Nanoparticle Tagging of Live Cells to Monitor Cell Migration

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Introduction

Monitoring cell migration through engineered tissue can be difficult. Tracking is more complicated when the tissue is composed of one cell type of the same gender because protein and chromosomal identification is not useful. Human umbilical vein endothelial cells (HUVEC) can be tagged with superparamagnetic iron oxide (SPIO) nanoparticles in order to track cell migration.

The medical imaging community has employed SPIO for decades. Small SPIO particles are approximately 20 nm in diameter and can be used for magnetic resonance imagining of lymph nodes, bone marrow, stem cells, and localization of gene therapy [1, 2]. Particles of SPIO can be used to image organs because the nanoparticles are not retained by tumor tissue in the liver and spleen [3]. These nanoparticles have also been used for pre-labeling cells for *in vivo* tracking of stem cells and the *in vivo* monitoring of macrophage activity [4]. It has been found that SPIO labeling has no short- or long-term toxic effects in tumor and stem cells [5]. Therefore, SPIO is a viable tool for tagging HUVECs to monitor migration in engineered microvascularized tissue.

Materials and Methods

The SPIO used in this procedure are nanoparticles of γ-Fe_2O_3 (Ferrornan 6503). The particle size range is 45–60 nm, which allows the SPIO to enter the endothelial cell membrane, which has pore sizes ranging from 0.8 to 3.0 µm [6].

Engineered microvascularized tissues were fabricated. HUVECs were suspended in collagen type I hydrogels. The HUVECs organized into capillary networks within 5 days as a result of daily supplementation of angiogenic factors (vascular endothelial growth factor, basic fibroblast growth factor) in the basal medium. This microvascularized tissue is a disc 3 cm in diameter and 1 cm thick with the capillary network at the bottom of the tissue.

The HUVECs seeded on the surface of the engineered microvascularized tissue were processed as follows. A flask of HUVECs was rinsed with phosphate buffer solution (PBS) for 5 minutes to remove serum from the cell surface. Gamma-Fe_2O_3 (0.3 µmol) was suspended in EGM-2MV (Clonetics, CC-4147). The SPIO-medium was pipetted into the flask and momentarily oscillated to uniformly disperse the nanoparticles over the HUVECs. The flask was incubated at 37°C for 5 hours.

After the SPIO incubation, the HUVECs were seeded on the microvascularized tissues. The flask of HUVECs was rinsed with PBS to remove the nanoparticles that did not enter the cells. The HUVECs were trypsinized from the flask, centrifuged, and then seeded onto the surface of the capillary tissues. The tissues were incubated for 12 hours.

The capillary tissues were prepared for microscopy. The medium was removed and 10% neutral buffered formalin was used for tissue fixation. Five ml each of 20% hydrochloric acid solution and 10% potassium ferrocyanide (w/v, Sigma, P3289) solution were mixed (fresh), and 1 ml was pipetted over each capillary tissue. The solution was incubated on the tissues at room temperature for 20 minutes, removed, and then three one- minute DiH_2O rinses were performed. The tissues were maintained in DiH_2O to prevent dehydration. The engineered tissues were imaged using brightfield microscopy on a Zeiss Axiovert.

Results and Discussion

Nanoparticle iron oxide provides the opportunity to differentiate between a pre-existing microvascularized network and migrating endothelial cells connecting with the network. The SPIO in the HUVEC and ferrocyanide react to form ferric ferrocyanide, which creates a bright blue pigment called Prussian blue. The HUVECs that migrated from the surface of the tissue to the pre-existing capillary network at the bottom were blue (Figure 1). Therefore, we were able to visualize the HUVECs associated with the established network at the bottom of the tissue and the blue HUVECs that migrated (seeded on top of the tissue) to connect with the pre-existing network. Figure 2 shows two blue HUVECs that migrated through 1 cm of collagen tissue to the top of a capillary, and the cells were anastomosing with this pre-existing capillary. This also shows that the γ-Fe_2O_3 nanoparticles entering the cells did not change the ability of the HUVECs

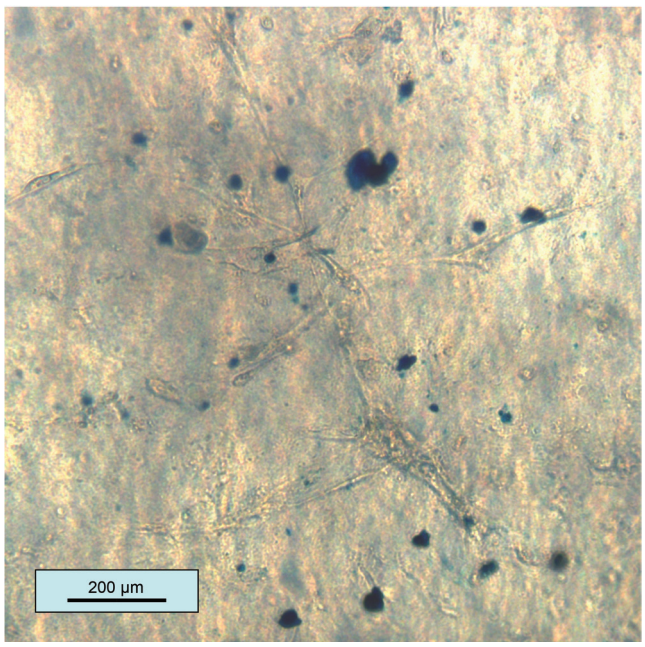

Figure 1: HUVECs were tagged with γ-Fe_2O_3 nanoparticles and then seeded onto the surface of a microvascularized tissue. HUVECs (blue spheres) have migrated 1 cm through the tissue to the pre-existing capillary network within 8 hrs of seeding.

