This step is important!*

3.1.1. Talent removes medium and rinses cells. **TEXT: MSC: mesenchymal stem cell** NOTE: take 2, cu at the end

3.1.2. Talent adds nanoparticle suspension to each culture flask.

3.2. Incubate the flasks at 37 degrees Celsius and 5 percent carbon dioxide for 30 minutes **[1]**.

3.2.1. Talent places flasks in incubator. NOTE: 2nd part

3.3. Then, discard the medium containing the nanoparticle suspension, and wash the cells once with serum free RPMI-1640 **[1]**. Add DMEM, supplemented with 10 percent FBS and 1 percent penicillin-streptomycin **[2]**. Incubate overnight at 37 degrees Celsius and 5 percent carbon dioxide to allow complete internalization of the nanoparticles **[3]**.

3.3.1. Talent discards medium.

3.3.2. Talent adds supplemented DMEM.

3.3.3. Talent places flasks in incubator.

4. Formation of Stem Cell-Spheroids

4.1. Add 10 milliliters of 0.05 percent trypsin-EDTA, pre-warmed to 37 degrees Celsius, to each flask of magnetically labelled MSCs **[1]**. After 2 to 3 minutes, when the cells are detached, immediately inactivate the trypsin by adding one-third of the volume of pre-warmed, supplemented DMEM **[2].**

4.1.1. Talent adds trypsin-EDTA to flasks.

4.1.2. Talent adds DMEM to flasks.

4.2. Centrifuge the detached cells at 260 times *g* for 5 minutes **[1]**. Aspirate the medium, and resuspend the cells in a small volume of cell-spheroid culture medium, at a concentration of approximately 200,000 cells per 50 microliters **[2]**.

4.2.1. Talent centrifuges cells.

4.2.2. Talent aspirates medium and resuspends cells. NOTE: take 2 , CU at the end

4.3. Add 1 milliliter of freshly prepared cell-spheroid culture medium to a 15-milliliter sterile conical centrifuge tube **[1]**. Then, add a volume of cell suspension corresponding to 200,000 cells **[2]**.

4.3.1. Talent adds culture medium to a 15-milliliter tube.
4.3.2. Talent adds cell suspension to tube.

4.4. Centrifuge these magnetically labeled cells at 180 times *g* for 3 minutes **[1]** in order to form a cell pellet at the bottom of the tube **[2]**, then place the tube in the incubator at 37 degrees Celsius and 5 percent carbon dioxide, keeping the cap slightly opened for gas exchange **[3]**. *Videographer: This step is important!*

4.4.1. Tube is placed in the centrifuge

4.4.2. The cell pellet at the bottom of the tube is shown after centrifugation

4.4.3. Talent placing the tube in the incubator.

5. Quantification of Magnetic Nanoparticles Using a Vibrating Sample Magnetometer (VSM)

5.1. Place either a given volume of magnetic nanoparticle or a fixed single cell-spheroid suspension into the vibrating sample magnetometer, or VSM, sample holder. If necessary, PTFE tape can be used to prevent any leakage **[1-TXT]**.

5.1.1. Talent places sample in sample holder. **TEXT: PTFE: Polytetrafluoroethylene**
NOTE: take 2, take 3 : cork it

5.2. Insert the sample holder into the VSM, and scan for sample offset. Place the sample at the position corresponding to the magnetization maximum **[1]**.

5.2.1. Talent inserts sample holder into VSM and scans it.
5.2.2. Talent adjusts the position of the sample. Author NOTE: For this step, we took a snapshot of the software, which could for example be shown on part of the screen in parallel to 5.2.1, while the sample is scanned

5.3. Perform the first measurement at a low magnetic field, between -1500 Oersteds *(pronounce er-stədz)* and +1500 Oersteds, at a rate of 20 Oersteds per second **[1]**. Perform a second measurement at a high magnetic field, between 0 Oersteds and +30 000 Oersteds, at a rate of 200 oersteds per second **[2]**.

