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Title: Fabrication of a Biomimetic Nano-Matrix with Janus Base Nanotubes and Fibronectin for Stem Cell Adhesion

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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? **No**

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

Introduction

1. Introductory Interview Statements

REQUIRED:

1.1. **Yupeng Chen:** We developed an injectable scaffold for stem cell growth. The Nano-Matrix scaffold is different from conventional semi-solid hydrogels. Nano-Matrix has a solid fibrous structure which more closely mimics the ECM.

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

1.2. **Libo Zhou:** The main advantage of this technique is that the Nano-Matrix can self-assemble in a few seconds without any additional assistance. When co-cultured with human mesenchymal stem cells, the Nano-Matrix exhibits excellent bioactivity in encouraging cell adhesion.

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

OPTIONAL:

1.3. **Anne Yau:** The Nano-Matrix mimics the extracellular matrix morphologically. Therefore, it has the potential to serve as an injectable scaffold to repair bone fractures by promoting cell adhesion and function at the target location.

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

Protocol

2. Observation of Lyophilized Specimens

2.1. Begin by fabricating the nanomatrix **[1]**. Add 80 microliters of 1 milligram per milliliter Janus base nanotubes to 40 microliters of 1 milligram per milliliter fibronectin and pipette the mixture gently and slowly several times **[2]**. After 10 seconds, visually inspect the tube for the presence of a white solid suspension **[3]**. *Videographer: This step is difficult and important!*

2.1.1. WIDE: Establishing shot of talent at the lab bench.

2.1.2. Talent adding JBNT solution to FN solution and pipetting up and down.

2.1.3. ECU: White solid suspension in the tube.

2.2. Next, prepare fibronectin, JBNT, and fibronectin-JBNT nanomatrix solutions for lyophilized materials observation according to manuscript directions **[1]**. Coat three wells of a 96-well clear round bottom microplate with solutions, adding 50 microliters to each well **[2]**. Freeze the plate at -80 degrees Celsius overnight **[3]**.

2.2.1. Tubes with prepared FN, JBNT, and NM solutions, all labeled.

2.2.2. Talent adding the solutions to the wells in the plate.

2.2.3. Talent putting the plate in the freezer and closing the door.

2.3. On the next day, turn on the lyophilized instrument and vacuum pump **[1]** and press the **cool down** button to reduce the temperature of the system to -50 degrees Celsius **[2]**. Put the frozen plate into the instrument and close all openings **[3]**.

2.3.1. Talent turning on the lyophilized instrument and pump.

2.3.2. Talent pressing the **cool down** button.

2.3.3. Talent putting the plate into the instrument and closing the openings.

2.4. Click the **start** button to reduce the system pressure to 0.9 millibar **[1]**. After 4 hours, press the **aerate** button and open the valve to increase the system pressure to the atmospheric pressure **[2]**, then take the plate out of the lyophilized instrument **[3]**.

2.4.1. Talent clicking the start button and pressure decreasing.

2.4.2. Talent pressing the aerate button.

2.4.3. Talent taking the plate out of the instrument.

3. Absorption Spectra Measurements

3.1. Prepare the JBNT-fibronectin nanomatrix solution as described in the text manuscript **[1]** and dilute the JBNT and fibronectin solutions with distilled water to make control solutions **[2]**.

3.1.1. Talent preparing the NM solution.

3.1.2. Talent diluting the JBNT or FN solution with water.

3.2. Measure the absorption spectra of the solutions with the spectrophotometer to characterize the assembly of nanomatrices **[1]**. Click the **UV-Vis** button **[2]**, then clean the test surface of the spectrophotometer and drop 2 microliters of distilled water on it **[3]**. Measure the water as blank from 190 to 850 nanometers **[4]**.

3.2.1. Talent walking to the spectrophotometer with the samples in hand.

3.2.2. Talent clicking the **UV-Vis** button.

3.2.3. Talent cleaning the surface of the spectrophotometer and adding water.

3.2.4. Talent taking the blank measurement.

3.3. For each sample, clean the test surface and drop 2 microliters of the solution on it **[1]**, then measure the UV-Vis absorption spectra from 190 to 850 nanometers **[2]**. *Videographer: This step is important!*

3.3.1. Talent cleaning the test surface.

3.3.2. Talent adding the sample and measuring absorbance.

4. In Vitro Biological Function Assay

4.1. Add 200 microliters of negative controls, JBNTs, fibronectin, and the nanomatrix solutions into the wells of an 8-well chambered slide **[1]**. Freeze dry the 8-well chambered coverglass with the lyophilized instrument to coat materials onto the bottom of the wells **[2]**. *Videographer: This step is important!*

