Cell Tracking and Imaging Technologies for CNS Regenerative Medicine Purposes

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Comparison

Imaging Modalities

Cells Surface Engineering

Introduction

Content

Translation Cell Therapies into Clinical

Repeatedly Noninvasively

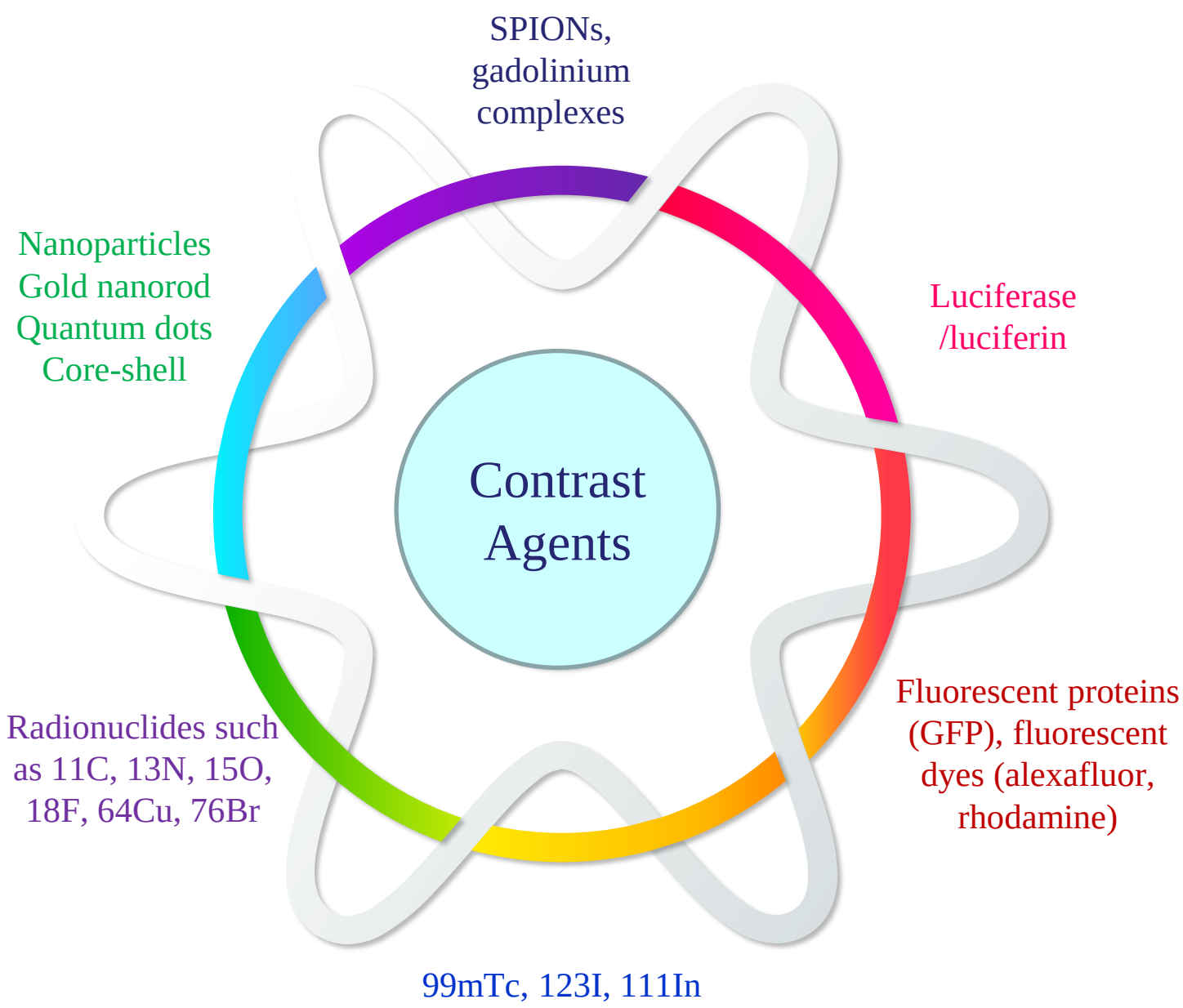
High Sensitivity Of Imaging Agent

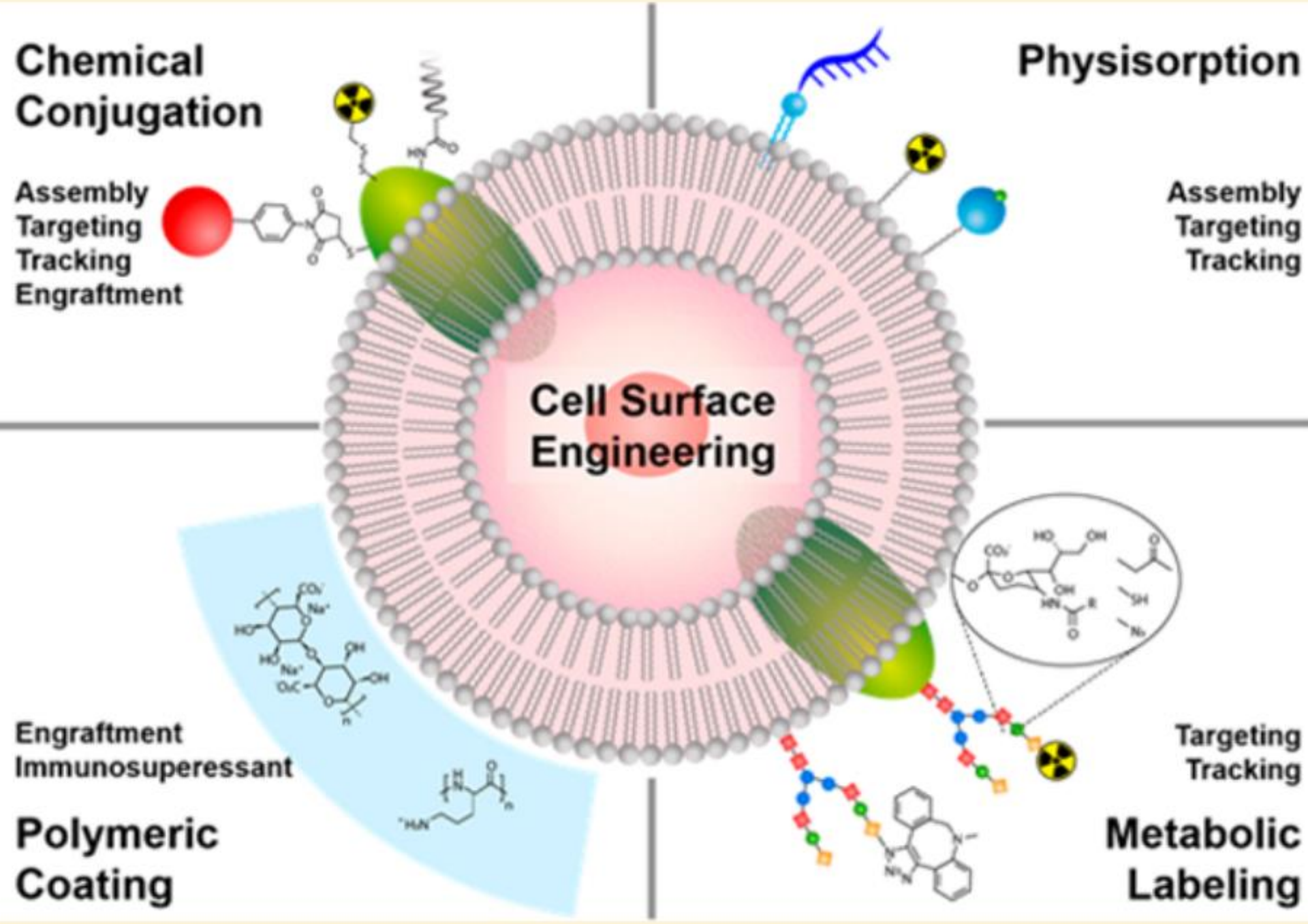
Final Distribution Longitudinally

Able To Image Deep Tissues high resolution

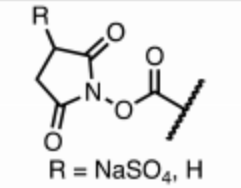
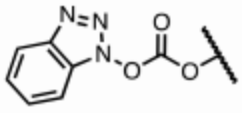
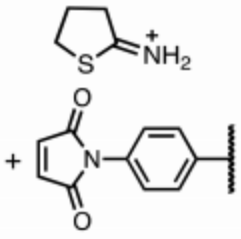
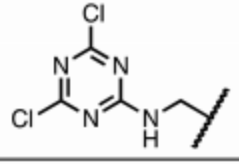
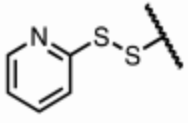
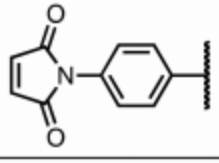
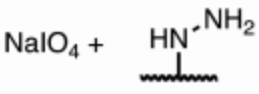
Migration Pathway

Tracking for a long time & very fast image acquisition


SPIONs, gadolinium complexes
Nanoparticles Gold nanorod Quantum dots Core-shell
Luciferase /luciferin
Contrast Agents
Radionuclides such as 11C, 13N, 15O, 18F, 64Cu, 76Br
Fluorescent proteins (GFP), fluorescent dyes (alexafluor, rhodamine)
99mTc, 123I, 111In

