Video Article

Labeling hESCs and hMSCs with Iron Oxide Nanoparticles for Non-Invasive in vivo Tracking with MR Imaging

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Abstract

In recent years, stem cell research has led to a better understanding of developmental biology, various diseases and its potential impact on regenerative medicine. A non-invasive method to monitor the transplanted stem cells repeatedly in vivo would greatly enhance our ability to understand the mechanisms that control stem cell death and identify trophic factors and signaling pathways that improve stem cell engraftment. MR imaging has been proven to be an effective tool for the in vivo depiction of stem cells with near microscopic anatomical resolution. In order to detect stem cells with MR, the cells have to be labeled with cell specific MR contrast agents. For this purpose, iron oxide nanoparticles, such as superparamagnetic iron oxide particles (SPIO), are applied, because of their high sensitivity for cell detection and their excellent biocompatibility. SPIO particles are composed of an iron oxide core and a dextran, carboxydextran or starch coat, and function by creating local field inhomogeneities, that cause a decreased signal on T2-weighted MR images. This presentation will demonstrate techniques for labeling of stem cells with clinically applicable MR contrast agents for subsequent non-invasive in vivo tracking of the labeled cells with MR imaging.

Protocol

Labeling of hMSC with Ferucarbotran

This is the technique for labeling human mesenchymal stem cells with Ferucarbotran, an iron oxide based contrast agent for MR imaging:

1. To begin, plate the cells 18-24 hours before the labeling procedure in T75 flasks at a confluence of 80%. If you use a different culture dish, this is equal to about 10000 cells per cm^2.
2. The next day, start with preparing the labeling media by adding 30 µl of Resovist to 8 ml of serum free media. This will label one T75 flask at 80% confluency, corresponding to a concentration of 100 µg Fe/ml media.
3. Take off the culture media and wash the cells once with PBS or serum-free media. This is done to get rid of residual serum proteins and other constituents of the media that could bind the contrast agent and influence labeling efficiency.
4. Add the labeling media to the flask and put the flask back in the incubator.
5. After 2 hours, add 2ml of FCS so that a final concentration of 20% FCS is achieved. This is done to ensure that the cells do not receive stimulus to differentiate, but instead grow in their familiar environment.
6. Incubate the cells over 18 hours. This is best done overnight.
7. The next day, rinse the cells with PBS, then trypsinize them as usual.
8. Once trypsinized, the cell suspension needs to be washed 3 times to get rid of residual, free contrast agent in the media. This is done with PBS centrifuging the cells at 400 rcf for 5 minutes.
9. The final cell pellet can be resuspended and is ready for further experiments.
10. Count the cells now, as some might have been lost during the previous washing steps. Also, perform viability testing at this point using the Trypan blue exclusion assay and take some samples for spectrometric analysis to measure the labeling efficiency.

Labeling of human embryonic stem cells with Ferumoxides

1. To begin, plate the hESC in 10cm dishes, as usual. These dishes are pre-coated with gelatin and have irradiated feeder cells on them. You can use this protocol for feeder-free cultures as well.
2. Let the hESC attach and grow for approximately 3-4 days so that they form medium sized colonies. During this time, ensure that the colonies do not grow to large, as larger colonies are more difficult to break up later.
3. Differentiated cells can contaminate these cultures, so depending on the cleanliness of the colonies, you may have to get rid of differentiated cultures by dissection.
4. Prepare the labeling media that consists of Ferumoxides at a concentration of 100µg/ml and full hESC media. For this, we mix 89µl Ferumoxides and 10 ml of full growth media.
5. Wash the dish once with complete media.
6. Add 10 ml of labeling media per dish and incubate the cells for 4 hours.
7. Now, the labeling is complete. In the next step, it is necessary to wash the cells and get a single cell suspension for further use. For this, first rinse the dish with PBS.
8. Then, replace the PBS with 5ml of 0.25% Trypsin and incubate in the incubator for 5 minutes, or until the cells detach. It helps to tap the dish frequently.
9. The cells have now detached. Keep in mind that if the dish contained larger colonies there might be some clumps left.
10. To get rid of these clumps, one can mix the whole suspension once using a serological pipette, then let it sit for another 2 minutes.
11. We do not use an Eppendorf pipette, as repeated sucking of the cells through the thin tip can break the cell membranes.
12. If residual clumps are present, one can run the cell suspension through a 40µm cell strainer.

13. If everything worked correctly, a single cell suspension now exists that is mixed with a lot of debris, originating from the gelatin coating, the ESC-matrix and dead cells. To get rid of this, wash the cells twice by spinning them down at 400 rcf for 5 minutes and resuspend them in full media.
14. Now, the ESC needs to be isolated from the irradiated feeder cells. This can be done efficiently by plating the cell suspension on a gelatin-coated dish.
15. If the dish is not manipulated, all cells will sediment down, but the feeders will attach faster than the ESC.
16. So, if the supernatant is taken off after 45 minutes, it should contain mainly ESC, while the feeders should mainly be attached to the dish.
17. This way, a single cell suspension of magnetically labeled human embryonic stem cells that can be used for further experiments is obtained.
18. As in the previous protocol, at this point, you need to count the cells and take samples for viability assessment and measuring of labeling efficiency.

Discussion

A non-invasive depiction of stem cells is crucial for monitoring virtually any stem cell based therapy. A better understanding of the in vivo kinetics of labeled stem cells, as visualized on MR images, could lead the way to a rational and a more effective use of stem cell-based therapies. Since we use clinically applicable contrast agents and imaging techniques, our presented protocols should be readily translatable to applications in patients. Potential clinical applications of our methods include comparative investigations of the engraftment process of various stem cell subtypes or genetically engineered stem cells, as well as, the assessment of therapy effects on the outcome of stem cell engraftment. Results should be immediately helpful in preclinical assessments of stem-based therapies, in the design of related clinical trials, and later, in the assessment of those stem cell-based therapies in clinical practice. Of note, our imaging technique is also applicable to other stem cell populations, such as hematopoietic stem cells or neuronal stem cells.

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References

1. Daldrup-Link, H.E., Rudelius, M., Oostendorp, R.A.J., Settles, M., Piontek, G., Metz, S., Heinzmann, U., Rummeny, E.J., Schlegel, J., Link, T.M. Targeting of Hematopoietic Progenitor Cells with MR Contrast Agents. Radiology 228, 760-767 (2003)

2. Daldrup-Link, H.E., Rudelius, M., Piontek, G., Metz, S., Bräuer, R., Debus, G., Corot, C., Schlegel, J., Link, T.M., Peschel, C., Rummeny, E.J., Oostendorp, R.A.J. Migration of iron oxide labeled human hematopoietic progenitor cells in a xenotransplant model: in vivo monitoring using clinical magnetic resonance imaging equipment. Radiology 234, 197-205 (2005)

3. Henning, T.D., Saborowski, O., Golovko, D., Boddington, S., Bauer, J.S., Fu, Y., Meier, R., Pietsch, H., Sennino, B., McDonald, D.M., Daldrup-Link, H.E. Cell labeling with the positive MR contrast agent Gadofluorine M. Eur Radiol 17, 1226-34 (2007)