4.1.1. Talent adding solutions to wells in a slide.

4.1.2. Talent using the lyophilized instrument.

4.2. Then, add 10,000 human mesenchymal stem cells to each well of the coverglass **[1]** and incubate it for 24 hours at 37 degrees Celsius with 5% carbon dioxide **[2]**. After the incubation, remove the cell culture medium from the wells **[3]** and rinse them with PBS **[4]**. *Videographer: This step is important!*

4.2.1. Talent adding cells to the wells on the slide.

4.2.2. Talent putting the slide in the incubator and closing the door.

4.2.3. Talent removing medium from wells.

4.2.4. Talent adding PBS to the wells and rinsing them, with the PBS container in the shot. *Videographer: Obtain multiple usable shots because this will be reused in 4.3.2 and 4.3.4.*

4.3. Add 100 microliters of the fixative solution to each well and leave it for 5 minutes at room temperature **[1]**. Then, gently rinse the cells with PBS twice **[2]** and add 100 microliters of 0.1% Triton-X 100 to each well **[3]**. After 10 minutes, repeat the rinses with PBS **[4]**.

4.3.1. Talent adding fixative to a few wells.

4.3.2. *Use 4.2.4.*

4.3.3. Talent adding Triton-X 100 to a few wells, with the Triton-X container in the shot.

4.3.4. *Use 4.2.4.*

4.4. Add 100 microliters of Rhodamine Phalloidin to each well **[1]** and incubate the chambered coverglass for 30 minutes, protecting it from light **[2]**. Rinse the cells twice with PBS and image them with a fluorescent microscope **[3]**.

4.4.1. Talent adding Rhodamine Phalloidin to a few wells.

4.4.2. Talent covering the slide to protect it from light.

4.4.3. Talent using the microscope.

Results

5. Results: Self-assembled JBNT/FN Nanomatrices

5.1. Nanomatrix formation is fast and completely biomimetic **[1]**. White floccule was visible 10 seconds after the JBNT solution was mixed with the fibronectin solution **[2]**.

5.1.1. LAB MEDIA: Figure 2.

5.1.2. LAB MEDIA: Figure 2. *Video Editor: Emphasize the white floccule that the arrow is pointing to in the 10 s image.*

5.2. Camera images of fibronectin, JBNTs, and the nanomatrix were captured and analyzed **[1]**. No long fibers were observed in the fibronectin group, indicating that the scaffold structure can't form without JBNTs **[2]**.

5.2.1. LAB MEDIA: Figure 3.

5.2.2. LAB MEDIA: Figure 3. *Video Editor: Emphasize A.*

5.3. JBNTs appear as long and thin fibers **[1]**. When crosslinked with fibronectin, the nanomatrix fiber can grow up to several centimeters in length **[2]**. The assembly of the nanomatrix was also confirmed by obtaining UV-Vis spectra **[3]**. The two absorption peaks of JBNTs alone diminish after nanomatrix formation **[4]**.

5.3.1. LAB MEDIA: Figure 3. *Video Editor: Emphasize B.*

5.3.2. LAB MEDIA: Figure 3. *Video Editor: Emphasize C.*

5.3.3. LAB MEDIA: Figure 4.

5.3.4. LAB MEDIA: Figure 4. *Video Editor: Emphasize the blue line.*

5.4. Transmission electron microscopy was used to characterize the morphology of the JBNTs and nanomatrices **[1]**. The JBNTs are slender tubes with uniform diameters **[2]**. When mixed with fibronectin, they form a long fibroid nanomatrix via charge interactions **[3]**.

5.4.1. LAB MEDIA: Figure 5.

5.4.2. LAB MEDIA: Figure 5. *Video Editor: Emphasize A.*

5.4.3. LAB MEDIA: Figure 5. *Video Editor: Emphasize B.*

5.5. When the pH is lower than the isoelectric point of the fibronectin, the nanomatrix bundles self-release due to the positively charged fibronectin **[1]**.

5.5.1. LAB MEDIA: Figure 5. *Video Editor: Emphasize C.*

5.6. The effect of the JBNT-fibronectin nanomatrix on cell adhesion was also explored **[1]**. Since nanomatrices are designed to morphologically mimic the extracellular matrix,

they provide a scaffold and increase cell adhesion **[2]**. Fluorescence microscopy confirmed that incubation with the nanomatrices increased cell adhesion **[3-TXT]**.

5.6.1. LAB MEDIA: Figure 6.

5.6.2. LAB MEDIA: Figure 6. *Video Editor: Emphasize the JBNT/FN bar.*

5.6.3. LAB MEDIA: Figure 7. *Video Editor: Emphasize D. Label A "***control hMSC***", B "***hMSC w/JBNT***", C "***hMSC w/FN***", and D "***hMSC w/NM***".*

Conclusion

6. Conclusion Interview Statements

6.1. **Yupeng Chen:** There are many types of injectable hydrogels but very few injectable scaffolds. The self-assembled Nano-Matrix brings a new strategy to fabricate injectable scaffolds.

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