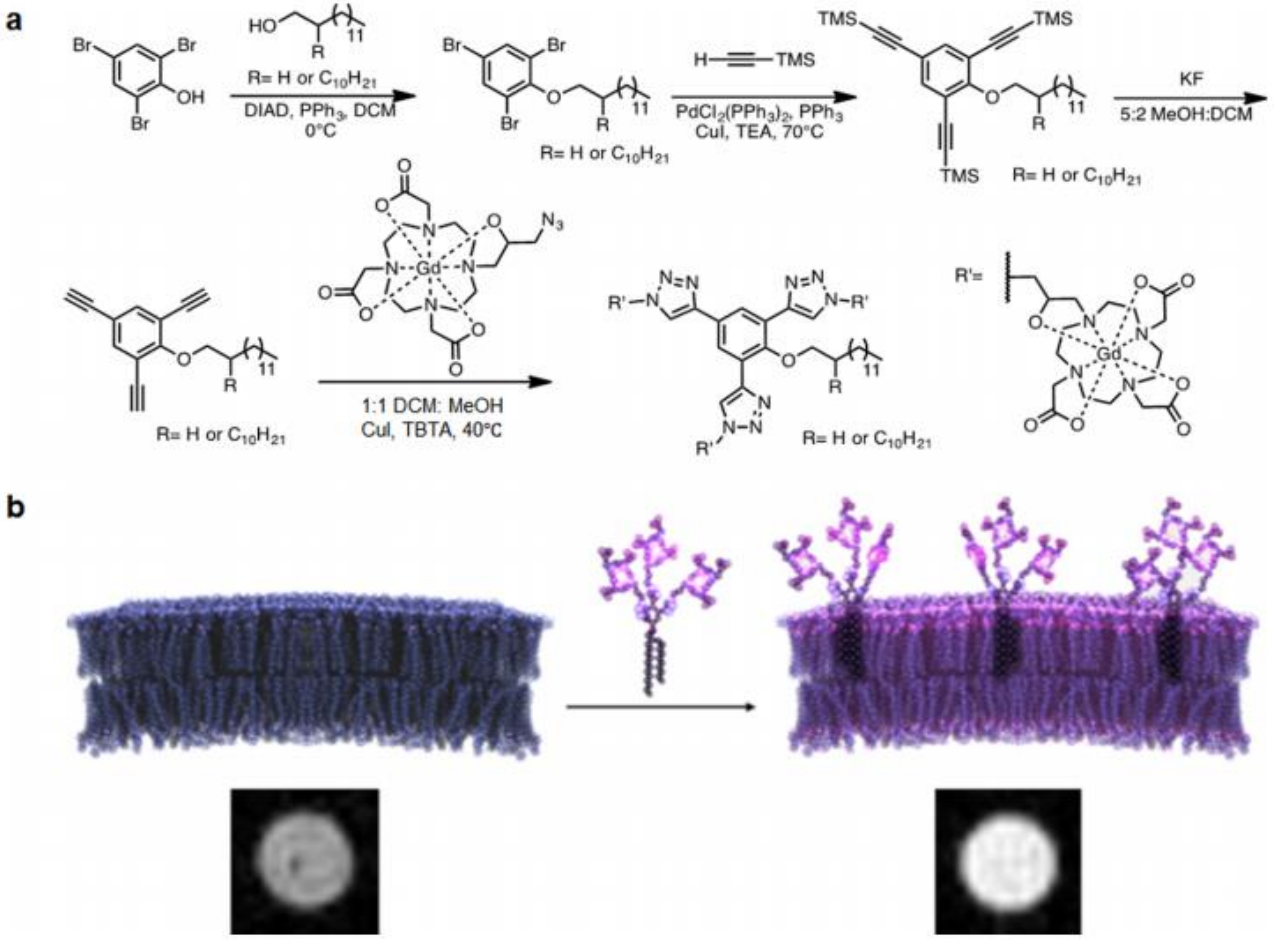


Engineering the Surface of Therapeutic "Living" Cells, Chem. Rev. 2018, 118, 1664−1690

ll Surface unctional Group	Cross-linker	Reaction Conditions	Benefits/ Limitations	Ref.
Native Amines	 R = $NaSO_4$, H	Incubation with NHS ester in pH 8.0 for 20 min - 1 hr at room temperature to 37 °C	Most common conjugation method Stable amide bond formation Slower reaction than cyanuric chloride	44,131,133, 136,138,144, 145,183,207
		Incubation with benzotriazole carbonate in pH 8.0 for 1 hr at room temperature	Stable amide bond formation Slower reaction than cyanuric chloride More reagent required	145
		Incubation with 2-iminothiolane and malimidophenyl in pH 7.4 for 2 hr at room temperature	Iminothiolane can reach buried amine groups Provides a 8 Å extension away from surface making attachment of bulky groups possible Will not alter net surface charge of functionalized surface	146
		Incubation with cyanuric chloride in pH 8.7 for 20 – 30 min	Highly reactive, shorter reaction times Introduction of bulky triazine group Possible over functionalization	143 - 5
Native Thiols		Incubation with 2-pyridyl-dithio group for 4 hr at 37 °C	Close to quantitative conjugation High cell viability (~ 85%) Limited to the number of exofacial protein thiols (cell dependent) Possible internalization of conjugated group	78
		Incubation with malimidophenyl group for 30 min at 37 °C	Cellular function maintained Limited to the number of exofacial protein thiols (cell dependent) Possible internalization of conjugated group	185
Glycans		Incubation with $NaIO_4$ for 15 min at 4 °C followed by Incubation with hydrazine linker for 30 min	Limited cytotoxicity ≤ 20% Maintained cell proliferation High incorporation of ligand	197-9

Gadolinium Contrast Agents

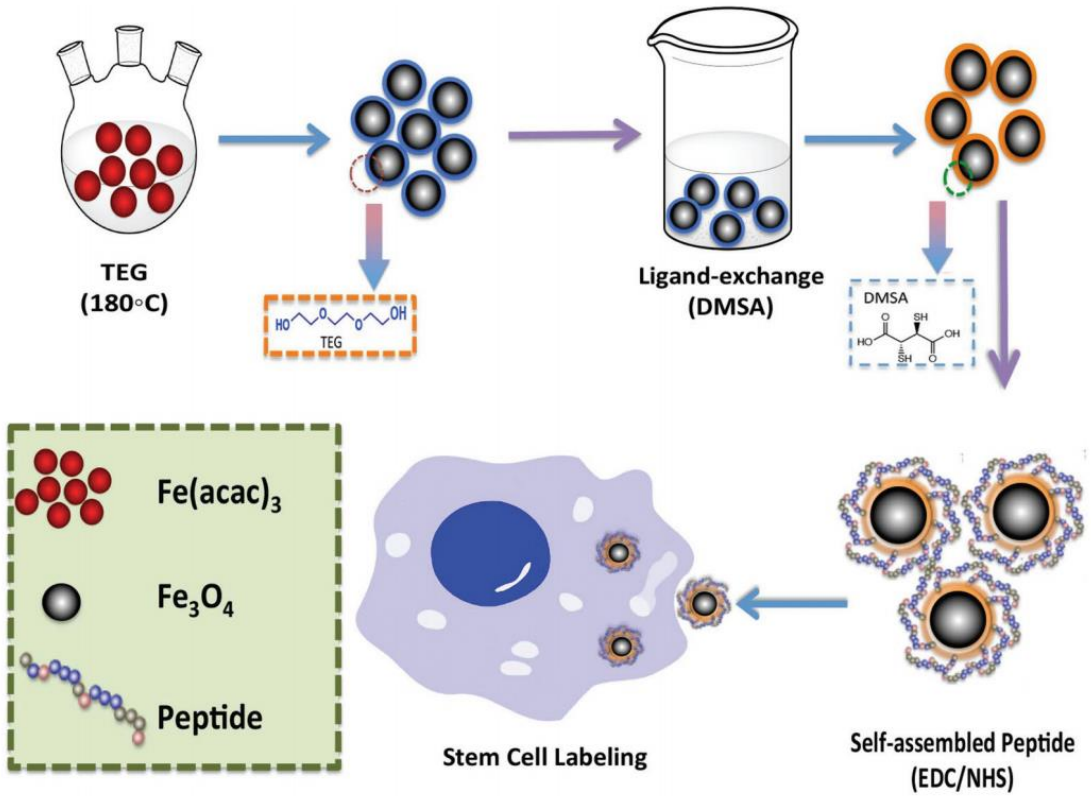
- ✓ **Intracellular Entry** With **Transfection** Agents or **Gadolinium-loaded Nanoparticles**.
- ✓ **Cell Surface** Immobilization



Cell Labeling Via MembraneAnchored Lipophilic Mr Contrast Agents. Bioconjugate Chem. 2014

Superparamagnetic Iron Oxide Nanoparticles

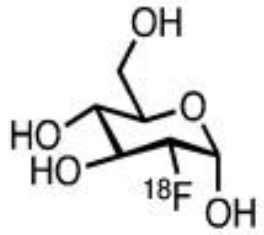
- ✓ Self-assembled Peptide Amphiphile (PA)
- ✓ PA Conjugated to the Surfaces of SPIONS to Label Rat (MSCS)



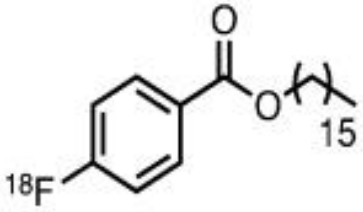
Stem cell tracking using effective self-assembled peptide-modified superparamagnetic nanoparticles, Nanoscale, 2018

^{18}F-radionuclide-based radiolabeling agents

- ✓ ^{18}Ffluorodeoxy-$_{D}$-glucose (^{18}F-FDG) and hexadecyl 4-[^{18}F]- fluorobenzoate (^{18}F-HFB).
- ✓ ^{18}F-HFB is designed to intercalate into cell surface membrane.



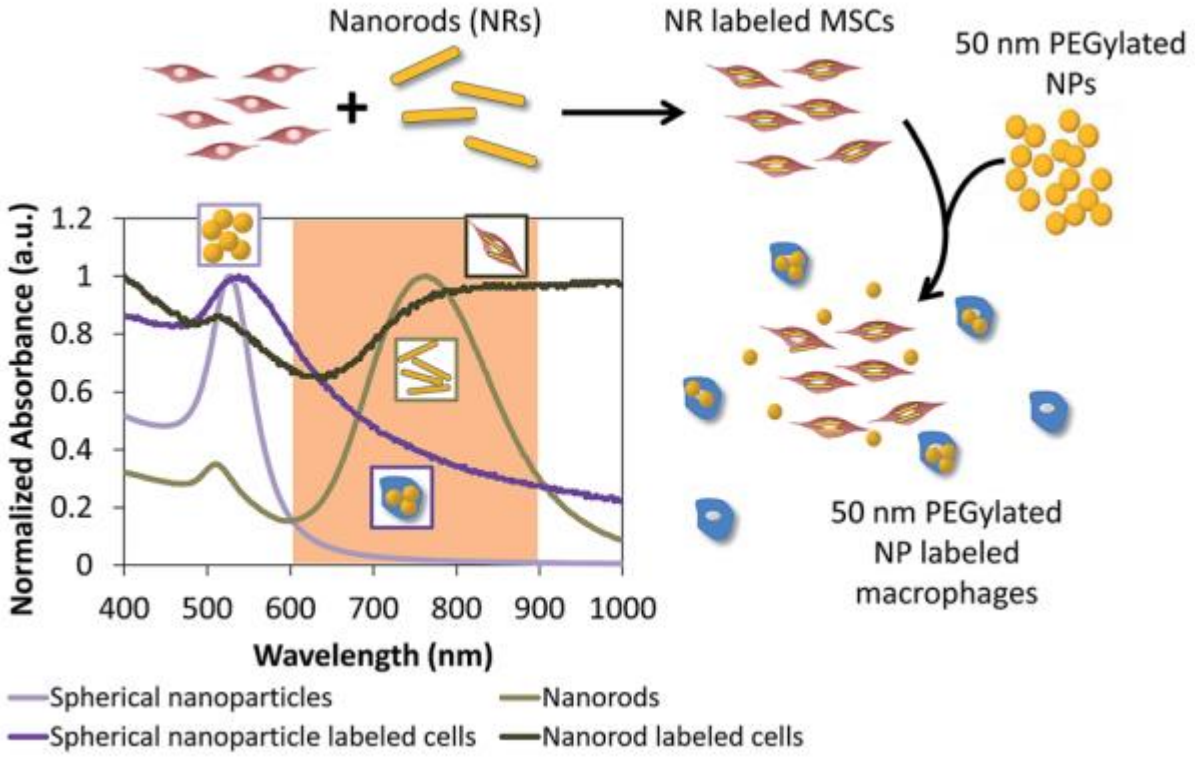
^{18}F-fluoro-deoxy-D-glucose (^{18}F-FDG)



