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Optimize myoblast monodisperse suspension in hydrogel precursor solution using C2C12 cells

Achieving a monodisperse cell population within the viscous hydrogel pre-cursor solution is a technical bottleneck that must be achieved in order to obtain reproducible results with the mini-MEndR platform. This is particularly challenging with myoblasts, which are very sticky cells that tend to clump. Use this practice protocol to check your performance before proceeding to make mini-MEndR tissues.
Tips that I found helpful for early users are outlined in blue text.

Monodisperse cell suspension in PBS

1. Trypsinize a plate of C2C12 cells and centrifuge to pellet.
2. After C2C12 cells have pelleted, count the number of cells in the pellet using a hemacytometer.
3. Transfer 500,000 cells to each microcentrifuge tube. Centrifuge the cells at 0.3 x g (300 rpm) for 6 minutes.
    - Orient the tube in the centrifuge so the hinge faces outward from the rotor; this ensures that the pellet is on the same side as the hinge.
4. After cells have pelleted in the microcentrifuge tube, aspirate the media in each of the microcentrifuge tubes. Store tubes on ice.
    - The media must be aspirated completely to ensure that the resuspension in the next step results in accurate cell numbers being suspended.
    - To avoid disturbing the pellet, do not touch the side of the tube that contains the pellet using the aspirating pipette. Instead, use a P100/P1000 which is gentler than a vacuum aspirator.
5. Critical step for monodisperse cell suspension: Resuspend cells in 80 µL PBS by pipetting up and down quickly (i.e. triturate), but without producing bubbles.
6. Pipette 10 uL of the PBS cell suspension into a hemacytometer (no trypan blue). Observe the cells under the microscope, single cells should be seen.
7. If cells are not monodisperse, repeat this PBS suspension practice with more vigorous pipetting and/or with more rounds of trituration. If single cells are seen, make note of the conditions that led to this result and proceed to the next section. Tips: Resuspend and evaluate the results of one tube at a time to be able to iterate quickly. You can re-spin these tubes to practice again if needed.

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Monodisperse cell suspension in Hydrogel Master Mix

1. Trypsinize a plate of C2C12 cells and centrifuge to pellet.
2. While C2C12 cells are pelleting, create fibrinogen solution: Reconstitute fibrinogen powder, pre-weighed and aliquoted into a 1.5 mL centrifuge tube, in 0.9% (w/v) NaCl solution such that you achieve a final concentration of 10 mg/mL fibrinogen.
3. After adding the NaCl solution to the fibrinogen tube, warm the tube at 37˚C for 1-2 minutes, or until the fibrinogen powder is fully dissolved.
4. Filter the warmed fibrinogen solution through a 0.22 µm filter attached to a syringe, into another tube. Store this final filtered solution on ice in the BSC.
5. After C2C12 cells have pelleted, count the number of cells in the pellet using a hemacytometer.
6. In a microcentrifuge tube, pellet out 500,000 cells. Centrifuge these cells at 0.3 x g for 6 minutes.
    a. Orient the tube in the centrifuge so the hinge faces outward; this ensures that the pellet is on the opposite side of the hinge.
7. While the cells are pelleting, prepare 80 µL hydrogel master mix: 40% DMEM, 40% filtered fibrinogen solution and 20% Geltrex.
8. After cells have pelleted in the microcentrifuge tube, aspirate the media in the microcentrifuge tube.
    a. The media must be aspirated completely to ensure that the resuspension in the next step results in accurate cell numbers being seeded.
    b. To avoid disturbing the pellet, do not touch the side of the tube that contains the pellet using the aspirating pipette. Instead, use a P100/P1000 which is gentler than a vacuum aspirator.
9. Critical step for monodisperse cell suspension: Resuspend cells in 80 µL Master Mix by pipetting up and down quickly (i.e. triturate), but without producing bubbles.
10. Pipette 10 uL of the Master Mix cell suspension into a hemacytometer (no trypan blue). Observe the cells under the microscope, single cells should be seen.
11. If cells are not monodisperse, repeat this PBS suspension practice with more vigorous pipetting and/or with more rounds of trituration. If single cells are seen, make note of the conditions that led to this result and proceed to the next section. Tips: Resuspend and evaluate the results of one tube at a time to be able to iterate quickly. You can re-spin these tubes to practice again if needed.

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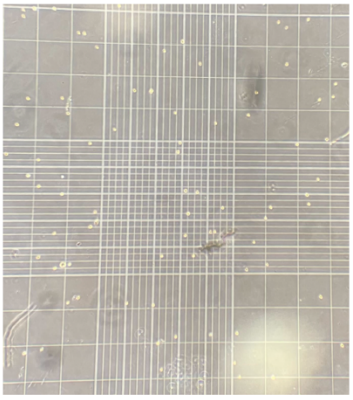
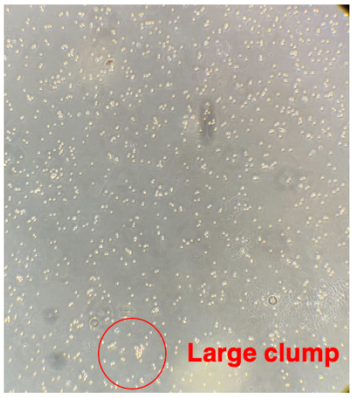
Example: Single cells after resuspension in PBS, clumping after resuspension in Master Mix, and decrease in clumping after refinement of resuspension technique



PBS
Single cells are seen (cells do not stick to each other)



Master Mix (1)
In Master Mix, cells exhibit more clumping than in PBS

Master Mix (2)
Correction to previous technique: pipette up and down more times than before