Imagerie Cellulaire

O Clément

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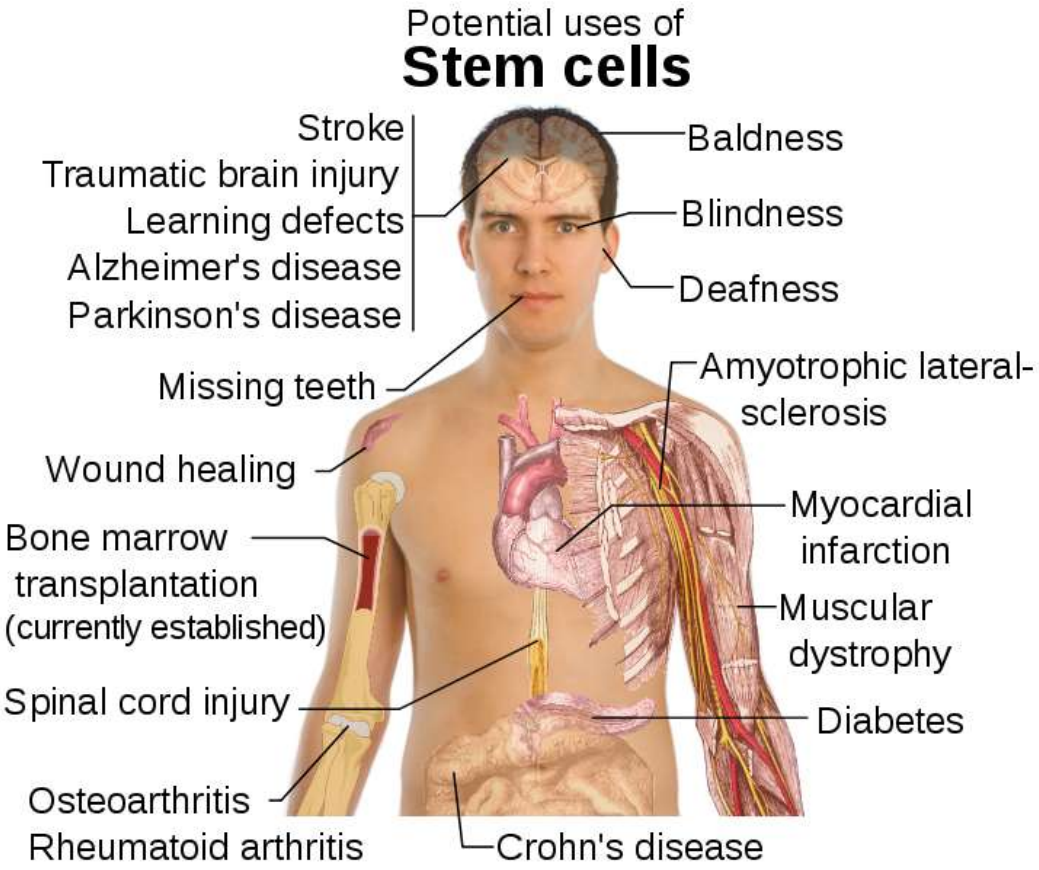
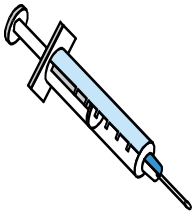
Tissue Regeneration

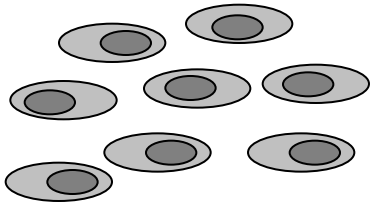
- **Bone marrow transplant (1957-1959)**
- **Cardio and Vascular diseases (1995)**
- **Diabetes**
- **Neurological (1988)**
- **Musculoskeletal**