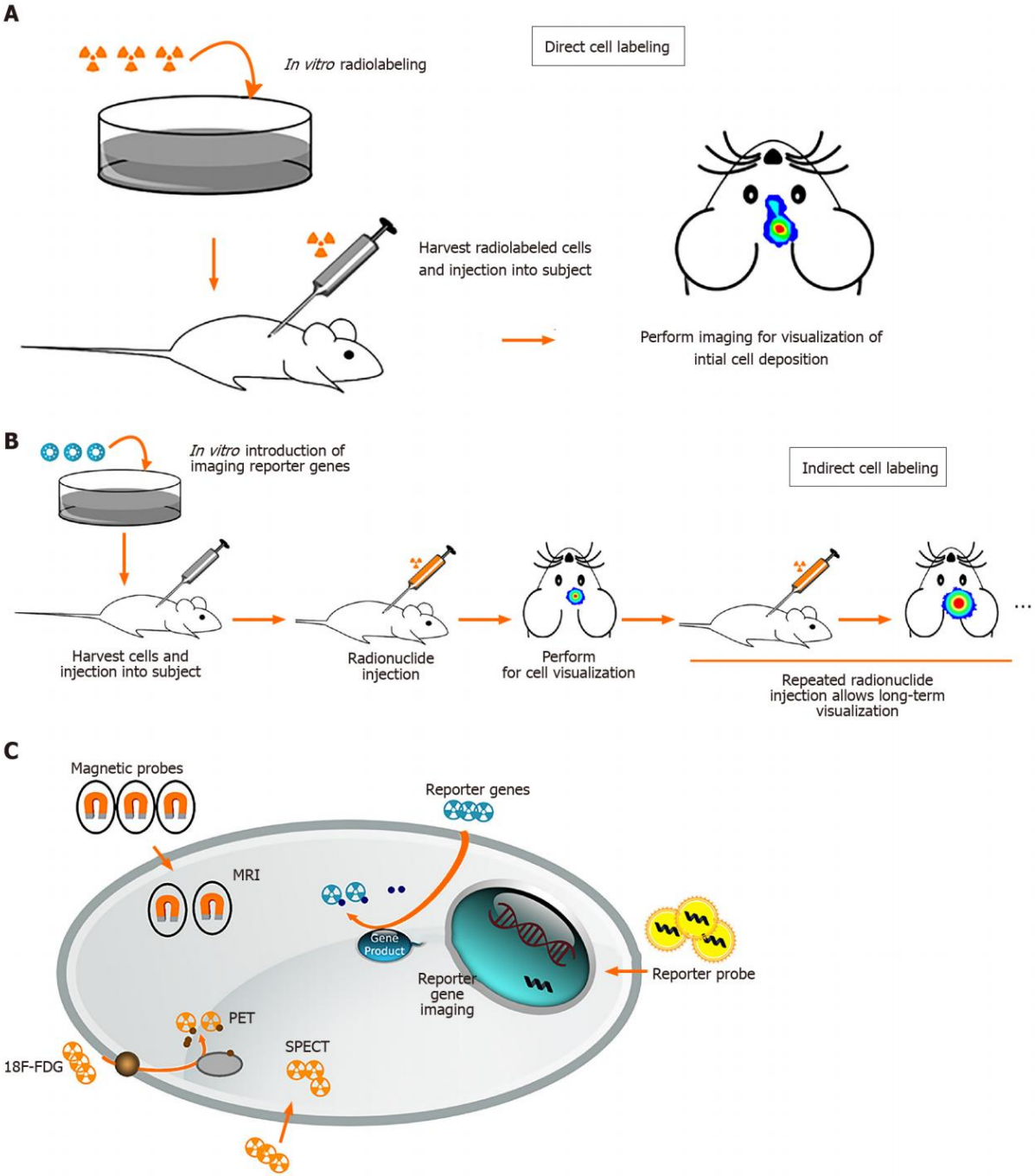
hexadecyl-4-[^{18}F]fluorobenzoate (^{18}F-HFB)

Gold nanoparticles

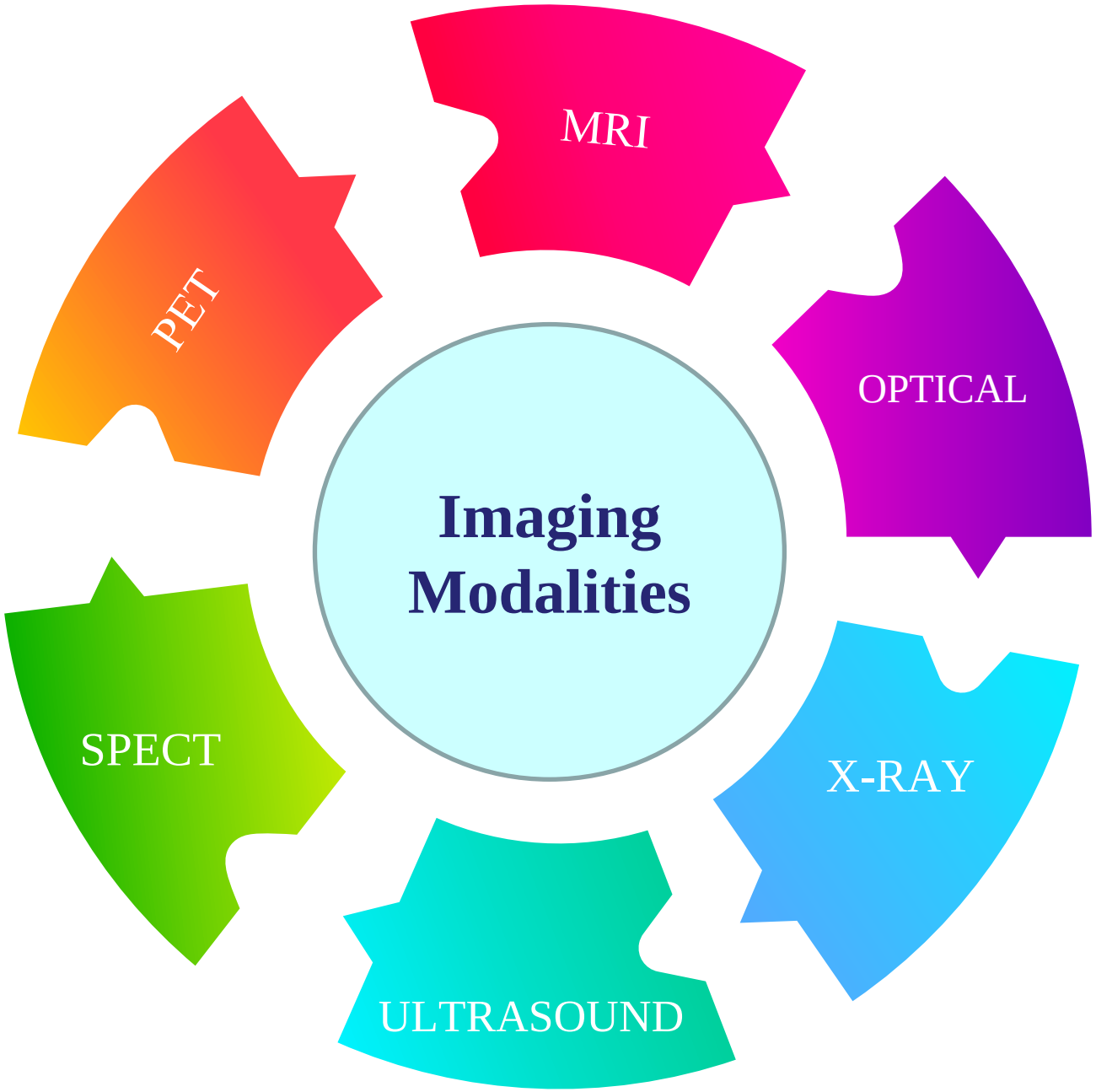
- ✓ Dual Gold Nanoparticle System Capable of Monitoring Both Delivered Stem Cells And Infiltrating Macrophages Using Photoacoustic Imaging.
- ✓ Tetraethyl Orthosilica (TEOS) & A Layer Of Poly-l-lysine



A dual gold nanoparticle system for mesenchymal stem cell tracking. Nanoscale, 2018



Cell tracking


MRI
OPTICAL
X-RAY
ULTRASOUND
SPECT
PET
Imaging Modalities

MRI

Monitoring cell death is still a challenge, Low sensitivity, agent dilution

MRI-guided cell injections in real time & temporal resolution on the order of a few seconds

Gadolinium delivered with labeled cell transplants (up to 10 pg gadolinium per cell per individual but 2.27 g for a 70-kg individual

Superior delineation of morphology, no exposure to radiation, monitoring over long periods of time

In the first study, the sensitivity of detection for a 3.0-T clinical imager was determined 1% of the total number of injected cells.

In the second study, patient-derived neural stem cells were labeled with Feridex and injected into the temporal lobe of a patient with brain trauma.

The third study, used autologous CD34+ bone marrow stem cells, which were injected into the spinal cord by means of a lumbar puncture.

Other clinical studies SPIO-labeled therapeutic cells can be detected in the spinal cord and occipital horns of patients with multiple sclerosis, amyotrophic lateral sclerosis and neonatal ischemic hypoxia.

