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Assessment of an Engineered Endothelium via Single-Photon Emission Computed Tomography

Bin Jiang, Ph.D.^{1,2,#}, Yidi Wu, M.S.^{1,3,#}, Chad R. Haney, Ph.D.^{1,4,5}, Chongwen Duan, M.S.¹, and Guillermo A. Ameer, Sc.D.^{1,2,4,6,7,*}

¹Biomedical Engineering Department, Northwestern University, Evanston, IL

²Department of Surgery, Northwestern University, Chicago, IL

³Master of Biotechnology Program, Northwestern University, Evanston, IL

⁴Chemistry of Life Processes Institute, Northwestern University, Evanston, IL

⁵Center for Advanced Molecular Imaging, Northwestern University, Evanston, IL

⁶International Institute for Nanotechnology, Northwestern University, Evanston, IL

⁷Simpson Querrey Institute for BioNanotechnology, Northwestern University, Chicago, IL

Abstract

The clinical translation of cell-based therapeutics often requires highly sensitive, non-invasive imaging tools to assess cell function and distribution *in vivo*. The objective of this research was to determine whether human Sodium-Iodide Symporter (hNIS) ectopic expression in endothelial cells (ECs) in combination with single-photon emission computed tomography (SPECT) is a feasible approach to non-invasively monitor the presence and viability of an engineered endothelium on expanded polytetrafluoroethylene (ePTFE). Human umbilical vein endothelial cells (HUVECs) were transduced with pLL3.7-hNIS via lentivirus with multiplicity of infection (MOI) of 0, 2, 5 and 10 (n=4). Ectopic expression of hNIS in HUVECs via optimized lentiviral transduction (MOI 5) enabled cell uptake of a radioisotope that can be detected by SPECT without affecting endothelial cell viability, oxidative stress, or antithrombogenic functions. The viability and distribution of an engineered endothelium grown on ePTFE coated with the biodegradable elastomer poly(1, 8 octamethylene citrate) (POC) and exposed to fluid flow was successfully monitored non-invasively by SPECT. We report the feasibility of a non-invasive, highly sensitive and functional assessment of an engineered endothelium on ePTFE using a combination of SPECT and X-ray computed tomography (SPECT/CT) imaging and hNIS ectopic expression in ECs. This technology potentially allows for the non-invasive assessment of transplanted living cells in vascular conduits.

Keywords

Endothelial Cells; Vascular Grafts; Tissue Engineering; Imaging; Sodium Iodine Symporter

*Corresponding Author: Guillermo A. Ameer, Sc.D., 2145 Sheridan Road, Tech B382, Evanston, IL 60208, g-ameer@northwestern.edu, Phone: 847-467-2992.

#These authors contributed equally to this work.

1. Introduction

The use of cell-based therapies to address vascular problems in humans will require technologies that allow the short- and long-term monitoring of the transplanted cells to properly assess safety and efficacy. For example, in vascular tissue engineering, vascular endothelial cells (ECs) are often seeded onto the lumen of vascular grafts to form an intact endothelium to prevent thrombosis and neointima hyperplasia.(Jiang et al. 2015c) While non-invasive vascular imaging techniques such as ultrasound and magnetic resonance angiography can be used to monitor the graft's patency over time,(Wolf et al. 2000) they do not provide information on the presence, distribution, and viability of seeded ECs. Imaging technologies continue to be developed mostly for diagnostic applications and to a lower extent to assess the performance of biomaterials *in vivo*.(Michalet et al. 2005)(Artzi et al. 2011) However, none of these methods have been shown to provide the required sensitivity, specificity, and depth to monitor live cell tracking on vascular grafts *in vivo*. Such an imaging methodology ideally should be non-invasive, quantitative, and capable of providing three-dimensional information to troubleshoot problems either *in vitro* for quality control or *in vivo* to predict patient outcome.(Appel et al. 2013)