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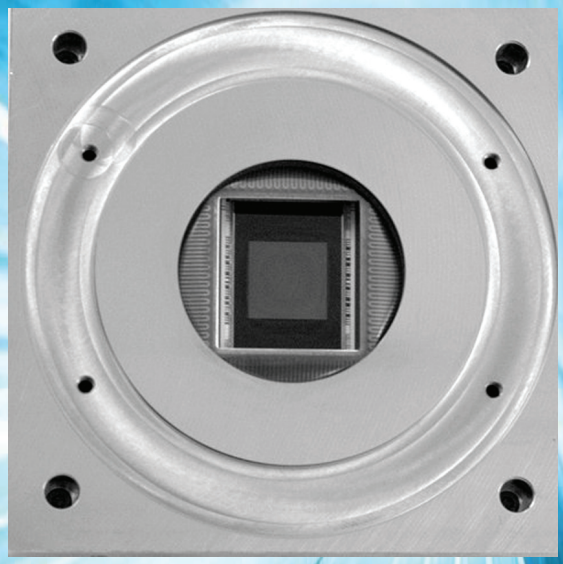

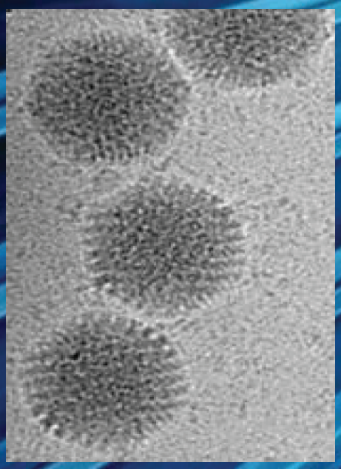

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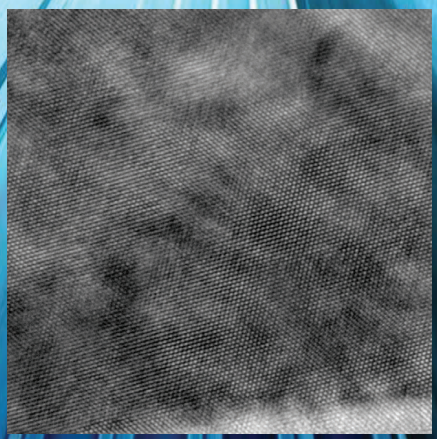

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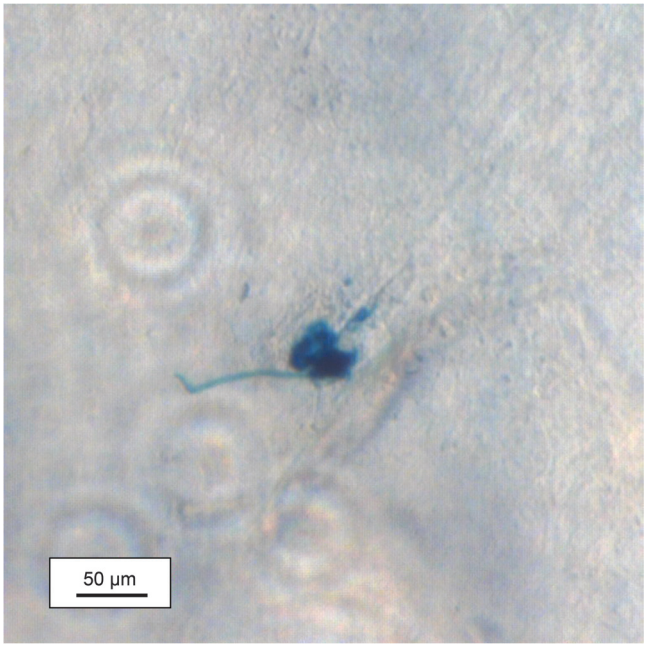

Figure 2: Two blue HUVECs connecting with the pre-existing microvascularized network within 10 hrs of seeding. Prussian blue diffuses into cell extension that has elongated to connect with the capillary network.

to migrate or connect with the microvascularized network. For best resolution, the tissue must be imaged immediately following treatment (20% hydrochloric acid and 10% potassium ferrocyanide solution) because the color intensity decreases over time. Tagged cells were maintained in culture for 7 days without a decrease in cell viability. The ferrocyanide treatment does kill the cells; it is not for live cell staining.

Conclusion

SPIO nanoparticles are routinely used for medical imaging of various parts of the human body and to track cell migration within the body. *In vitro* use of SPIO nanoparticles to monitor cell activity and migration could have many applications. This research shows that 45–60 nm γ-Fe_2O_3 particles can penetrate the cell membrane of endothelial cells and be used to track cell migration through an *in vitro* tissue. This method could also be used to tag stem cells and determine stem cell homing to specific cell types in 2- or 3-dimensional *in vitro* environments.

Acknowledgement

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References

[1] J Frank et al., *Radiology* 228(2) (2003) 480–87.
[2] M Taupitz et al., *Rofo* 175(6) (2003) 752–65.
[3] J Ferrucci and D Stark, *AJR Am J Roentgenol* 155(5) (1990) 943–50.
[4] M Modo et al., *Mol Imaging* 4(3) (2005) 143–64.
[5] A Arbab et al., *Radiology* 229(3) (2003) 838–46.
[6] Y Wang et al., *Forensic Sci Int* 178(2) (2008) 204–06.

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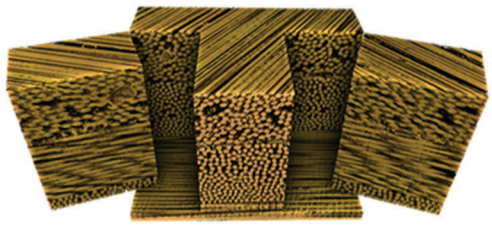

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