Table 1: Clinical MRI Cell Tracking Case Studies

Study and Year of Publication	MRI Nucleus	MRI Label	Adjunct Labeling Agent	Cell Type	Disease Target	No. of Patients
de Vries et al (12), 2005	^{1}H	Endorem	…	Dendritic cells	Melanoma	8
Zhu et al (13), 2006	^{1}H	Feridex	Effectene	Neural stem cells	Brain trauma	1 (plus one control subject)
Callera and de Melo (14), 2007	^{1}H	Dynabeads	Anti-CD34 monoclonal antibody (mab)	Hematopoietic bone marrow stem cells	Spinal cord injury	10 (plus six control subjects)
Toso et al (15), 2008	^{1}H	Resovist	NA	Pancreatic islet cells	Type 1 diabetes	4
Saudek et al (16), 2010	^{1}H	Resovist	NA	Pancreatic islet cells	Type 1 diabetes	8
Karussis et al (17), 2010	^{1}H	Feridex	NA	Mesenchymal stem cells	Multiple sclerosis and/or amyotrophic lateral sclerosis	9 (plus 39 control subjects)
Jozwiak et al (18), 2010; Janowski et al (19), 2014	^{1}H	Endorem	NA	Umbilical cord blood–derived neural progenitors	Neonatal ischemic brain injury	1
Richards et al (20), 2012	^{1}H	Endorem	Protamine sulfate	Peripheral blood mononuclear cells	Healthy volunteers and/or cutaneous inflammation model	20
Ahrens et al (21), 2014	^{19}F	CS-1000	NA	Dendritic cells	Colorectal adenocarcinoma	5
Malosio et al (22), 2015	^{1}H	Endorem	NA	Pancreatic islet cells	Type 1 diabetes and/or pancreatitis	4
Rose et al (23), 2015	^{19}F	CS-1000	NA	Stromal vascular fraction cells	Radiation-induced fibrosis	NA (in progress)

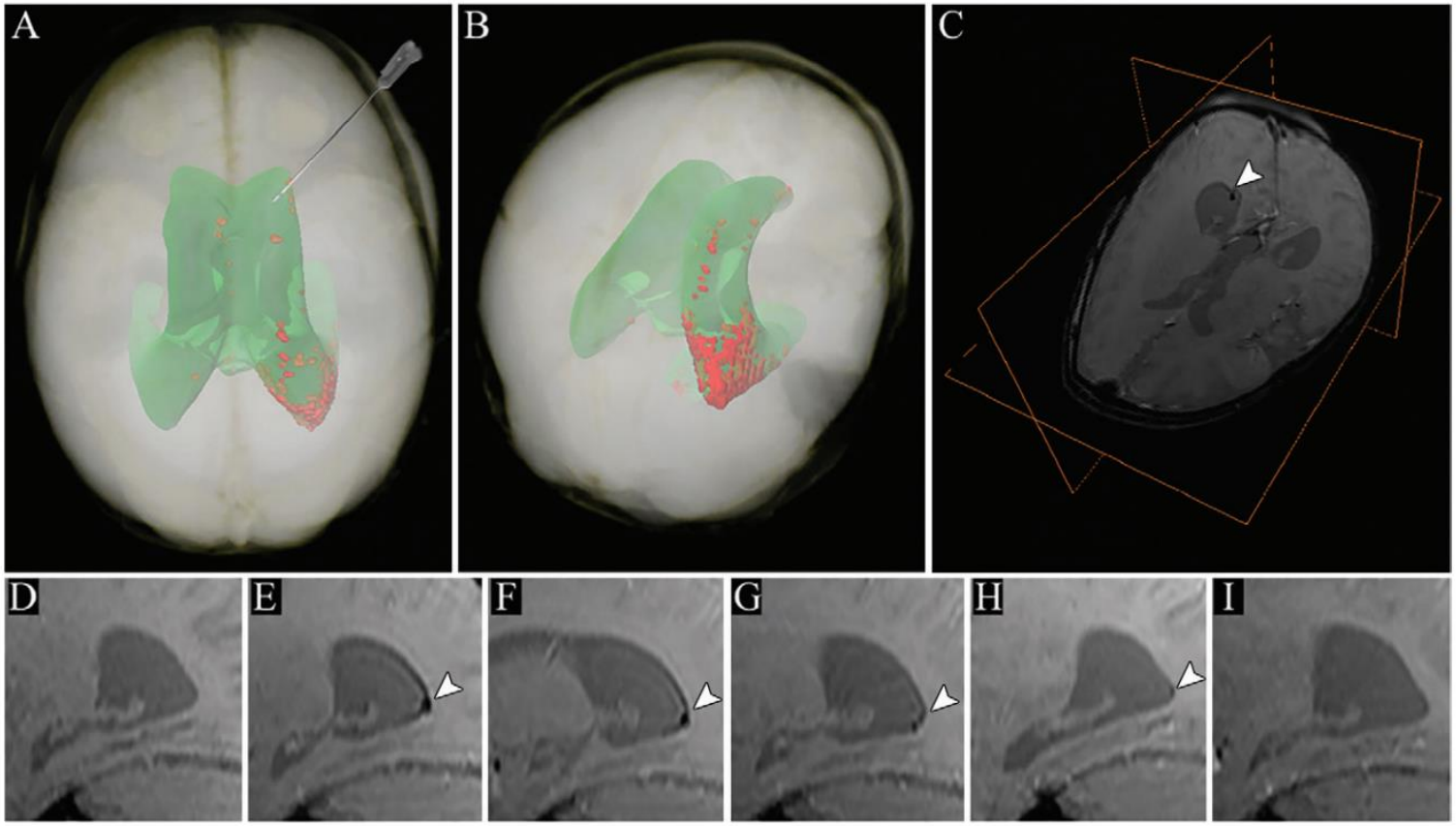
Note.—^{19}F = fluorine 19, ^{1}H = hydrogen 1, NA = not applicable.

Clinical Tracking of Cell Transfer and Cell Transplantation: Trials and Tribulations, Radiology 2018

Superparamagnetic iron oxide (SPIO)–labeled autologous cord blood derived cells were injected in patient with global cerebral ischemia.

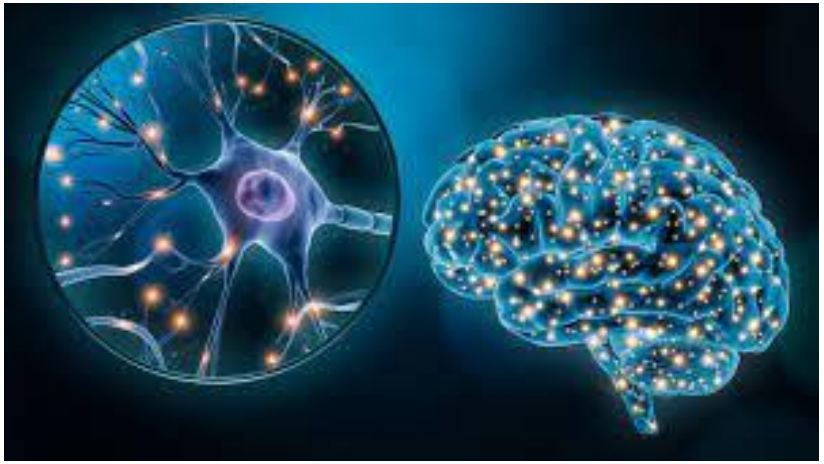
Volume rendering of MRI data of patient's head obtained 24 hours after transplant.

Ventricular system (green) and cell-derived SPIO signal within occipital horn of right ventricle (red).



Clinical Tracking of Cell Transfer and Cell Transplantation: Trials and Tribulations, ***Radiology 2018***

New Drug approval for a first-inhuman use of **ferumoxytol to label neural stem cells** before transplant into patients with brain tumor. However, **a few clinical trial** using ferumoxytol-labeled stem cells has **been reported on.**

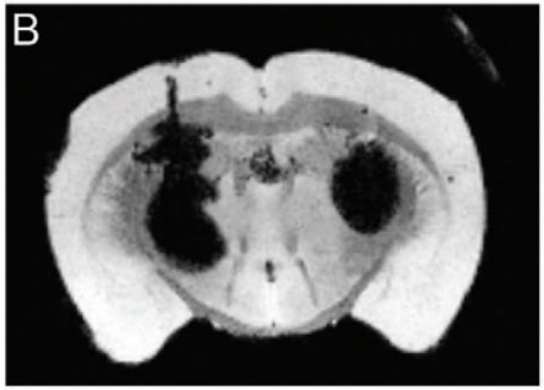


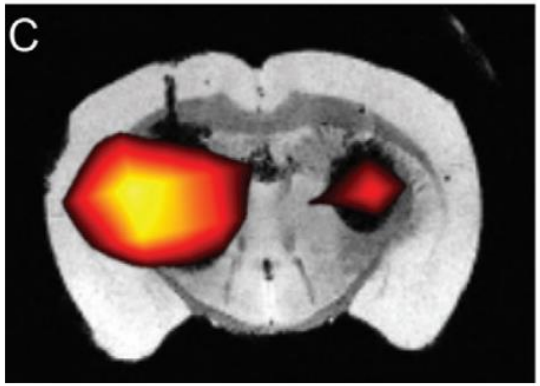
Tribulations

- SPIO-based MRI Cell Tracking Cannot Report Directly on Cell Viability
- SPIO-based MRI Cell Tracking Is Not Inherently Quantitative
- SPIO-based MRI Cell Tracking Cannot Be Used for Long-term Tracking of Rapidly Dividing Cells and in Areas of Hemorrhage and/or Traumatic Injury
- Spio-based MRI cell tracking cannot help differentiate labeled cell transplants from invading host macrophages

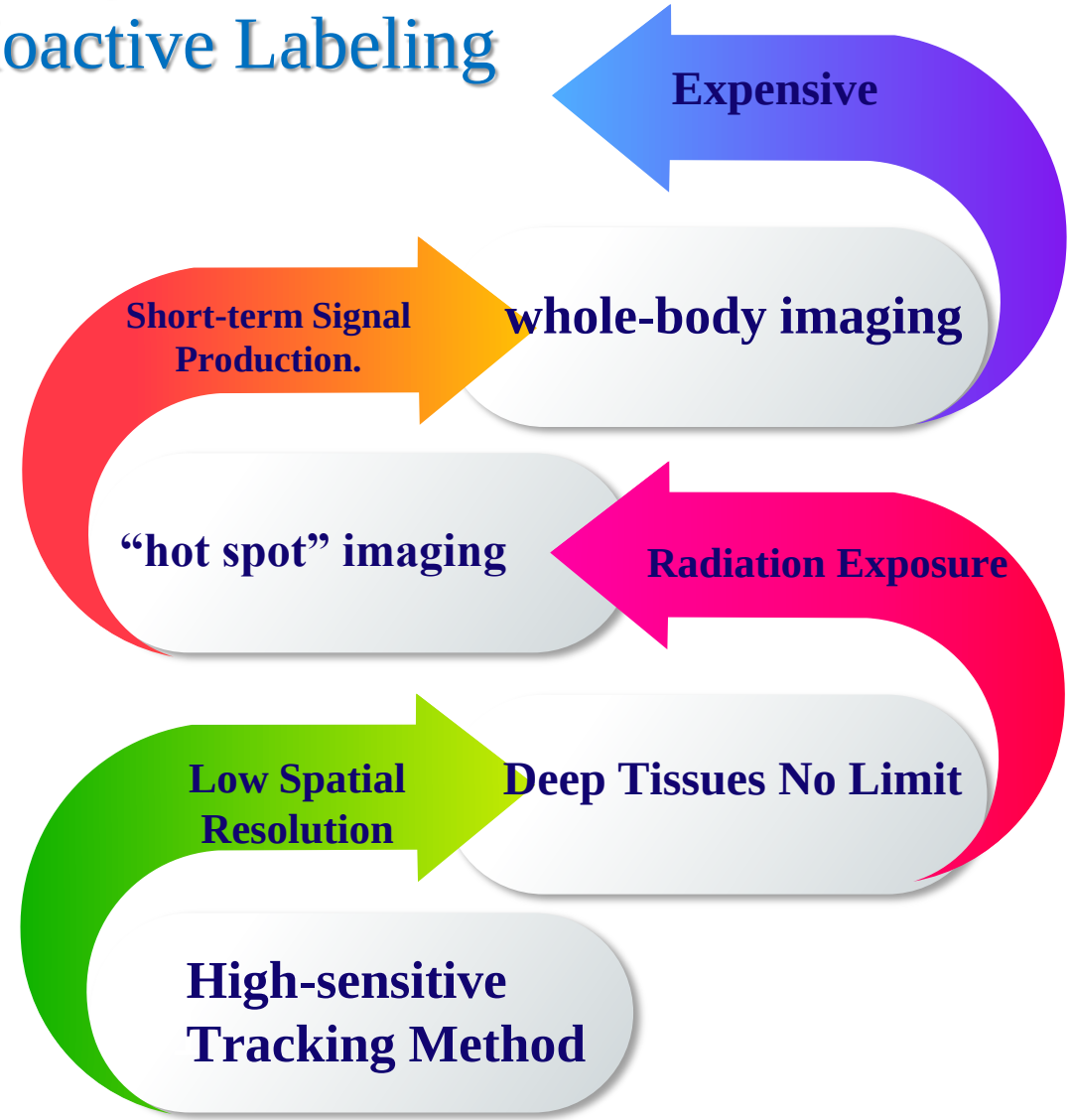
Magnetic particle imaging (MPI) cell tracking