In this study, we investigate the use of human sodium-iodide symporter (hNIS) as a reporter gene(Dingli et al. 2004) for live EC tracking by single-photon emission computed tomography with x-ray computed tomography (SPECT/CT) in tissue engineered vascular constructs. NIS is an integral plasma membrane glycoprotein that mediates active iodine transport into the thyroid follicular cells.(Dohán et al. 2003) Ectopic expression of NIS has enabled the imaging of transplanted cells such as human induced pluripotent stem cells (Templin et al. 2012) and cardiac stem cells (Terrovitis et al. 2008) in tissue engineering and regenerative medicine applications by non-invasive nuclear imaging techniques such as SPECT and positron emission tomography (PET). This is the case because cells expressing NIS can take up radioisotopes such as technetium 99m (^{99m}Tc) or iodine 124 (^{124}I).(Chung 2002) However, there are no reports on the use of NIS expression to visualize and characterize cell monolayers such as the endothelium. We report the effect of hNIS ectopic expression on EC viability and functions and show that hNIS-positive cells can uptake enough radioisotopes to allow the imaging of an endothelium within ePTFE tubes. We also demonstrate how this technology can be used to assess an engineered endothelium *in vitro* using ePTFE tubes coated with poly(1, 8 octamethylene citrate) (POC).

2. Materials and Methods

2.1 hNIS lentiviral transduction of ECs

Human umbilical vein endothelial cells (HUVECs) were purchased from Lonza CloneticsTM (Walkersville, MD). HUVECs from passage 3 to passage 8 were cultured in endothelial growth medium-2 (EGMTM-2 BulletKitTM, Lonza) in 5% CO₂ in air at 37°C. Lentivirus with pLL3.7-hNIS (Imanis Life Sciences, Rochester, MN) was added to cultured HUVECs (passage 3) at a multiplicity of infection (MOI) of 2, 5 or 10 and kept in culture for an additional 48 hours. Cells that were not exposed to the virus (MOI 0) were used as a negative control. The cells were passaged every 5 to 7 days after lentiviral transduction.

Lentiviral transduction efficiency was evaluated with immunofluorescence staining for hNIS. After paraformaldehyde fixation and serum blocking, the cells were incubated with primary antibody (hNIS goat polyclonal IgG, Santa Cruz, Dallas, TX) overnight at 4°C followed by a fluorescent secondary antibody for 2 hours at room temperature. The cells were counter stained with DAPI to visualize nuclei. Fluorescence microscopy (Nikon TE2000U, Japan) was used to image the hNIS and DAPI staining with a 20x objective. Transduction efficiency was calculated by quantifying the percentage of cells that stained positive for hNIS within an area of interest using ImageJ (National Institute of Health).

2.2 Assessment of endothelial function after hNIS transduction

HUVECs (passage 4–8) with hNIS lentiviral transduction at MOI 0, 2, 5 and 10 were seeded onto multi-well plates at 25,000 cells/cm² for all EC functional assays. EC viability was evaluated using the MTT assay (Sigma-Aldrich, St Louis, MO), following the manufacturer's protocol. Nitric oxide (NO) production and reactive oxygen species (ROS) formation within cells were assessed using cell-permeable 5,6-diaminofluorescein diacetate (DAF-2 DA, Santa Cruz Biotechnology, Dallas, TX) and 2', 7'-dichlorodihydrofluorescein diacetate (DCF-DA) (Life Technologies, Carlsbad, CA), respectively. DAF-2 DA (10 µM in PBS) or DCF-DA (1 µM in PBS) was added to cells and incubated for 1 h at 37°C before reading with a fluorescence spectrometer (Ex 495 nm, Em 520 nm). All results were normalized with cell number per DNA concentration measured by Pico Green DNA quantification kit (Thermo Fisher Scientific, Waltham, MA).

EC inflammatory status was assessed using cell-based ELISAs for ICAM-1, VCAM-1 and E-selectin following the manufacturer's protocols (ScienCell™ Research Laboratories, Carlsbad, CA). Tissue factor expression was assessed by measuring human coagulation factor III with a tissue factor ELISA kit (R & D Systems, Minneapolis, MN) in EC lysates. Tissue plasminogen activator (tPA) expression in cell culture media was measured via active human tPA functional assay ELISA kit (Molecular Innovations, Novi, MI) according to the manufacturer's protocol.