Imaging human cell repair *In vivo*



Implantation

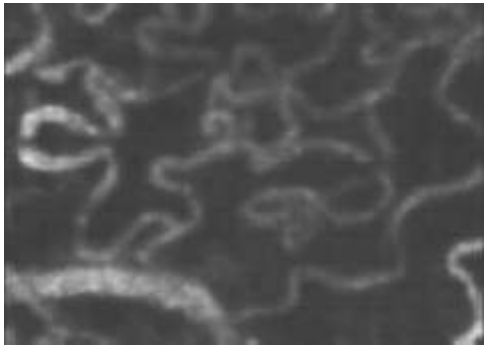


Cell Labeling

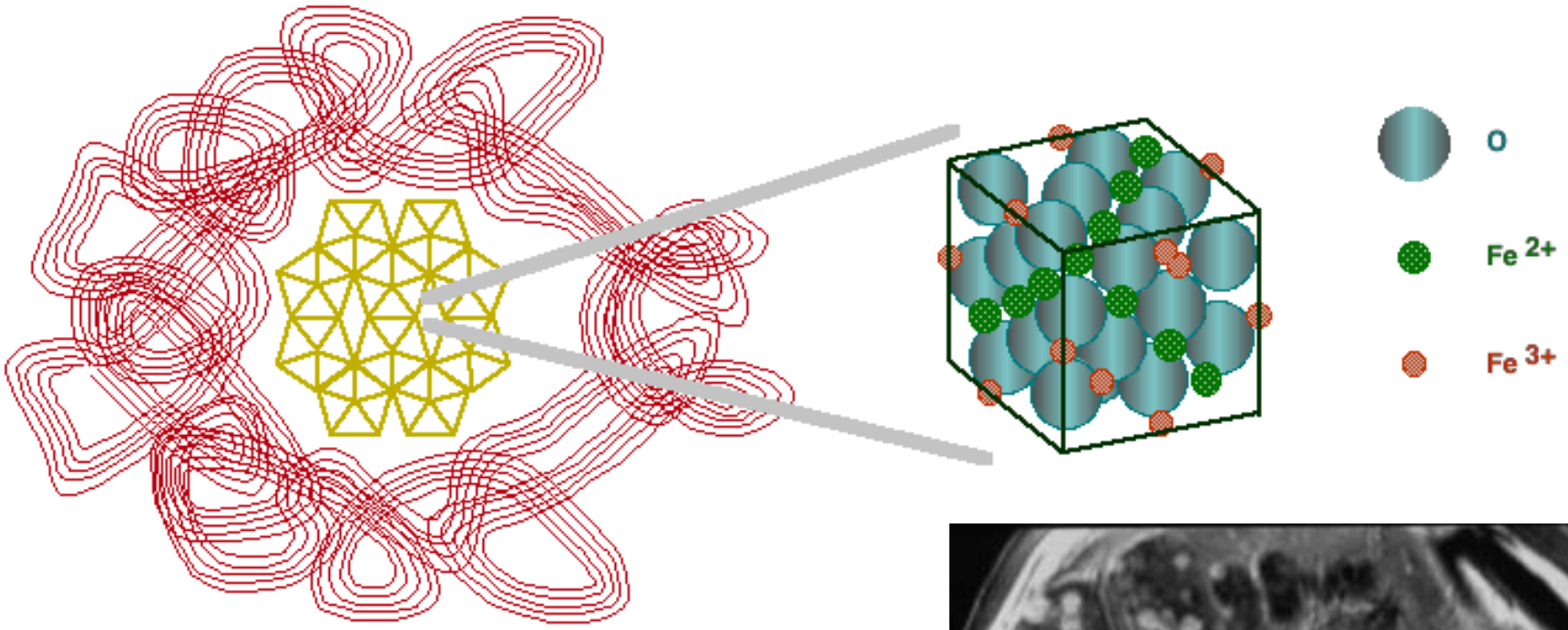
Nucl Med

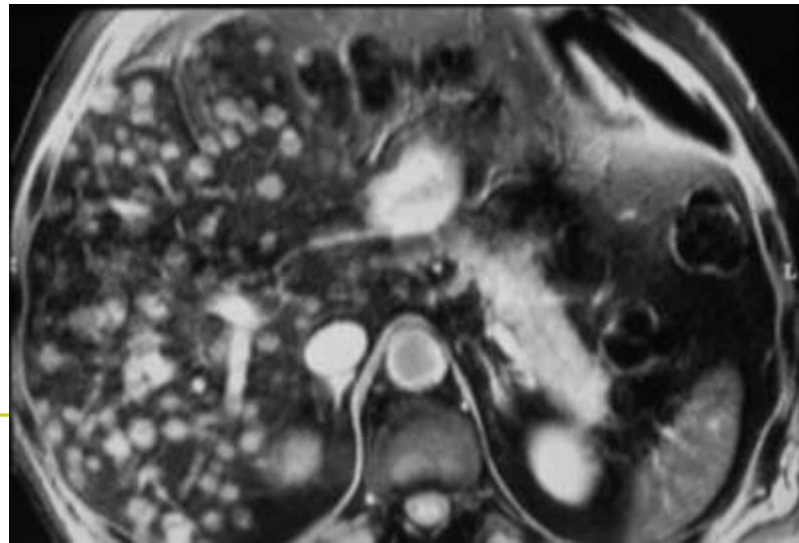
Microscopy Optics

MRI