- MPI, developed nearly 15 years ago.
- principle that SPIO nanoparticles which can be moved around in three-dimensional space.

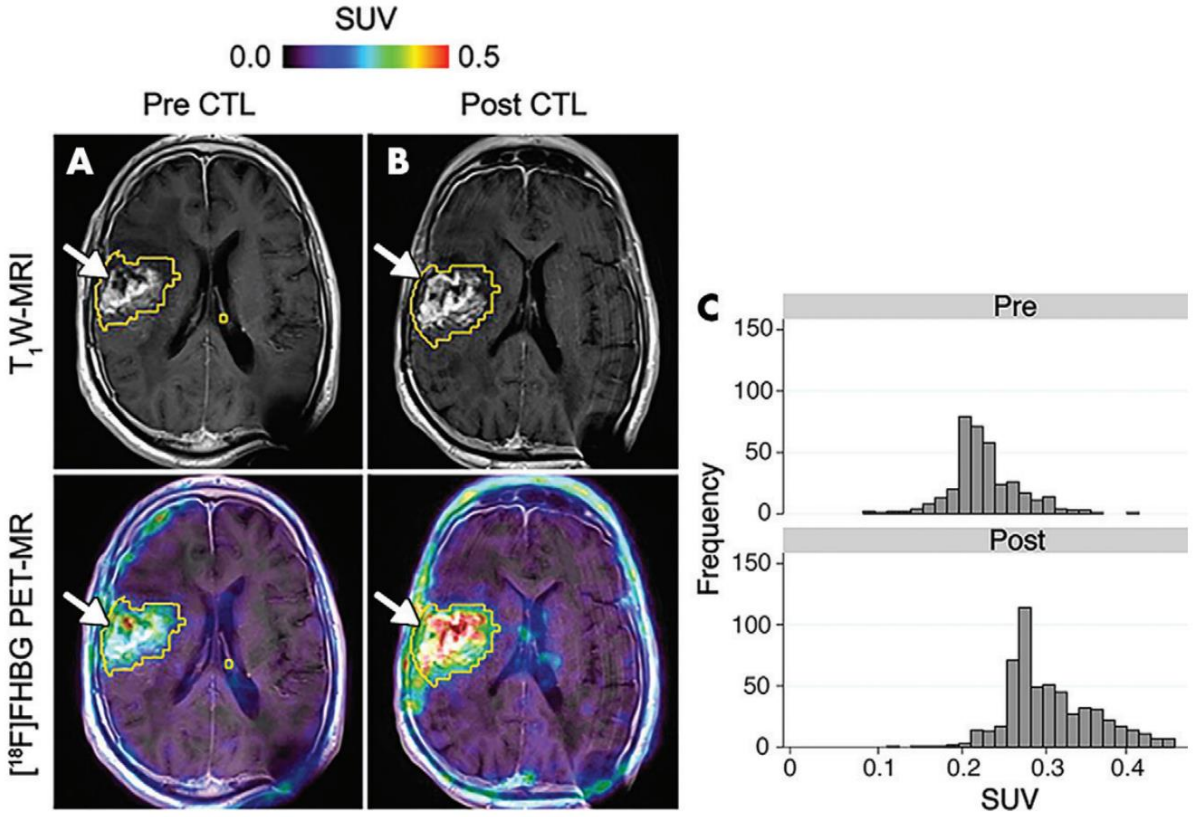




PET/SPECT Cell Tracking With Direct Radioactive Labeling



- ✓ Determine the amount of time that cells can be monitored noninvasively after cell labeling.
- ✓ SPECT has a nearly 10-fold lower sensitivity compared with PET.
- ✓ 111In labeling of cells requires high doses of radioactivity, which could induce cellular damage.



$_{18}$FHBG standardized uptake value

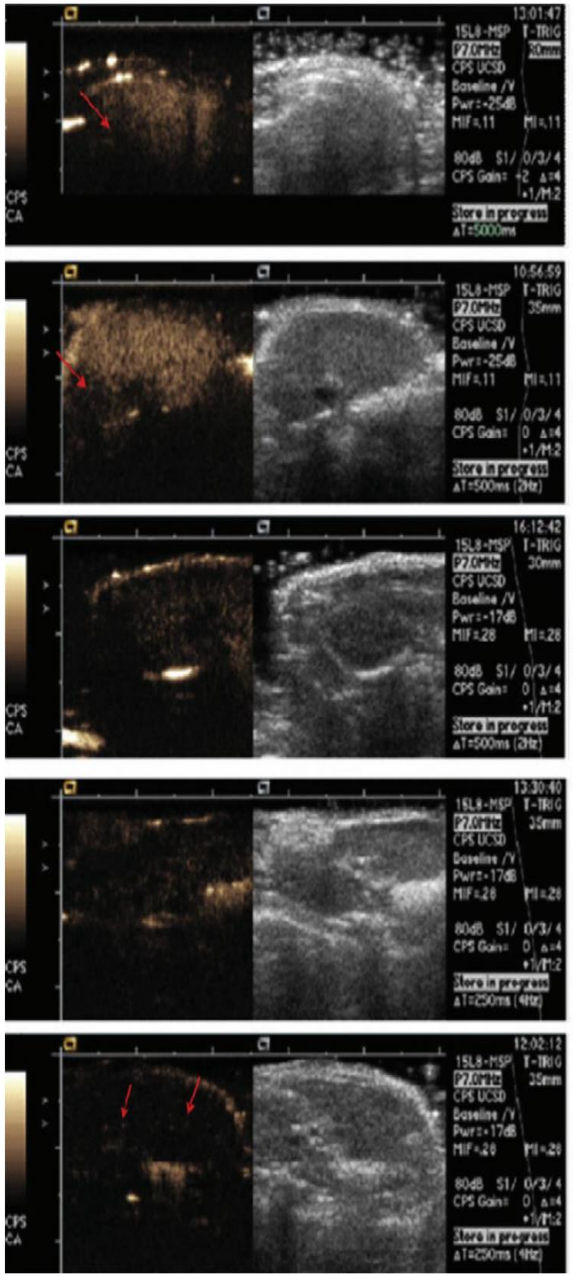
US cell tracking with direct labeling.

US cell tracking are relatively cheap, are fast, and come in a portable form.

US is used For imaging-guided cell injection procedures.

Clinically, US contrast agents consists of gas-filled microbubbles..

Large size, to image A single microbubble, this innocuous material appears to be the best candidate for future clinical US cell tracking.



X-ray Based Imaging

- Whole-body imaging,
- Low contrast agent sensitivity,
- High doses of radiopaque agents.

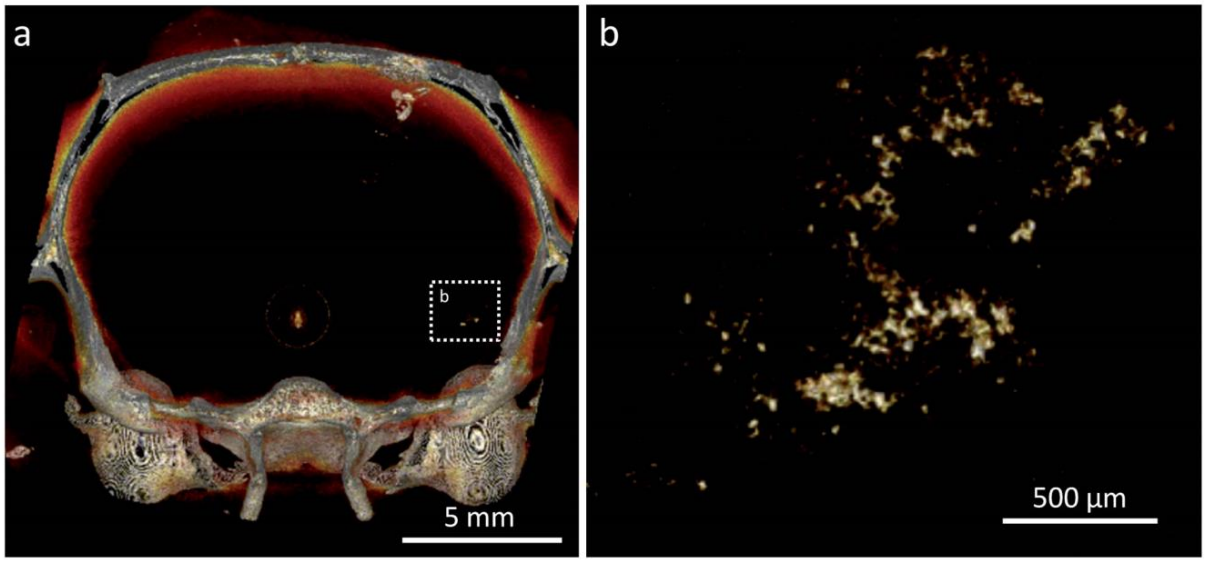
Whole gram of gold needed for in vivo cell tracking in patients is more than 4000 times higher than the total amount of gold in the body.

- Repeated CT scanning poses cumulative radiation.
- Exposure increases the risk of developing cancer.

- Gnps, an average 70-kg human individual contains 0.229 mg of gold versus 4000 mg of iron.