EC thrombogenicity (MOI 0 and 5) was assessed using the re-calcified whole blood clotting assay. Cells were seeded in 12-well plates and bare tissue culture polystyrene served as a control surface. All cells were cultured until 100% confluency was achieved prior to the assay. Porcine whole blood was collected into Acid Citrate Dextrose (ACD) tubes (BD Biosciences, Franklin Lakes, NJ) from a local slaughterhouse and recalcified with 10% (v/v) 0.1 M CaCl₂. Immediately thereafter, 200 µL of recalcified blood was added to each well. At incremental time points (0, 10, 20 and 30 min), 3 mL of milli-Q water were added to each well for 5 minutes. The concentration of hemoglobin was measured for absorbance at 540 nm using spectrometer. The data were converted to percent clotting by normalizing to the maximum absorbance reading (0% clotted) and the minimum absorbance reading (100% clotted).

2.3 Preparation and evaluation of ePTFE tubes seeded with ECs expressing hNIS

Aeos® ePTFE tubing (5 mm inner diameter) was obtained from Zeus (Orangeburg, SC) as a model for ePTFE vascular grafts. POC prepolymer was synthesized with equal molar

concentrations of citric acid and 1,8 octanediol as previously described (Yang et al. 2004) and dissolved in ethanol. Prepolymer solutions at 0%, 2.5%, 5% and 10% (% polymer mass in ethanol) were perfused through ePTFE tubing using a syringe pump at 1 ml/min flow rate, followed by post-polymerization at 80°C for 4 days and acid leaching in PBS at 37°C for 3 days. The POC coating on ePTFE tubes was assessed with Toluidine blue staining as previously described (Hoshi et al. 2013).

HUVECs (MOI 0 and 5) were seeded onto the lumen side of POC-coated ePTFE tubes (3–3.5 cm long, 5 mm inner diameter) with various POC coating concentrations at a density of 100,000 cells/cm² initially, followed by a subsequent seeding of another 100,000 cells/cm² after overnight incubation. After a 5-day static culture, cells were lysed with 1% Triton X-100 and cell number was measured with Pico Green DNA quantification kit to evaluate the number of adhered cells.

The cell-seeded ePTFE tubes (10 cm long, 5 mm inner diameter) were also connected to a bioreactor system previously described by us (Allen et al. 2010) and subjected to fluid flow using a Masterflex L/S programmable pump (Cole-Parmer Model 7550-30, Vernon Hills, IL): (1) starting with 12 hours at a low flow rate of 50 ml/min representing a shear stress of ~0.9 dynes/cm², (2) followed by a constant shear stress ramp rate over 24 hours (0.38 dynes/cm² increase/hour) to reach 10 dynes/cm², and (3) holding at 10 dynes/cm² for 24 additional hours. Dextran (6 %) was added to EGM-2 to increase the viscosity of the fluid from 0.75 cp to 3.75 cp.

2.4 SPECT/CT imaging of the endothelium on ePTFE tubes

All SPECT images were acquired at the Center for Advanced Molecular Imaging (CAMI) of the Chemistry of Life Processes Institute at Northwestern University (Evanston, IL), using microSPECT, MILabs U-SPECT+/CT (MILabs, Netherlands). For all samples, the radioisotope, technetium-99m, in the form of technetium pertechnetate (Na[^{99m}TcO₄]) at 0.500 mCi was diluted using EGM-2 and added to each sample (petri-dish or ePTFE tube) for 60 min at room temperature. The samples were washed twice with PBS to remove excess radioisotopes prior to SPECT imaging. Cell-seeded or POC-coated petri dishes with 2 ml of EGM-2 per dish were imaged (4 dishes in a row). Cell-seeded or POC-coated ePTFE tubes were added to Eppendorf tubes containing EGM-2 and arranged to image 6–12 tubes simultaneously. The following parameters were used for data acquisition: 2 frames of 20 min each and General Purpose Rat and Mouse (GP-RM, 1.5 mm pinhole) collimator were used for SPECT (spatial resolution 0.25mm), followed by CT (60 kV, 615 μ A, normal gantry speed, 360 projections). The data were reconstructed using OS-EM with 4 subsets, 6 iterations (24 Maximum Likelihood – Expectation Maximization, or ML-EM equivalent) and post filtered with 0.8 full width at half maximum (FWHM) filter. The voxel size was 0.4 mm³. CT was used for attenuation correction. A calibration factor of 634.6 MBq/(CPM*mL) was used to convert counts per minute to actual activity.