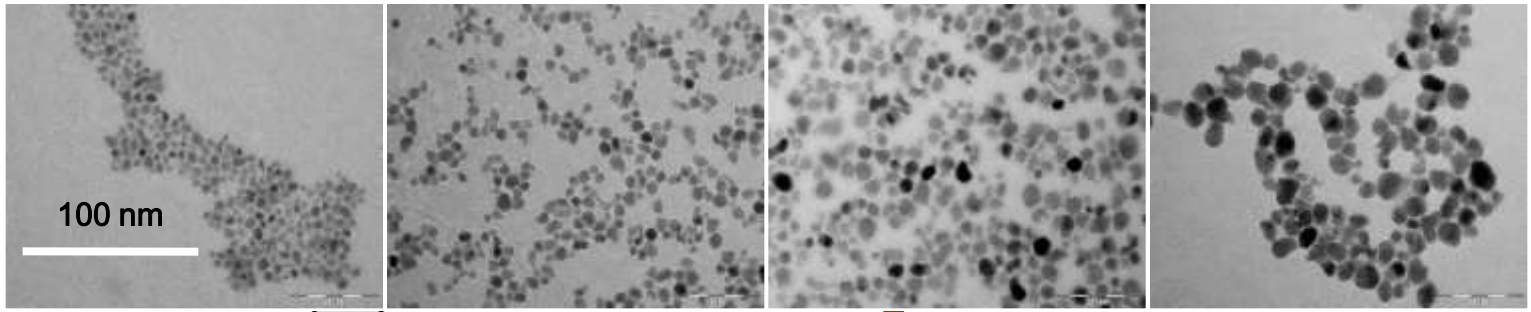


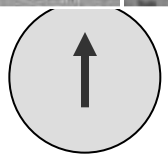
Approved drugs : Feridex®, Resovist® for liver MRI





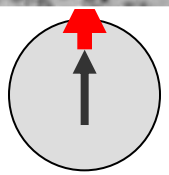
Chemically synthesized nanomagnets (5-20 nm)