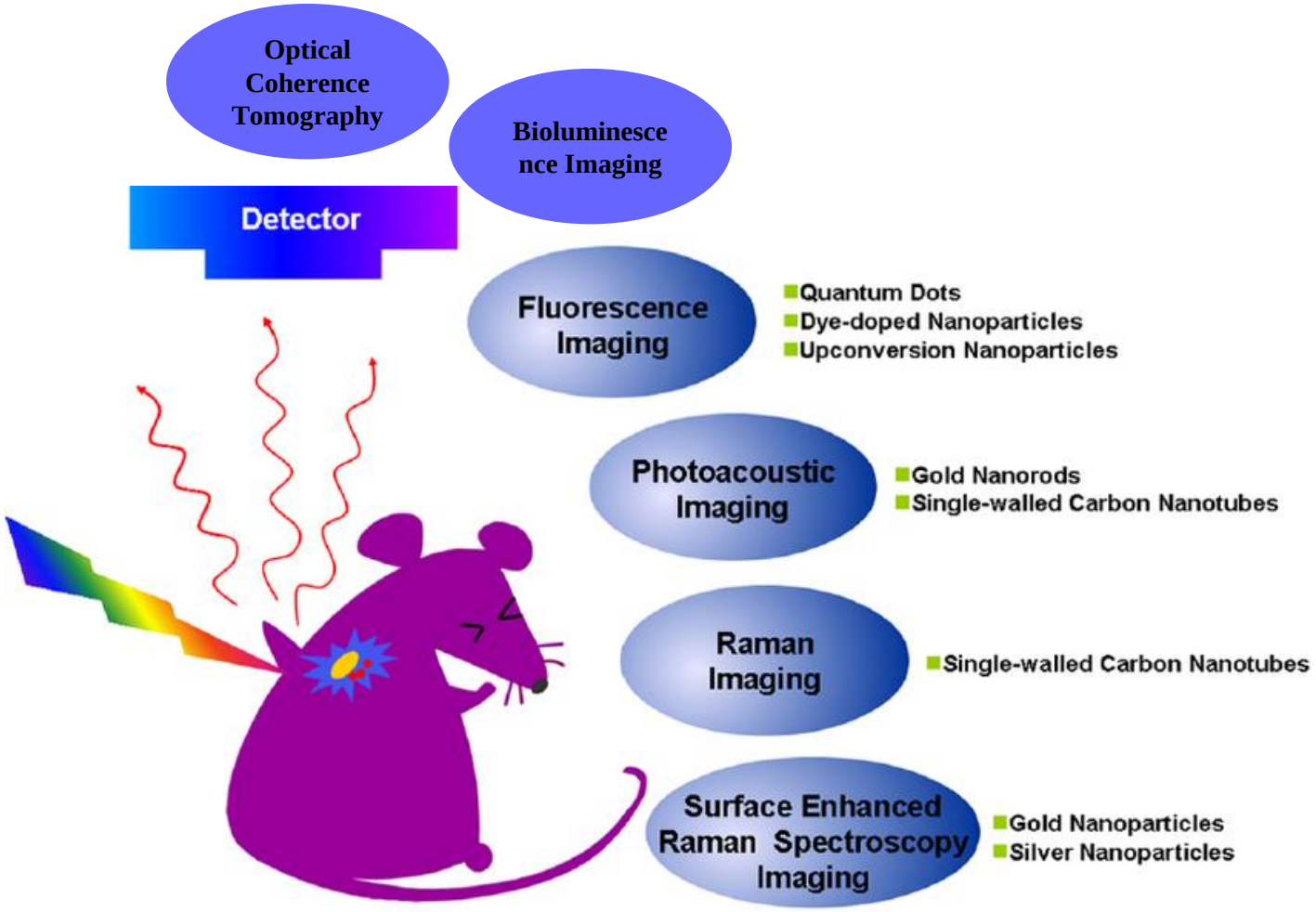
Computed Tomography (CT)

- For target locations, measurements were made using **CT-scan** images fused with previous MR images.
- **Bilateral parasagittal incisions** and corresponding **14 mm burr holes** were made in preparation for **cell** suspension injections.



DOI: 10.1039/c3nr34089a, nanoscale

Optical imaging



Favorable pharmacokinetic profile, access a target molecule with high affinity, separate the target from the bloodstream., The probe must be detectable with high sensitivity, quickly, and at high resolution.

Optical imaging, which can track stem cells for a long time without radiation.

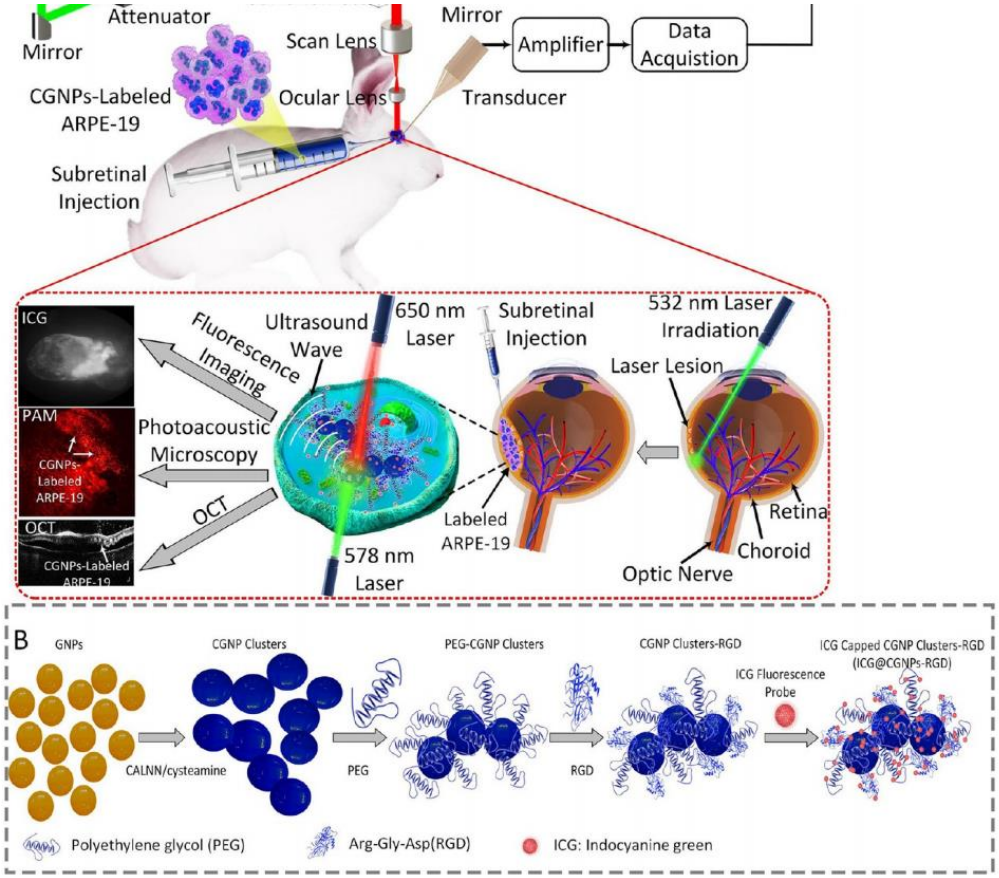
Optical imaging requires the injection of an imaging probe able to produce a detectable and targeted signal.

Is not feasible for clinic application as the limited penetration depth and low spatial resolution

Nontoxic & Noninvasive Visualizatio

Optical Coherent Tomography

- Transplantation of human mesenchymal stem cells in patients with Traumatic Optic Neuropathy.



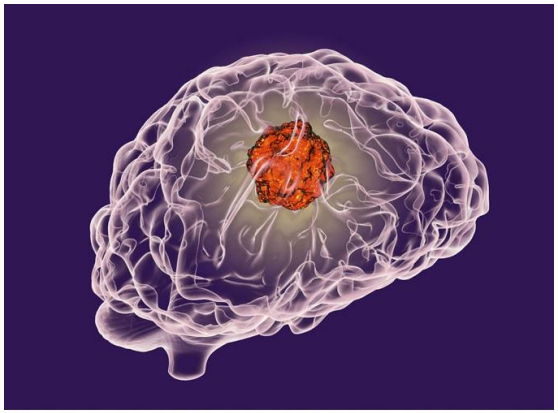
https://doi.org/10.1021/acsnano.1c03035 ACS Nano 2021, 15, 13289−13306

Fluorescence imaging

Near-infrared- (NIR-) emitting QDs may be especially useful to track transplanted cells in the human brain because their longer wavelengths allow easier penetration of tissue such as bone and skin.

NIR fluorescence labeling allows noninvasively tracking of transplanted cells engrafted in the infarction region as long as 8 weeks after transplantation.

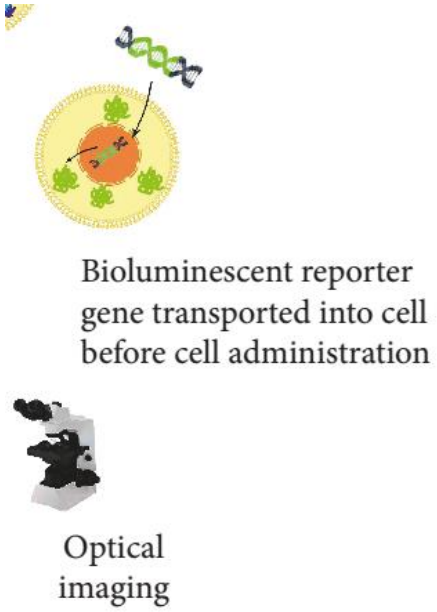
Cell tracking is limited by the short wavelengths as it is unable to obtain fluorescence signal through the bone and skin.

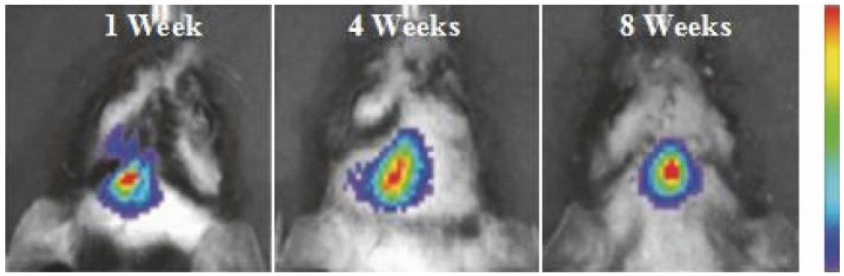


Fluorescent imaging with cetuximab safely used in glioblastoma patients

Bioluminescence imaging (BLI)

- Widely applied in preclinical studies of stem cell imaging to in the brain for years.
- Light emission is directly proportional to the number of cells.
- Considerable long term due to the luciferase gene that is stably integrated into the genome of stem cells.