The following samples were imaged: (1) *Cell seeded petri dishes*. On 35 mm petri-dishes, HUVECS at MOI 0, 2, 5 and 10 were seeded at a density of 10,000/cm² and cultured for 1 week to reach confluency prior to SPECT imaging. (2) *POC-coated ePTFE tubes*. ePTFE tubes (3–3.5 cm in length, 5 mm in diameter) coated with 0, 0.8, 2, and 3 mg/cm² POC were

incubated in radioisotope solution and scanned for a background signal due to the POC coating. (3) *Cell-seeded tubes under static culture*. HUVECs (MOI 0 and MOI 5) were seeded onto ePTFE tubes (3–3.5 cm in length, 5 mm in diameter) coated with 2 mg/cm² of POC as previously described and cultured for 5 days prior to SPECT imaging. (4) *Cell seeded tubes exposed to fluid flow*. HUVECs (MOI 5) were seeded onto ePTFE tubes coated with 2 mg/cm² (10 cm in length, 5 mm in diameter) and subjected to a flow schedule as described above. The tubes were cut into three pieces from the proximal to distal end and placed in Eppendorf tubes containing EGM-2 before SPECT/CT imaging.

2.5 Statistical analysis

All data were expressed as mean $\pm$ standard deviation. Data were analyzed using one-way ANOVA with a Tukey–Kramer post-test. For all comparisons, $p < 0.05$ was considered statistically significant.

3. Results

3.1 HUVECs expressing hNIS take up the radioisotope ^{99m}Tc and hNIS expression can be maintained throughout several passages without loss of endothelial phenotype

Transduction of hNIS in HUVECs resulted in the ectopic expression of hNIS on the cell membrane (Figure 1.A). hNIS expression increased as the lentiviral concentration increases from MOI 2 to MOI 10 (transduction efficiency at MOI 2: $21.9 \pm 4.6\%$, MOI 5: $76.2 \pm 8.9\%$, and MOI 10: $80.5 \pm 7.5\%$. Figure 1.A, C). Cells expressing hNIS took up the radioisotope ^{99m}Tc and were detected by SPECT imaging (Figure 1.B, D). Both the hNIS expression and the radioisotope uptake increased with increasing lentiviral concentration up to MOI 5, with no significant increase from MOI 5 to MOI 10. The hNIS expressing cells (MOI 2, 5 and 10) continued expressing hNIS from passage 4 to 9 (the entire time course of the study) after the one-time transduction at passage 3. Cells with hNIS ectopic expression continued to express mature endothelial markers such as CD31 and CD144 and maintained the typical endothelial cobble-stone morphology (Supplementary Figure S1). Throughout the entire cell culture, there were no signs of EC dedifferentiation or apoptosis, with EC morphology and phenotype well maintained when compared to control cells (MOI 0) at the same passage number.

3.2 hNIS expression on ECs does not affect key cellular functions

Lentiviral transduction of hNIS did not affect cell viability at low lentiviral concentrations (MOI 2 and 5) when compared to control cells (MOI 0). However, a significant decrease of $51.6 \pm 28.4\%$ ($p=0.0258$) in cell viability was observed for cells exposed to lentivirus at MOI 10, suggesting cytotoxicity at high viral concentrations (Figure 2.A). A significant decrease in nitric oxide production was observed within ECs exposed to lentiviral concentration at MOI 5 ($30.0 \pm 8.4\%$, $p<0.05$) and at MOI 10 ($41.3 \pm 18.1\%$, $p<0.05$) (Figure 2.B). No significant increase in the formation of ROS was observed with lentiviral transduction of hNIS at any lentivirus concentration (MOI 2, 5 and 10) (Figure 2.C). There was a small increase in ICAM-1 expression (Figure 2.E) (73.5 ± 7.7 pg/5,000 cells at MOI 0 to 86.4 ± 7.0 pg/5,000 cells for MOI 10, $p<0.05$), with no changes observed in VCAM-1 (Figure 2.D) or E-selectin (Figure 2.F) expression at any MOI. Given that lentiviral

concentration at MOI 10 resulted in significant impairment in cell viability and function when compared to control cells (MOI 0) with no substantial improvement in radiation signal enhancement (Figure 1. B.D), cells transduced with MOI 10 were eliminated from further investigation. MOI 5 was determined to be the optimal concentration for use in subsequent studies.

