# Tracking of Endothelial Cell Response to Angiogenic Factors Using an Intracellular T2 Contrast Agent

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**Introduction:**

Understanding endothelial cell response to chemoattractants, angiogenic factors, and antiangiogenic drugs in microenvironments that mimic tumors is critical in assessing the effects of these factors in angiogenesis and antiangiogenic therapy. We developed an assay with Human Umbilical Vein Endothelial Cells (HUVECs), ECM gel and MDA-MB-231 breast cancer cells to longitudinally and non-destructively observe the effects of angiogenic factors secreted by MDA-MB-231 cells, on tubulogenesis and three-dimensional endothelial network response, *in vitro*. HUVECs were labeled with a $T_2$ contrast agent (Feridex) and placed in ECM gel-containing chambers with and without cancer cells, to observe invasion of HUVECs under these two conditions.

**Methods:**

*Cell culture and maintenance*: MDA-MB-231 cells, originally derived from the pleural effusion of a breast cancer patient, were maintained in RPMI 1640 supplemented with 9 % fetal bovine serum, 90 U/ml Penicillin, and 90 µg/ml Streptomycin. HUVECs, obtained from Clonetics (USA), were grown in EGM-2 (Clonetics, USA). Cells were maintained in 5 % $CO_2$ and 90 % humidity at 37ºC until the MR experiments. *HUVEC labeling*: HUVECs (day 17) were incubated with 2.5 µg Feridex per ml of EGM-2 in the presence of 0.0375 µg/ml Poly-L-lysine for 24 h prior to seeding on ECM gel. *Chamber preparation*: The invasion assays were housed in non-cell-culture Millipore inserts with 0.4 µm membrane pore size. In each insert, 100 µl of ECM gel was added and allowed to polymerize at 37ºC in a $CO_2$ incubator. Then 5 x $10^4$ MDA-MB-231 cells suspended in 100 µl EGM-2 were seeded in the invasion chambers, whereas 100 µl EGM-2 was added to the control chambers. After 4 h incubation, another 100 µl layer of ECM gel was placed in each chamber, followed by a 30 min incubation period. Finally, 1.5 x $10^5$ labeled HUVECs were seeded in all chambers approximately 24 h prior to imaging, allowing for attachment and lumen-like formation. *MRI*: All MR images were obtained on a 500 MHz (11.74 T) wide-bore imaging system with a Bruker Avance spectrometer. Saggital, coronal, and axial images of the chambers were obtained using a $T_2$-weighted MSME_TOMO spin-echo sequence. All chambers were imaged by MRI in approximately 24 h increments observed over four days, and checked for cell presence and structure by phase contrast light microscopy.

**Results:**

HUVECs in chambers containing MDA-MB-231 breast cancer cells traversed the ECM gel toward the cancer cells within 72 h of seeding (Fig. 3a) whereas HUVECs in control chambers did not invade (Fig. 3b). Feridex labeling appeared to have no detrimental effect on HUVEC survival over the period of observation, as was concluded from phase contrast light micrographs, MR images, and verified by MTT assay.

**Conclusions:**

We have developed a non-destructive assay capable of dynamic and longitudinal study of the invasive response of endothelial cells to changes in the microenvironment caused by factors secreted by epithelial cancer cells. This will be useful in the three-dimensional study of the effects various angiogenic factors, growth factors and chemoattractants on tubulogenesis and endothelial cell migration and invasion, and the efficacy of antiangiogenic drugs, *in vitro*.

**Acknowledgements:**

The authors thank Dmitri Artemov for useful discussion and expert technical assistance. We acknowledge support from NIH 1 RO1 CA 82337.

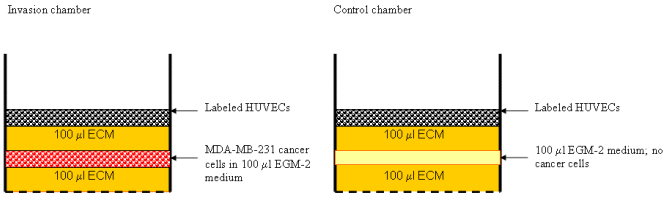


Figure 1: Schematic of the MR invasion assay - Left panel: Invasion chamber containing MDA-MB-231 breast cancer cells sandwiched between two layers of ECM gel, with Feridex-labeled HUVECs on top; Right panel: Control chamber with no cancer cells between the two layers of ECM gel and HUVECs on top.

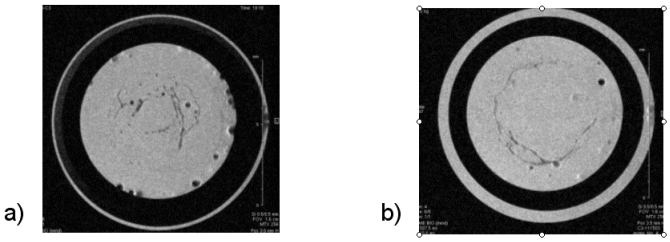


Figure 2: Axial images showing Feridex-labeled HUVECs 24 h after seeding, with a square field-of-view = 1.6 cm, acquisition matrix 256 x 256, slice thickness = 0.5 mm TR = 507.5 ms, TE = 15 ms, number of averages = 4. HUVEC network formation is seen clearly in the two contiguous slices shown above.

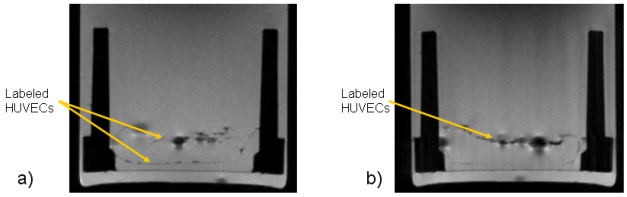


Figure 3: Coronal images showing Feridex-labeled HUVECs 72 h after seeding in an a) invasion chamber and b) a control chamber. The images are obtained using a $T_2$-weighted spin-echo sequence with field-of-view = 2 cm, acquisition matrix 256 x 256, slice thickness = 1 mm, TR = 530 ms, TE = 60 ms, number of averages = 4.