(d) BLI

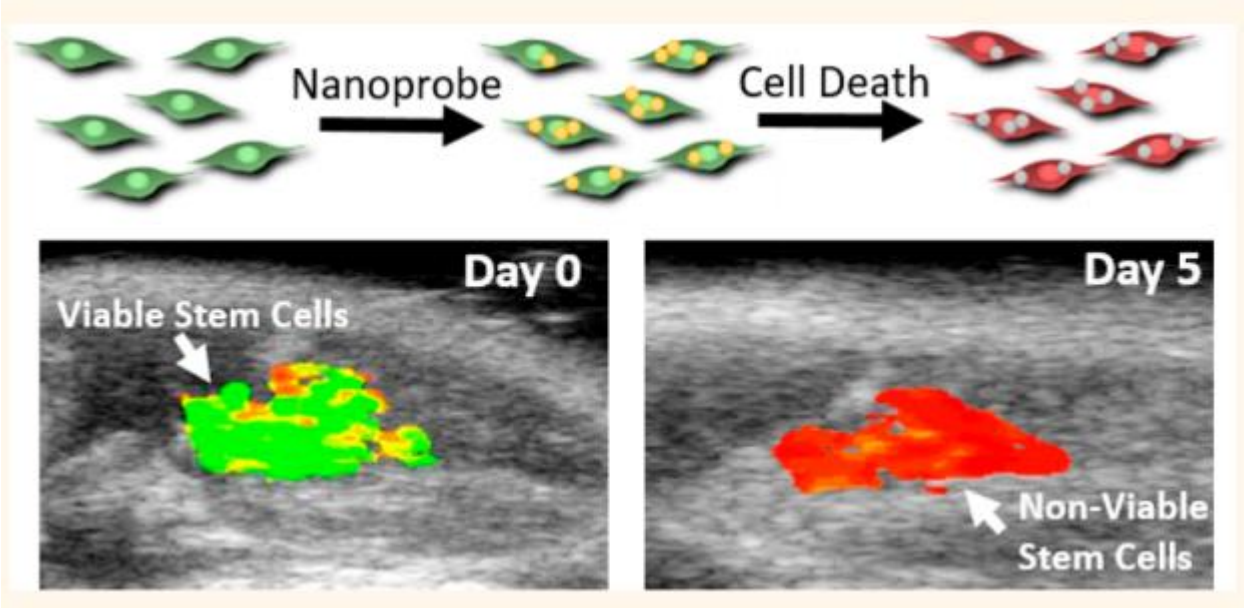
Luciferase photon emission detected through bioluminescence imaging (BLI) 1 week to 8 weeks after transplanting of neural progenitor cells (NPCs)

Stem Cell Tracking Technologies for Neurological Regenerative Medicine Purposes, Stem Cells International, 2017

Photoacoustic

- Photoacoustic imaging is not sensitive toward implanted stem cells alone, but through the use of exogenous contrast agents such as gold nanoparticles.

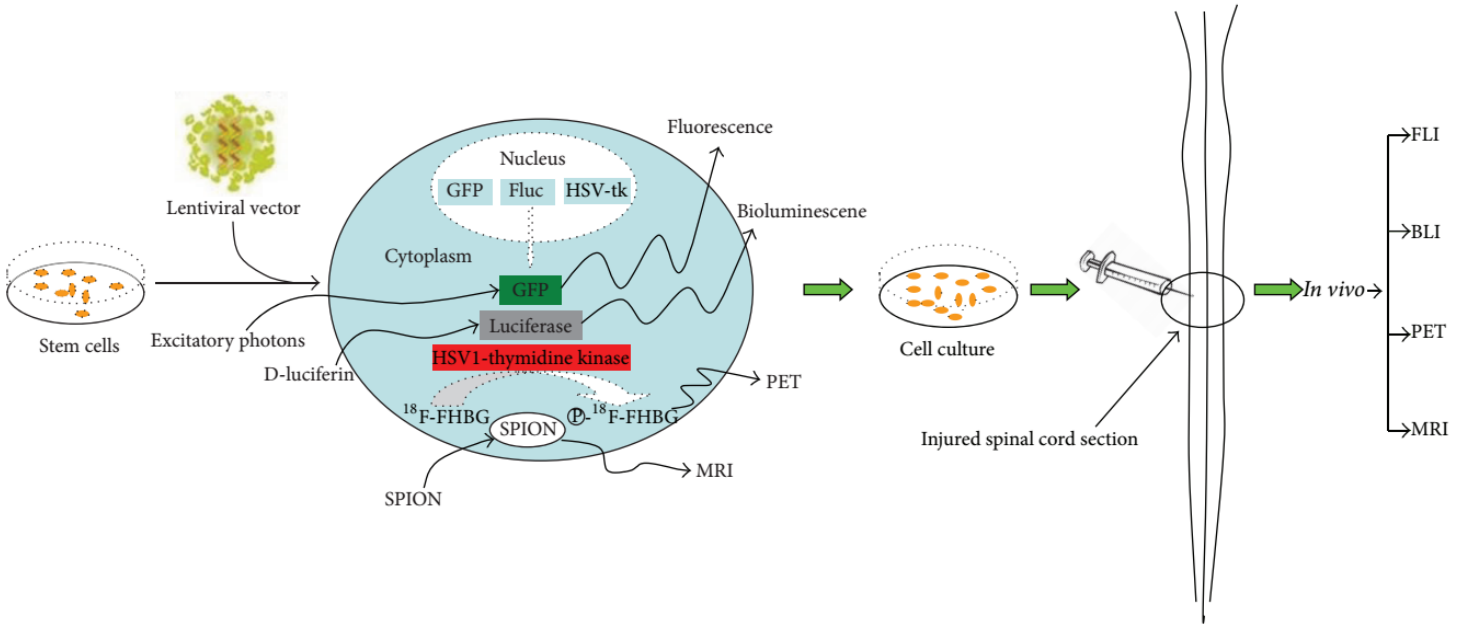
- Combine one of these sensitive near-infrared photoacoustic dyes with the labeling capability of gold nanoparticles to develop a viability probe using photoacoustic imaging.



DOI: 10.1021/acsnano.9b01802, ACS Nano 2019, 13, 7791−7799

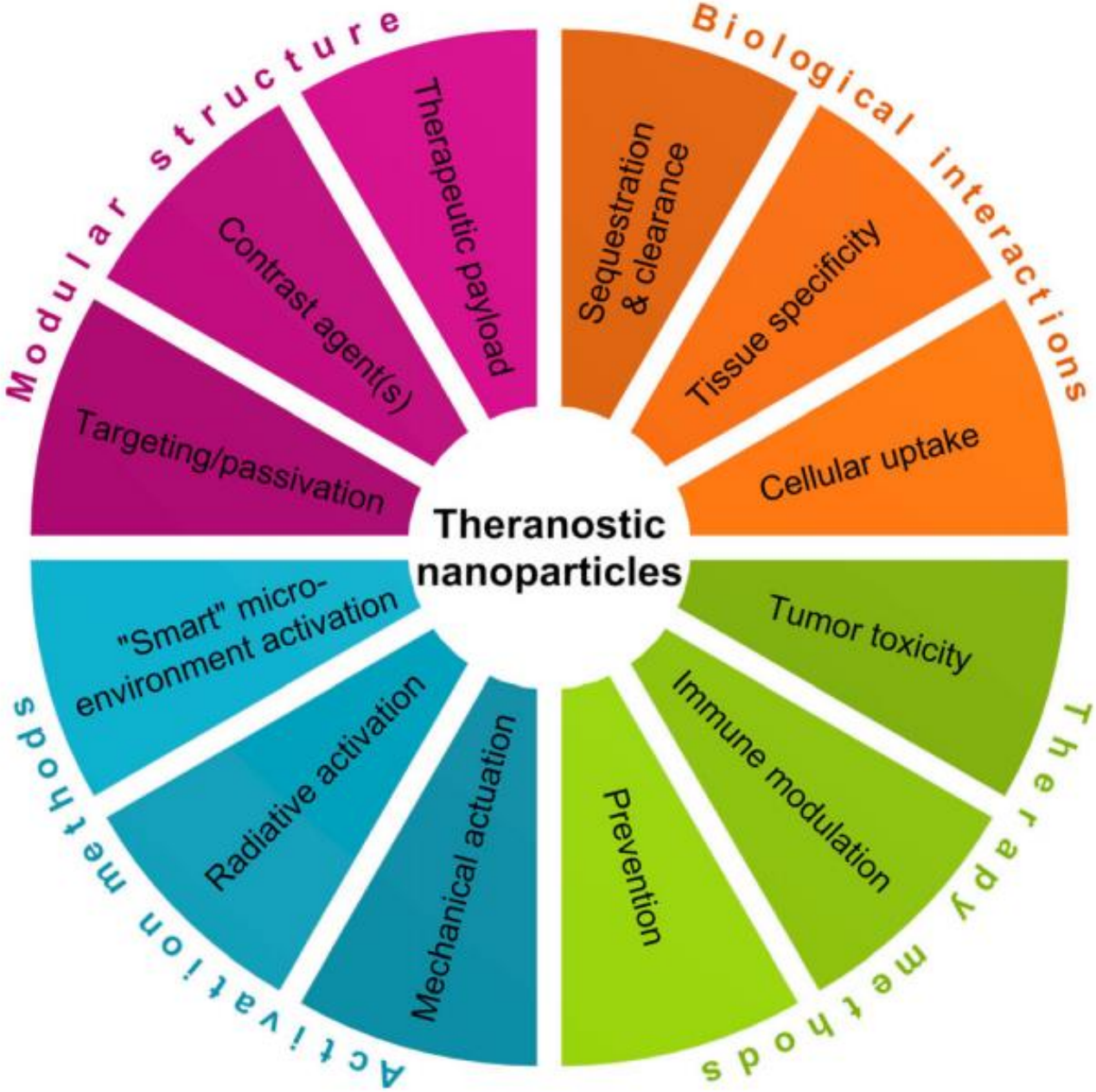
Multi-modal Imaging

- Multi-modal multi-scale in vivo cell tracking integrates medical and optical imaging.
- Technological advances providing enhanced resolution, sensitivity and multiplexing capabilities.