ECs with or without hNIS both express tissue factor (Figure 3.A) and secrete tPA (Figure 3.B) at similar concentrations (MOI 0 vs. MOI 5, $p>0.05$), indicating the retention of antithrombogenic and fibrinolytic functions after hNIS ectopic expression. The clotting kinetics of re-calcified whole blood on bare tissue culture plastic (TCP) and monolayers of HUVECs with and without hNIS expression showed a significant delay in the onset of clot formation in the presence of ECs (both MOI 0 and MOI 5) when compared to bare TCP (Figure 3.C). These data further demonstrate that the antithrombogenic activity of ECs expressing hNIS is preserved.

3.3 The use of POC to enhance cell adhesion is compatible with SPECT imaging

Coating ePTFE tubes with POC pre-polymer concentrations of 0%, 2.5%, 5% and 10% (% mass in ethanol) resulted in polymer surface densities of 0 mg/cm², 0.8 mg/cm², 2 mg/cm², and 3 mg/cm², respectively. POC-coated ePTFE tubes exhibited dark blue color on the lumen side with Toluidine blue staining, indicating the presence of carboxylic groups, as opposed to the white color on the bare ePTFE tubes (Figure 4.A). ECs expressing hNIS (MOI 5) seeded onto ePTFE tubes with various POC concentration showed significantly increased cell adhesion on the ePTFE tubes when POC coating density increased from 0 to 2 mg/cm² (Figure 4.B). Increased background signal in SPECT was observed with 2–3 mg/cm² POC when compared to bare ePTFE tubes (Figure 4.C). However, the SPECT/CT background signal due to surfaces coated with 2 mg/cm² POC, blank, and MOI 0 was negligible when compared to hNIS⁺ cell (MOI 5)-seeded tubes (Figure 5.A). Quantitative analysis of SPECT data shows that the background signals generated on POC-coated ePTFE tubes (2 mg/cm²) either without cells or with MOI 0 cells were significantly lower than the signals generated from POC-ePTFE tubes seeded with hNIS⁺ endothelial cells (MOI 5). (Figure 5. B)

3.4 SPECT imaging can be used to assess cell seeded vascular constructs in vitro

As a proof of concept, we assessed EC-seeded ePTFE tubes (10 cm in length, 5 mm in diameter, coated with 2 mg/cm² POC) (MOI 5) *in vitro*, after subjecting it to flow for 3 days. After exposing the cells to flow, the distribution of the cells was heterogeneous throughout the entire length of the tube. Most cells remained at the proximal region, however, cells detached from the middle and the distal regions of the tube (Figure 6.A). Quantitative analysis of cell distribution showed significantly higher radioisotope uptake at the proximal region, compared to the middle and distal regions (Figure 6.B). Histological assessment of the lumen side using phalloidin staining revealed a confluent cell layer at the proximal region (Figure 6. C), a reduced number of cells at the middle region (Figure 6. D), and no cells at the distal region (Figure 6. E) in agreement with the results obtained from SPECT.

4. Discussion

ECs are important to proper vascular function. The endothelialization of vascular grafts has been shown to significantly improve the clinical outcome of bypass procedures involving small diameter vascular grafts.(Meinhart et al. 2001),(Melchiorri et al. 2013),(Deutsch et al. 1999) In this study, HUVECs were chosen as a model EC because this cell line is readily available, easy to culture, and exhibits typical EC phenotype and functions. However, in clinical practice, patient-specific autologous ECs are needed for *in vitro* endothelialization to avoid immune rejection caused by allogeneic cells. Autologous ECs derived from subcutaneous veins,(Deutsch et al. 1999) peripheral blood-derived endothelial progenitor cells (EPCs)(Allen et al. 2010) and induced pluripotent stem cells (iPSCs)(Jiang et al. 2015b) are among the cell sources currently under investigation for endothelialization of vascular constructs. We expect the results of our study to be applicable to those efforts as our study demonstrates the feasibility of engineering ECs to express hNIS to enable their detection by nuclear imaging. This is a first step towards non-invasive imaging of an engineered endothelium *in vivo*.

