Cardiac Stem Cell Imaging

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민 정 준

Unresolved Issues of Stem Cell Study

- What is the optimal cell type, cell dosage, and delivery route?
- Who are the ideal patient populations?
- How long do cells survive after transplant?
- Do these cells integrate, proliferate, and differentiate?
- Can these physiologic processes be monitored *in vivo* by noninvasive imaging?

2000 National Heart Lung & Blood Institute Workshop

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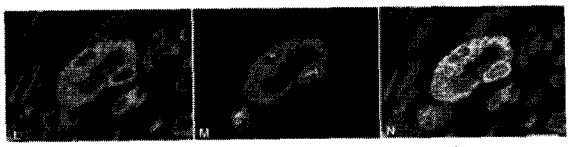
Conventional Methods of Tracking Cells



GFP | Antibody to GATA-6 | Overlay Image

**BMSC from transgenic mice (eGFP) injected into recipient mice with LAD infarction. Survival of transplanted cells identified by eGFP on fluorescence microscopy and differentiation into endothelial cells or smooth muscle cells by respective antibody stainings.

Katstura J et al, Circ Res 2005

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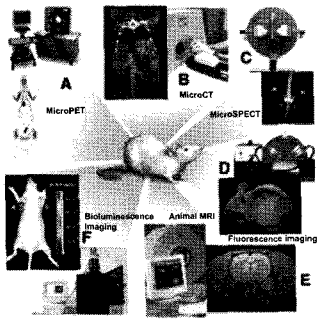
Newer Methods of Tracking Cells



Massoud T & Gambhir S. Genes & Dev 2003

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Ideal Imaging Modality

1. Biocompatible, safe, and nontoxic
2. Least perturbation to the stem cell
3. Detailed anatomic location
4. Quantification of cell survival
5. No signal dilution with cell proliferation

**Comparison of radionuclide, ferromagnetic, and reporter gene approaches

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First Approach, Radionuclide Imaging (1)

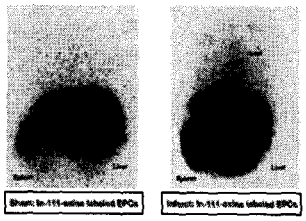
- Many radiotracers are FDA approved
- Many years of imaging experience by nuclear medicine specialists using clinical SPECT and PET scanners
- Tomographic resolution, functional & metabolic assessment of varying organs

Radionuclide Labeling

- Culture stem cells *ex vivo*
- Incubate cells with radioactive compound (eg, ^{111}In, ^{123}I, ^{99m}Tc).
- Labeling efficiency is ~90% with ^{111}In. Labeling retention is only ~50% after 1 hour and ~25% after 48 hours
- Inject cells directly in the specific organ or intravenously to study stem cell homing.

Scintigraphic Pinhole Imaging

Human endothelial progenitor cells in rat MI model



Aicher A et al, Circulation 2003

Limitations

1) Half-life of [^{111}In]-indium oxine is 2.8 days; can follow cells for 5-7 days at most.

2) Poor retention rate of radiotracers to cells. *Leakage of ^{111}In from* labeled cells can account for radioactivity signals in the liver and kidney.

Second Approach, Magnetic Resonance Imaging (2)

- Superparamagnetic particles are relatively safe, non-toxic, and biocompatible.
- Many years of imaging experience by radiologists using clinical MR scanners.
- Detailed anatomic resolution, myocardial perfusion and wall motion.

Ferromagnetic Labeling

- Culture stem cells *ex vivo*
- Incubate cells with Feridex® (mixture of SPIO and lipofectamine transfection agent). Endocytosis results in magnification of T2 MR contrast.
- Amount of cellular iron uptake is ~15 pg Fe per cell. Cell viability is ~95% by trypan blue exclusion.
- Inject cells directly or under X-ray fluoroscopy

Cardiac 1.5T MR Imaging

Porcine mesenchymal stem cells labeled with Feridex for 24-48 hours. Injected intramuscularly into LAD artery infarcted pigs under X-ray fluoroscopy guidance.

Kraitchman DL, et al. Circulation 2003

Potential Issues

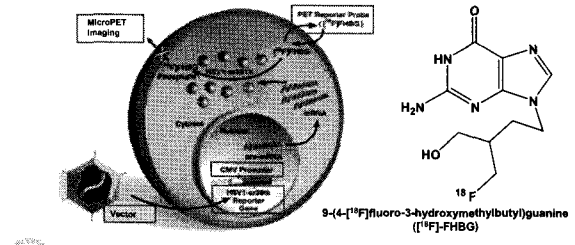
- Ferromagnetic particles inside the stem cells still register a MR signal even if cells are dead.
- Engulfment of dead cells with Feridex by tissue macrophages leads to signal degradation after 1-2 weeks.
- Intracellular Feridex is a fixed physical quantity so dilution of MR contrast with cell divisions.
- Susceptibility artifact causes difficulty for signal quantitation by MR.