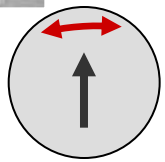
B_0

Magnetic field source



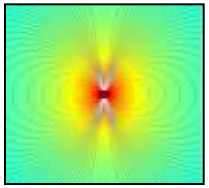
Grad B

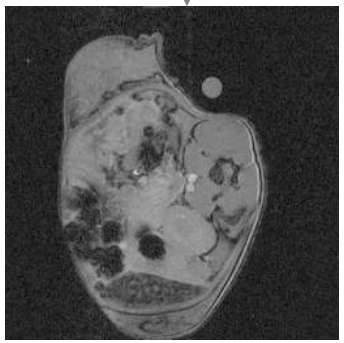
Remote magnetic forces



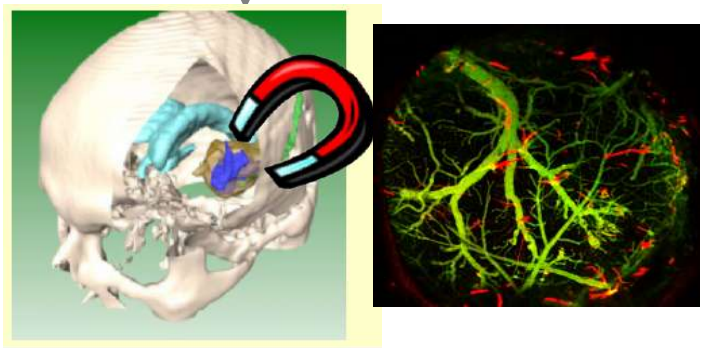
B(ω)

Nanoheaters

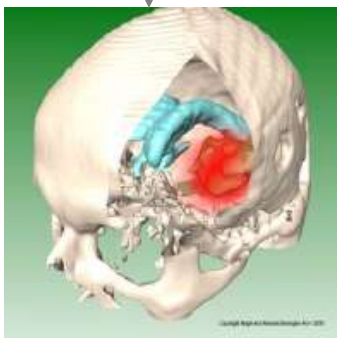




Detection
Imaging



Manipulation and Targeting



Thermally activated therapy

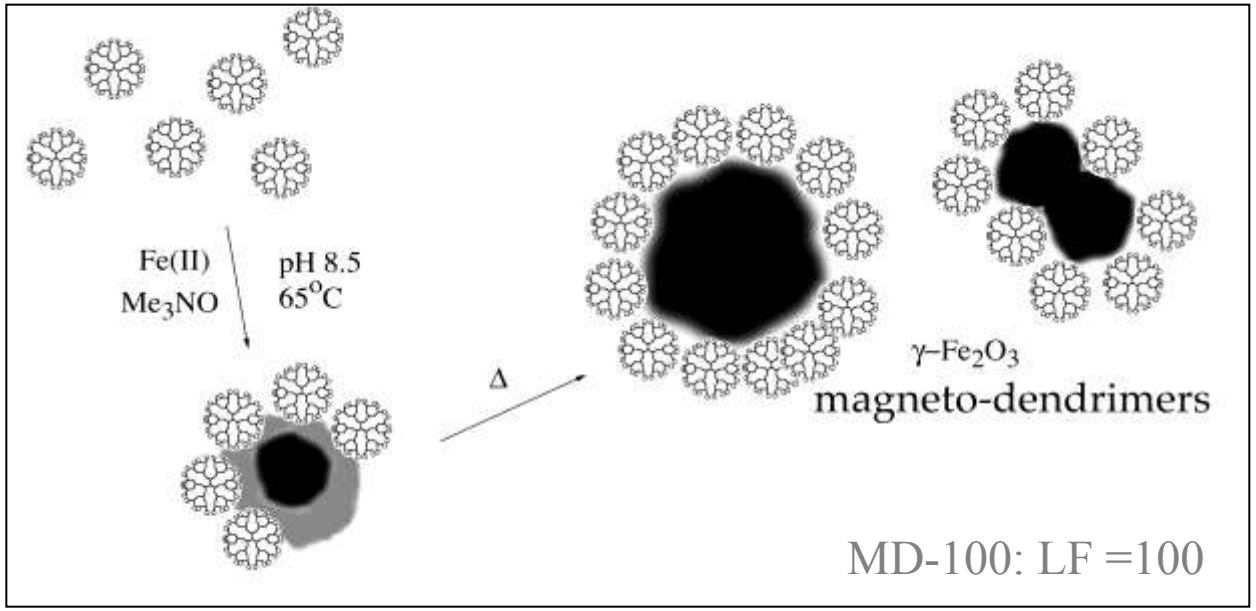
Feridex®, Resovist®

- Unlabeled use
- Spontaneous endocytosis is limited
- Especially with non-phagocytic cells