Endothelial dysfunction, characterized by a reduction in nitric oxide bioavailability, an increase in ROS, and elevated inflammatory status, is a major mediator of cardiovascular diseases.(Cai and Harrison 2000) Therefore, it is important to ensure that the modification of ECs with hNIS to enable non-invasive imaging with SPECT does not negatively impact key endothelial functions. It is encouraging that hNIS transgene expression in ECs using lentivirus concentration at MOI 5 did not significantly impact cell viability, redox status, inflammatory status or antithrombogenic functions and only caused a 30% decrease in nitric oxide production. Given that the use of lentiviral vectors as an efficient tool for gene delivery has always been associated with a number of safety concerns such as mutagenesis and host immune responses (Thomas et al. 2003), the development of novel non-viral vectors for gene delivery will be necessary to overcome potential endothelial dysfunction caused by lentiviral infection. (Vasir and Labhasetwar 2006)

POC is a biodegradable elastomer that has been shown to be biocompatible,(Yang et al. 2006; Yang et al. 2004) antioxidant,(Jiang et al. 2015c; van Lith et al. 2014) and support endothelialization when coated onto ePTFE vascular grafts (Hoshi et al. 2013) and extracellular matrix (ECM)-based vascular tissues.(Jiang et al. 2015a) Therefore it was important to assess whether POC would have an impact on SPECT signal due to non-specific adsorption of the radioisotope. POC-coated surfaces exhibited concentration-dependent binding towards the radioisotope ^{99m}Tc that resulted in SPECT background signals. This increase in background signal due to non-specific binding may give rise to a false positive signal in SPECT. For instance, a significant SPECT background signal can be detected on petri dishes coated with 5 mg/cm² of POC in the absence of cells or in the presence of cells not expressing hNIS (Supplementary Figure S2). Therefore, the concentration of POC used for coating ePTFE tubes must be taken into consideration in order to improve EC adhesion without interfering with SPECT/CT imaging due to non-specific radioisotope binding.

The use of POC as a modifying agent for commercially available ePTFE vascular grafts and decellularized vascular tissue has been shown to improve graft endothelialization *in vitro*, reduce oxidative stress, and provide a mechanism to functionalize the graft with anticoagulant molecules.(Hoshi et al. 2013; Jiang et al. 2015a; Jiang et al. 2015c; Yang et al. 2006) The results of this study confirm that cells expressing hNIS adhere well to POC (MOI 5, Figure 4.B), similar to what has been observed with ECs that do not express hNIS.(Hoshi et al. 2013) When seeded within the lumen of POC-coated ePTFE tubes at the same density, ECs with or without hNIS expression attach to the surface and form a near-confluent endothelium (Supplementary Figure S3). However, the use of microscopy to assess cell coverage on the lumen side of the vascular constructs is destructive and is not amenable to continuous monitoring. Therefore, ectopic expression of hNIS in ECs seeded in the graft allows the non-destructive assessment of an engineered endothelium within a vascular construct both *in vitro* and *in vivo*.

This current study illustrates how SPECT can be used to detect inconsistencies in cell coverage on cell-seeded vascular constructs. The Aeos® ePTFE tubes (Zeus) used in this study, though similar to ePTFE vascular grafts as they have characteristic nodes and fibrils, are not medical grade vascular grafts for use in humans. Their wall thickness is significantly smaller than that of a ePTFE graft giving it the consistency of a tape and the node and fibril architecture is different. Therefore, EC retention in these tubes when exposed to fluid shear stress has to be further investigated. Nevertheless, the strategy described herein is a new tool to non-invasively assess an engineered endothelium within a vascular graft. Future work will focus on using this method to assess EC-seeded POC-ePTFE vascular grafts *in vivo*.(Yang et al. 2006)

We have previously described the use of iron oxide-based contrast agents to label ECs before seeding them onto POC-coated ePTFE grafts to assess a single layer of engineered endothelium with magnetic resonance imaging (MRI) technique.(Jiang et al. 2015c) We were able to distinguish the endothelium using MRI. However, MRI in combination with contrast enhancement does not provide any functional information on the status of cells. ECs with low viability that are attached to the vascular grafts would still be detectable by MRI as normal cells. Although the resolution of SPECT/CT is not high enough (0.25 mm spatial resolution) to distinguish a single layer of cells or provide any structural/anatomical data, it provides highly sensitive and quantitative information on the status of cells used to engineer a neoendothelium. Other imaging modalities for cell tracking such as optical imaging based on fluorescence (Hadjantonakis and Papaioannou 2004) or bioluminescence(Wang et al. 2003) may provide quantitative information on metabolic function of cells, with high sensitivity. However, they are typically limited by the depth and volume that can be reached and therefore cannot be used to assess vascular grafts implanted beyond subcutaneous tissue. (Sutton et al. 2008) Furthermore, optical imaging is two dimensional, which further limits assessment of live cell tracking. The future of live cell tracking for vascular tissue engineering will most likely combine multiple imaging modalities for the assessment of cell-seeded vascular grafts *in vivo*.

