Video Article

Labeling Stem Cells with Fluorescent Dyes for non-invasive Detection with Optical Imaging

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Abstract

Optical imaging (OI) is an easy, fast and inexpensive tool for in vivo monitoring of new stem cell based therapies. The technique is based on ex vivo labeling of stem cells with a fluorescent dye, subsequent intravenous injection of the labeled cells and visualization of their accumulation in specific target organs or pathologies. The presented technique demonstrates how we label human mesenchymal stem cells (hMSC) by simple incubation with the lipophilic fluorescent dye DiD (C67H103CIN2O3S) and how we label human embryonic stem cells (hESC) with the FDA approved fluorescent dye Indocyanine Green (ICG). The uptake mechanism is via adherence and diffusion of the lypophilic dye across the phospholipid cell membrane bilayer. The labeling efficiency is usually improved if the cells are incubated with the dye in serum-free media as opposed to incubation in serum-containing media. Furthermore, the addition of the transfection agent Protamine Sulfate significantly improves contrast agent uptake.

Protocol

Labeling of mesenchymal stem cells with the fluorescent dye DiD

1. To begin the procedure for labeling mesenchymal stem cells, trypsinize and count the cells to get a suspension with a defined number of cells.
2. Take the cells out of the incubator and aspirate out old media from the flasks containing the cells to be labeled
3. Wash the cells with 10 ml of Mg/Ca-free PBS. Aspirate out the PBS. The Mg and Ca would inhibit the Trypsin, therefore we use PBS without these.
4. Add pre-warmed 0.05% Trypsin, for a T75 flask we use 5ml. Ensure that the entire surface of the flask is covered.
5. Incubate at 37°C in the incubator for about 5 minutes.
6. Confirm the detachment under the microscope. If the cells still adhere to the flask, tap it a few times and wait a little longer until the trypsinization is successful.
7. Now, it is necessary to neutralize the Trypsin by adding media containing 10% FCS. We use an equal amount of media as there is Trypsin.
8. Pipette up and down a few times to ensure that all the cells are re-suspended in the media.
9. Transfer the cell solution to a sterile 15ml capped polypropylene tube.
10. Centrifuge at 400 rcf for 5 minutes.
11. Aspirate out the supernatant ensuring not to disturb the cell pellet.
12. Resuspend the cells in DMEM and proceed with the cell count.
13. Suspend the cells to be labeled at a density of 1x10^6 per ml in serum-free culture medium (DMEM).
14. This was all preparation of the cell suspension. Now, it is ready to start the labeling.
15. First, add 5 µL of DiD contrast agent per ml of cell suspension.
16. Then, mix the solution by gently pipetting.
17. Incubate the cells with the labeling solution at 37°C for 20 minutes in a 6-well low-attachment dish.
18. Once the simple incubation is complete, it is necessary to transfer the cell solution to a 15 ml capped polypropylene tube.
19. Centrifuge it down at 400 rcf for 5 minutes.
20. Aspirate out the labeling medium, ensuring not to disturb cell pellet.
21. Wash the cells with PBS. Pipette the cells up and down, making sure to break up the cell pellet.
22. Repeat the latter two steps two more times, so there is a total of 3 washing steps.
23. Count the cells and perform a Trypan blue test to determine cell viability. These are standard cell culture protocols that we are not going to explain here.
24. Your cells are now ready to be imaged in optical imager.

Labeling of human embryonic stem cells with the fluorescent dye ICG

1. To begin the procedure for labeling human embryonic stem cells, prepare the contrast agent Indocyanine Green. Mix it with the transfection agent Protamine.
2. Measure out 1mg of the Indocyanine Green powder. Dissolve the ICG powder in 100 µL of Dimethyl Sulfoxide (DMSO).
3. Add 400 µL of Dulbecco's Modified Eagles Medium (DMEM + 10% Fetal Calf Serum) media to the mixture and shake it well. This results in a final concentration of 2mg/ml of Indocyanine Green.
4. Add the transfection agent Protamine. Protamine acts as a shuttle for the contrast agent, so that it gets into the cell more efficiently.
5. Mix 5 µL Protamine Sulfate, which is supplied at a concentration of 10mg/ml, with 300 µL ICG and 300 µL serum free Dulbecco's Modified Eagles Medium.
6. Gently shake the new transfection solution for 5 minutes to allow complex to form.
7. The labeling solution is ready.
8. Aspirate out the old media from hESC 10mm Petri dish.
9. Add 5ml of pre-warmed serum-free DMEM.

10. Add the previously prepared Protamine/ICG solution to the cells. Start the 1 hour incubation by placing the dish in an incubator at 37°C.
11. After the incubation is complete, remove the dish from the incubator and aspirate out the labeling solution.
12. Wash the cells by rinsing the dish with 5 ml PBS.
13. Aspirate out the PBS and replace with 5ml of 0.25% Trypsin. Incubate the dish at 37°C for 5 minutes to allow trypsinization to occur. It helps to shake the dish a little every once in a while.
14. Gently pipette up and down, to break up the remaining colonies.
15. Neutralize the Trypsin by adding an equal amount of KSR to the dish.
16. Transfer the cell solution to a 15ml tube and centrifuge the solution at 400 rcf for 5 minutes.
17. Resuspend the cells in full media.
18. If there still are clumps at this point, trypsinize and centrifuge again.
19. Once a clump-free cell solution is achieved, aspirate out the old media and resuspend the pellet in 10ml of pre-warmed full ESC media.
20. At this point, it is necessary to separate the mouse feeder cells from the hESCs. This is done to ensure that, later, only imaging the stem cells occurs.
21. For this, transfer the cell solution to a gelatin coated 10 cm dish.
22. Put the dish into the 37°C incubator and let it sit for 45 minutes. During this time, make sure not to disturb the dish. Now, the feeders will adhere to the dish and the stem cells will not.
23. Transfer the solution out of the Petri dish. and now we have a labeled single cells solution of human embryonic stem cells.
24. The cells can now be counted and a Trypan blue test can be performed on them.
25. The cells are ready to be imaged!

Discussion

OI is a relatively new imaging technique, based on the detection of fluorescence. OI is as sensitive as radiotracer-based imaging techniques, but not associated with any irradiation exposure. OI provides an effective means of tracking cells non-invasively and repetitively, thereby providing insight into cell migration to the target site. One major limitation of the technique is the limited tissue penetration of fluorescent probes in vivo. Thus, a tracer accumulation in deep tissues, more than about 5-10 cm from the skin surface, may not be detected. Current clinical applications are limited to superficial techniques such as endoscopic, cardiovascular and retinae imaging (Funovics et al. 2003) but this domain of research will continue to expand through the recognition of the vast possibilities offered by in vivo cell tracking.

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