Third Approach, Reporter Gene Labeling (3)

- Culture stem cells *ex vivo*.
- Stably transfect or transduce cells with reporter genes by plasmids or lentivirus.
- Reporter probe needs to be injected prior to each imaging session
- Transfection efficiency is ~20-40% with lentivirus. No adverse effects on cell proliferation rate but more rigorous studies are needed.

Imaging Reporter Gene Approach for PET

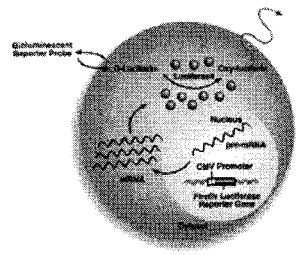
HSV1-tk / FHBG (Reporter Gene / Probe) System



Wu J et al, J Nuclear Cardiology 2004.

Imaging Reporter Gene Approach for Optical Imaging

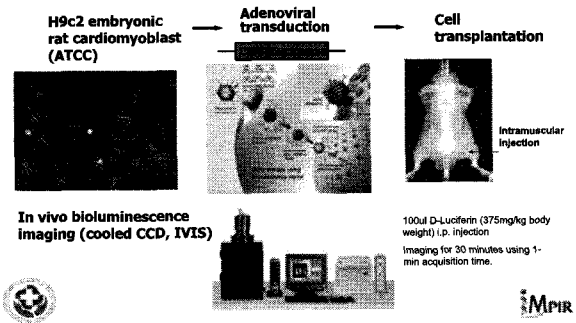
Firefly Luciferase / D-Luciferin Reporter Gene/Probe System



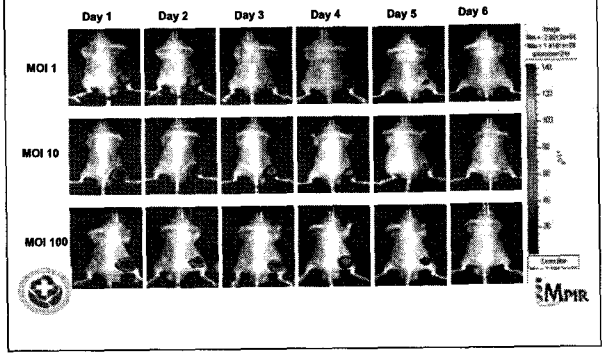
Multiplex System

- **Vector:** plasmid, lentivirus, adenovirus, AAV, gutless adenovirus, electroporation
- **Promoter:** constitutive (CMV), tissue specific promoter (MLC_{2v}), inducible (Tet-on)
- **Reporter gene:** HSV1-sr39tk, sodium iodide symporter, transferrin, firefly luciferase,
- **Reporter probe:** $[^{18}F]$-FHBG, $[^{99m}Tc]$-pertechnetate, iron oxide, D-luciferin
- **Imaging modality:** PET, SPECT, MRI, optical

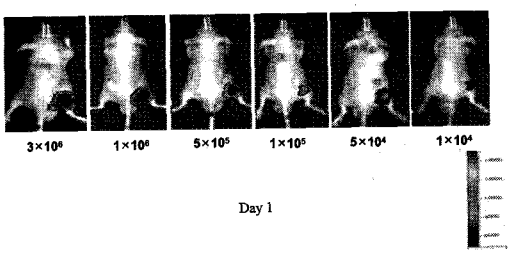
Intraskeletal Muscular Implantation of Cardiomyoblast



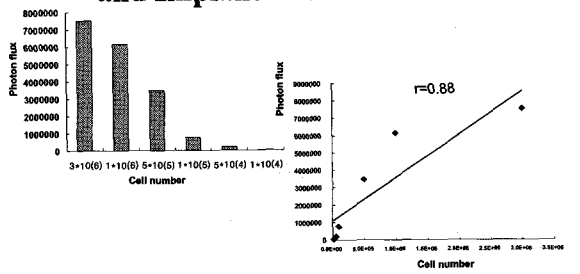
Signal Intensity according to MOI



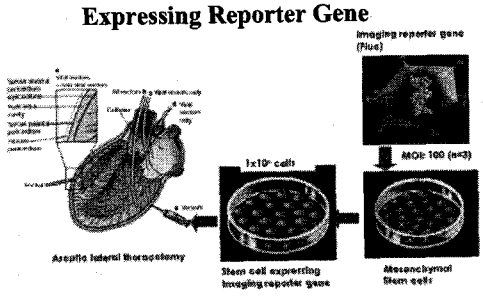
Signal Intensity according to Implanted Cell Numbers