Molecular Imaging in Stem Cell Therapy for Spinal Cord Injury, *BioMed Research International, 2014*

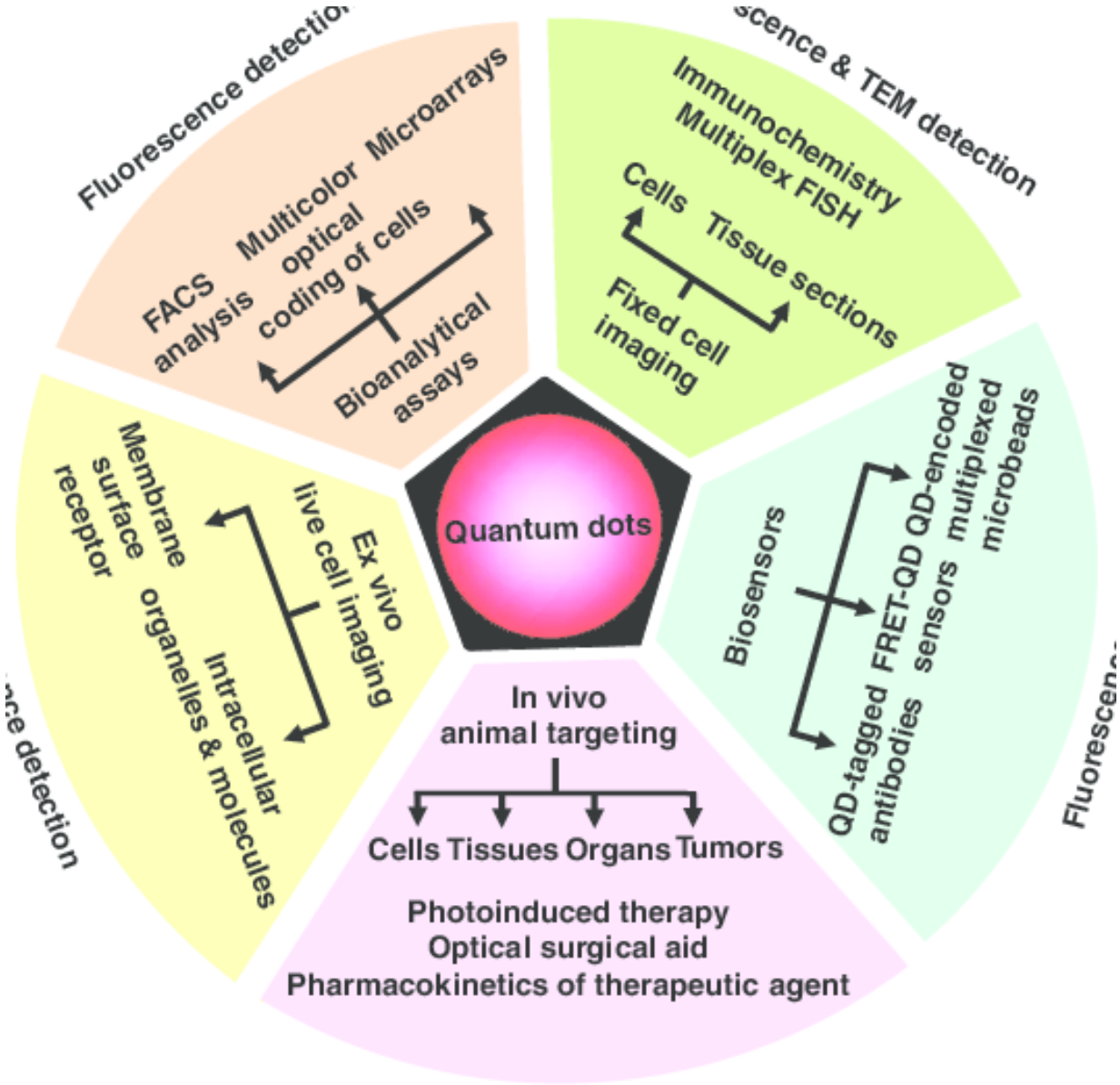
	MRI	PET	SPECT	US	CT Scan	FI	BLI	OCT	PA
Source Of Imaging	Radiowave	High-energy γ-ray	Low-energy γ-ray	Ultrasound wave	X-Ray	Visible light	Visible light	Visible light	Visible light/NIR/Ultrasound wave
Spatial Resolution	Small animals: >24 μm, clinic: 300 μm	Small animals: 41 mm, clinic: 44 mm (aided by simultaneously acquired CT images)	Small animals: 40.4 mm, clinic: 48 mm (aided by simultaneously acquired CT images)	50–500 μm	>50 μm	41 mm (depends heavily on depth of tissue, dye/ nanoparticle used)	1–20 mm (depends heavily on depth of tissue)	<10 μm	<45 μm
Tissue Depth	No limit	No limit	No limit	mm- cm	No limit	<1-3 cm	<1 cm	<2 cm	<7 cm
Sensitivity	μM	pM	pM	Not well	pM	nM-pM	pM	μm	μM–nM
Quantitative Degree	Poor	Fair	Fair	Poor		Fair	good	poor	poor
Long-term imaging	>2 month	Dependent on half-life of radioisotope, which can range from 110 minutes (18F) to 12.7 hours (18F). Effectively o2 days	Dependent on half-life of radioisotope, which can range from 6 hours (99mTc) to 2.8 days (111In). Effectively a few days	Poor	>1 month	>1 month	>1 month	Not well	Not well
Current Clinical Use	Yes	Yes	Yes	Fair	Fair	Fair	No	Fair	No
Advantages	No radiation, very good tissue contrast, high resolution	High sensitivity, deep tissues	High sensitivity, able to image deep tis	Real time, low cost	High resolution	Cheap, simple, high sensitivity, activatable	Deep tissue limited, low resolution, tissue damaging	High resolution	Multispectral imaging, real time,
Disadvantages	Low sensitivity, agent dilution	Radiation, radiotracer dilut	Radiation, low resolution, radiotracer dilution	Limited spatial resolution	Requires ionizing radiation	Simple, high sensitivity	Deep tissue limited, low resolution	Limited penetration depth, high cost	Insufficient resolution for cellular imaging

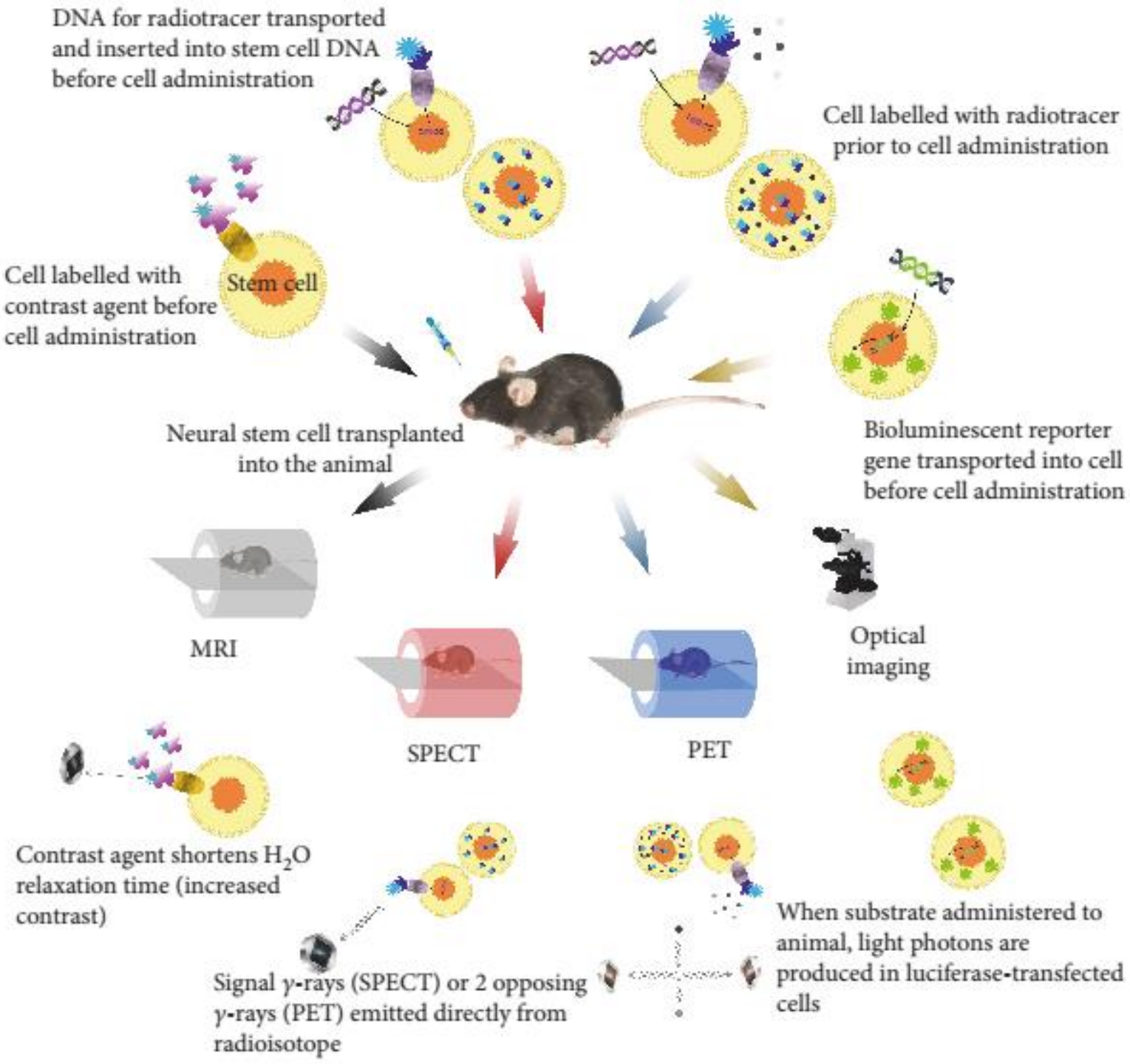

Modular structure
Therapeutic payload
Contrast agent(s)
Targeting/passivation
Biological interactions
Sequestration & clearance
Tissue specificity
Cellular uptake
Theranostic nanoparticles
"Smart" micro-environment activation
Radiative activation
Mechanical actuation
Activation methods
Tumor toxicity
Immune modulation
Prevention
Therapy methods

Conclusion

- Cell therapy is coming of age, medical imaging to become an integral part of it.
- New opportunities may present performing real-time imaging-guided cell injections.
- With a variety of clinically translatable cellular imaging techniques available or in development, these can affect clinical outcomes.


Thanks


Fluorescence detectio
FACS analysis
Multicolor optical coding of cells
Microarrays
Bioanalytical assays
scence & TEM detection
Immunochemistry
Multiplex FISH
Cells
Tissue sections
Fixed cell imaging
Quantum dots
Biosensors
FRET-QD sensors
QD-encoded multiplexed microbeads
QD-tagged antibodies
Fluorescence
In vivo animal targeting
Cells Tissues Organs Tumors
Photoinduced therapy
Optical surgical aid
Pharmacokinetics of therapeutic agent
ce detection
Membrane surface receptor
Ex vivo live cell imaging
Intracellular organelles & molecules


DNA for radiotracer transported and inserted into stem cell DNA before cell administration
Cell labelled with radiotracer prior to cell administration
Cell labelled with contrast agent before cell administration
Stem cell
Neural stem cell transplanted into the animal
Bioluminescent reporter gene transported into cell before cell administration
MRI
Optical imaging
SPECT
PET
Contrast agent shortens H_2O relaxation time (increased contrast)
When substrate administered to animal, light photons are produced in luciferase-transfected cells
Signal γ-rays (SPECT) or 2 opposing γ-rays (PET) emitted directly from radioisotope


STEP 01
STEP 02
STEP 03
STEP 04
STEP 05
STEP 06
STEP 07
STEP 08
STEP 09
STEP 10