5.3.1. SCREEN: Talent scans at a low magnetic field
5.3.2. SCREEN: Talent scans at a high magnetic field Author NOTE: Both these screen shots are on a same video file.
5.3.1. is from minute 0 to 7:04
5.3.2 is from minute 7:05 to the end

Results

6. Results: VSM Measures Uptake and Biodegradation of Nanoparticles

6.1. Ten microliters of nanoparticles dispersed in an aqueous solution were measured in the vibrating sample magnetometer **[1-TXT]**. The slope of the second part of the curve is the diamagnetic constant, representing the diamagnetic signal from the water and the sample holder **[2]**.

6.1.1. LAB MEDIA: Figure 2. *Video editor, please show only Figure 2A. Show both graphs, but emphasize the one on the left.* **TEXT: VSM: vibrating sample magnetometer**

6.1.2. LAB MEDIA: Figure 2. *Video editor, please show only Figure 2A. Show both graphs, but emphasize the one on the left, and highlight the boxed area.*

6.2. This diamagnetic constant was subtracted from the magnetic moment of the solution to obtain the magnetic moment of the nanoparticles only **[1]**. The magnetic moment at saturation was then determined **[2]**.

6.2.1. LAB MEDIA: Figure 2. *Video editor, please show only Figure 2A. Show both graphs, but emphasize the one on the right.*

6.2.2. LAB MEDIA: Figure 2. *Video editor, please show only Figure 2A. Show both graphs, but emphasize the one on the right, and highlight the letters M_s on the vertical axis.*

6.3. Coated nanoparticles were internalized in stem cells. Electron microscope images show the citrate-coated and PAA-coated nanoparticles confined in the endosomes **[1]**. After the cells were pelleted by centrifugation, they formed a cell-spheroid that can be kept in culture for extended time periods **[2]**.

6.3.1. LAB MEDIA: Figure 1. *Video editor, show only Figure 1B.*

6.3.2. LAB MEDIA: Figure 3. *Video editor, show only Figure 3A.*

6.4. Individual cell-spheroids were also measured using the VSM, and the saturation magnetic moment was used to calculate the quantity of nanoparticles in a cellular sample **[1]**. Uptake of the nanoparticles was found to depend on the citrate incubation concentration. **[2]**.

6.4.1. LAB MEDIA: Figure 2. *Video editor, please show only Figure 2B and 2C.*

6.4.2. LAB MEDIA: Figure 2. *Video editor, please show only Figure 2B and 2C, and emphasize Figure 2C.*

6.5. A decrease in magnetism of the cell-spheroids over time indicated the biodegradation of the nanoparticles, which was confirmed by electron microscopy **[1]**. Degradation

was more substantial in the citrate-coated nanoparticles **[2]** than in the PAA-coated nanoparticles **[3]**.

6.5.1. LAB MEDIA: Figure 3. *Video editor, show only 3B and 3C.*
6.5.2. LAB MEDIA: Figure 3. *Video editor, show only 3B and 3C, and emphasize 3B.*
6.5.3. LAB MEDIA: Figure 3. *Video editor, show only 3B and 3C, and emphasize 3C.*

Conclusion

7. Conclusion Interview Statements

7.1. **Aurore Van de Walle:** Using magnetometry, the degradation of magnetic nanoparticles within cells can be precisely quantified and the impact of the features of nanoparticle on their intracellular behavior can be assessed.

7.1.1. INTERVIEW: Named author says the statement above in an interview-style statement while looking slightly off-camera. NOTE: take 2

7.2. **Aurore Van de Walle:** In this protocol, the magnetometry measurements were performed on cell-spheroids, but they can be extended to cells in suspension or organs.

7.2.1. INTERVIEW: Named author says the statement above in an interview-style statement while looking slightly off-camera.

7.3. **Yoann Lalatonne:** With the described method, you are able to explore the interactions of newly designed magnetic nanoparticles in different cell systems of interest, but also you can follow their *in vivo* biodistribution and fate.

7.3.1. INTERVIEW: Named author says the statement above in an interview-style statement while looking slightly off-camera.