Correlation between Signal Intensity and Implanted Cell Numbers



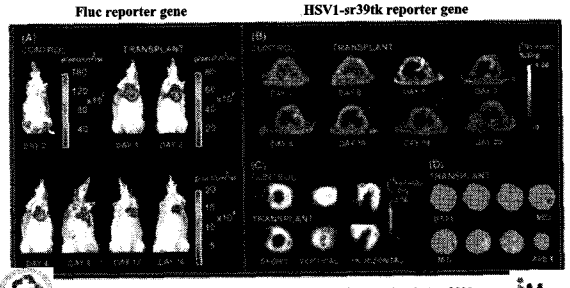
Cardiac Transplantation of Stem Cell Expressing Reporter Gene



Bioluminescence & MicroPET Imaging

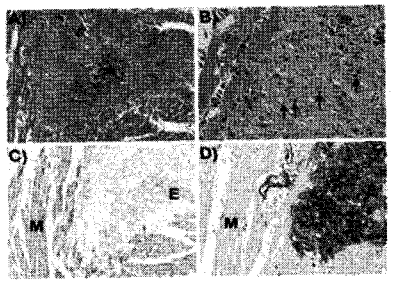
Embryonic H9c2 cardiomyoblasts in rat model

Fluc reporter gene HSV1-sr39tk reporter gene



Wu JC, Chen I, Min JJ et al, Circulation 2003

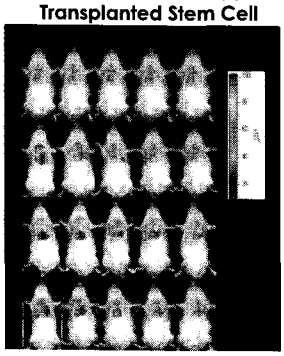
Immunohistochemistry



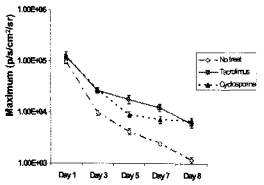
A) H&E (low)
B) H&E (high)

C) troponin
D) α-SMA.

Effect of Immunosuppressive Agents on Survival of Transplanted Stem Cell

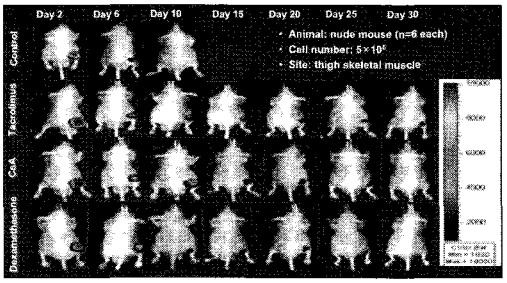


Human Umbilical Cord Blood derived Mesenchymal Stem Cells (1x10⁶)
Adenovirus mediated Fluc expression (MOI=100)
A: negative control
B: To Tx
C: Tacrolimus (1mg/kg/d)
D: Cyclosporine (5 mg/kg/d)

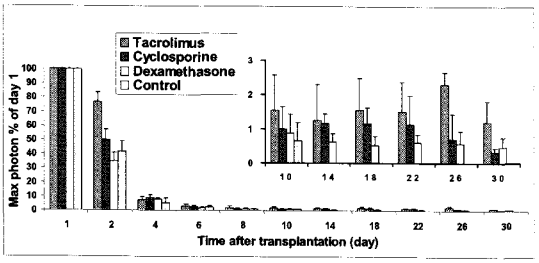


Min JJ et al. *Ann Nucl Med* in press

Effects of tacrolimus, cyclosporine, and dexamethasone on cell survival



Immunosuppressive therapy in nude mice



Imaging Murine Embryonic Stem Cell Transplantation in Living Mice

Beating Cardiomyocytes | Fluorescence microscopy | IVIS imaging

**Lentiviral transduction of murine ESC with ubiquitin promoter driving eGFP and firefly luciferase. Advantage of ESC is their robust self-renewal and pluripotency which allows isolation of differentiated cardiomyocytes

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Summary

	Radiolabeling Labeling	Ferromagnetic Labeling	Reporter Gene Labeling
Biocompatible, safe, non-toxic	++	++	?
Least perturbation to stem cells	++	++	?
Detailed anatomic location	++	+++	++
Quantification of cell survival	?	?	+++
No dilution with cell proliferation	?	?	+++

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Perspectives of Stem Cell Imaging

Noninvasive imaging can evaluate important parameters relevant to clinical protocols

- Maximal local delivery (% injected cells) to the area of interest
- Long term survival of the delivered cells
- Optimal cell type, dosage, routes of administration
- Efficacy of repeated interventions
- Screening for pharmaceutical agents capable of prolonging cell survival

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