5. Conclusion

We report, for the first time, the feasibility of a non-invasive, highly sensitive and functional assessment of an engineered endothelium in ePTFE tubes using a combination of SPECT/CT imaging and hNIS ectopic expression in ECs. This methodology may also provide a novel imaging tool for regenerative medicine and other cell-based therapy where non-invasive, live cell tracking is desirable.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1.

(A) Immunofluorescence staining for hNIS (red) expressed on HUVECs transduced with lentivirus at various MOIs. Scale bar = 100 μ m. (B) SPECT imaging of radioisotope uptake by HUVECs transduced with hNIS at various MOI. Purple dash lines indicate the outline of cell-seeded 35 mm petri dishes. (C) Quantitative analysis of hNIS lentiviral transduction efficiency based on immunofluorescence images (n=4). (D) Quantitative analysis of radioisotope uptake by cells seeded on 35 mm petri dishes (n=4). $p < 0.05$ indicates a statistically significant difference.

Figure 2.

Cellular functions of HUVECs transduced with hNIS. (A) MTT assay shows a significant decrease in cell viability at MOI 10 (n=4). (B) DAF2-DA assay shows a significant decrease in nitric oxide production at MOI 5 and 10 (n=4). (C) DCF-DA assay shows no significant difference in ROS formation (n=4). (D) Cell-based ELISA shows no significant difference in VCAM-1 expression (n=3). (E) Cell based ELISA shows a significant increase in ICAM-1 expression at MOI 10 (n=3). (F) Cell based ELISA shows no significant difference in E-selectin expression (n=3). $p<0.05$ indicates a statistically significant difference, whereas n.s. indicates no significant difference per ANOVA.

Figure 3.

Impact of hNIS transduction of EC on their antithromobogenic properties. (A) Tissue factor expression in ECs shows a slight decrease with no statistically significant difference (MOI 0 vs. MOI 5, $p > 0.05$) ($n=3$). (B) Secreted tissue plasminogen activator (tPA) from ECs shows slight increase with no statistically significant difference (MOI 0 vs. MOI 5, $p > 0.05$) ($n=3$). (C) Kinetics of whole blood clotting show that surfaces covered with EC (MOI 0 and MOI 5) inhibit clot formation when compared to tissue culture plastic (TCP) ($n=3$). N.s. indicates no significant difference per ANOVA. * indicates $p < 0.05$ whereas *** indicates $p < 0.01$.

Figure 4.

Impact of the concentration of POC (0, 0.8, 2, and 3 mg/cm²) coated on ePTFE tubes on cell adhesion and SPECT signal. (A) Toluidine blue staining shows increased blue color density on the lumen side with increasing POC coating concentration, indication the successful addition of the polymer to the tubes. (B) Cell number analysis based on DNA concentration shows increased adhesion of ECs (hNIS+, MOI 5) with increasing POC coating concentration. (C) Background signal in SPECT increases as POC coating concentration increases. $p < 0.05$ indicates a statistically significant difference per ANOVA.

Figure 5. SPECT/CT assessment of POC-coated ePTFE tubes (Blank) and ePTFE tubes seeded with EC (MOI 0 and MOI 5) cultured for 5 days. (A) SPECT/CT imaging shows very little background signal from POC-coated ePTFE tubes and tubes seeded with ECs (MOI 0). (B) SPECT data demonstrating significantly higher ($p < 0.05$) radioactive signal from tubes seeded with ECs expressing hNIS ($n=3$).

Figure 6.

Use of SPECT/CT to assess cell retention and distribution on ePTFE tubes exposed to flow.

(A) SPECT/CT imaging at cross sections show stronger signals from the proximal region.

(B) Quantification of the SPECT data shows a significantly higher ($p < 0.05$) radioactivity in the proximal region of the tube relative to middle and distal regions.

(C-E) Microscopic assessment of phalloidin stained cells on the lumen side of ePTFE tubes at the proximal (C), middle (D) and distal (E) regions.