- 24 hrs labeling

Transfection agents

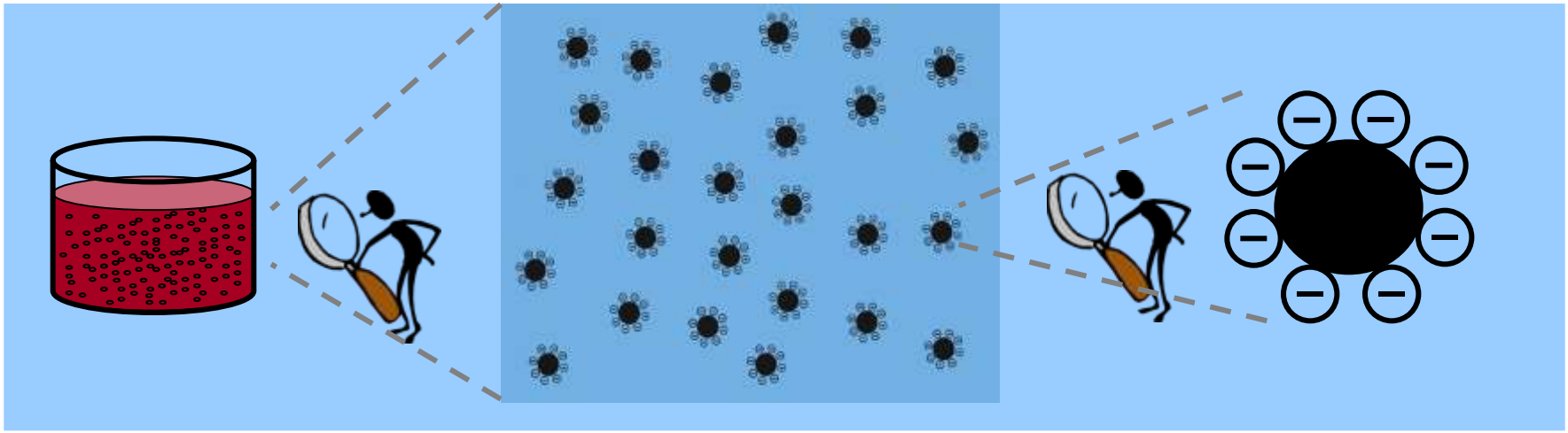
- Non specific
- Electrostatic interactions (cationic peptides, lipids, polyamines, dendrimers, protamin)



Bulte et al, Nature Biotech, 2001

Anionic Nanoparticles

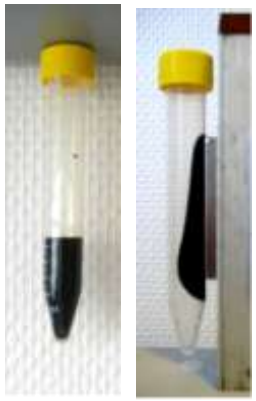
Lab PECSA, C MENAGER



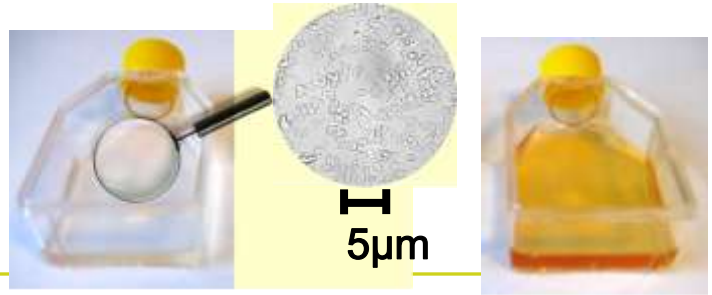
Ferrofluid : particles suspension

- **[Fe] 50 to 100 mM**
- **Maghemite Fe_2O_3 nanoparticles**
- **Magnetic diameter : 10 nm**
- **Negative surface charges**
- **Stable at pH 7**

Magnetic liquids



in cell culture medium



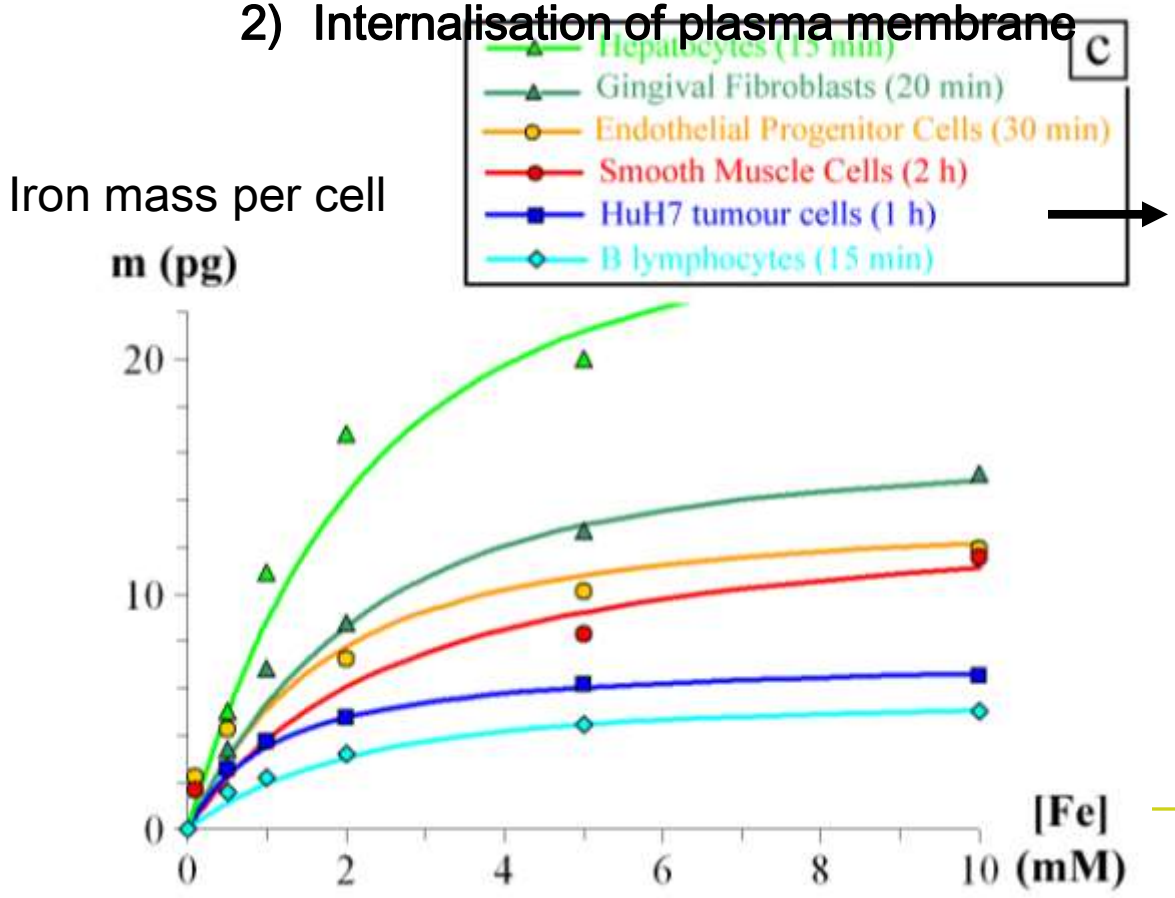
Wilhelm, Langmuir 2002

Universal cell uptake

1) Electrostatic non specific adsorption on plasma membrane (affinity : K)

limited by cell surface area

2) Internalisation of plasma membrane



Label any cell type

- Predictible cell iron load
- High labeling efficiency
- Short incubation time (10 min – 2 hrs)

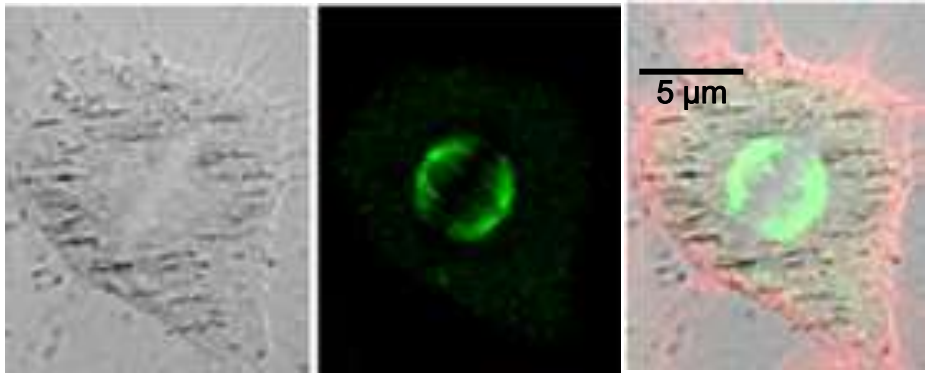
Wilhelm & Gazeau, Biomaterials 2008

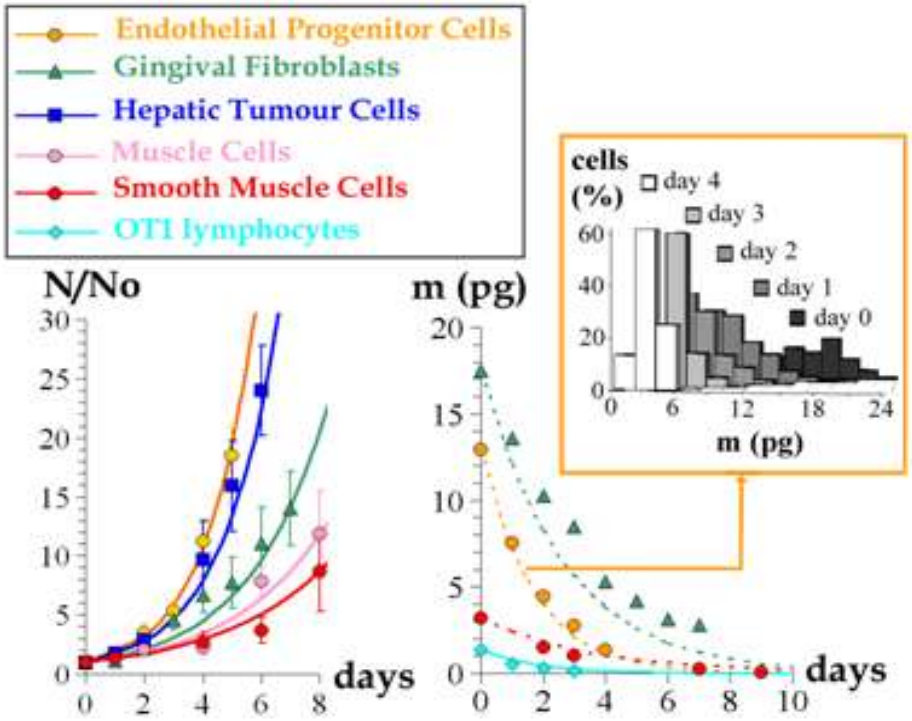
Biocompatibility : Cell Proliferation is not affected

Labelled cells share their magnetic load when dividing

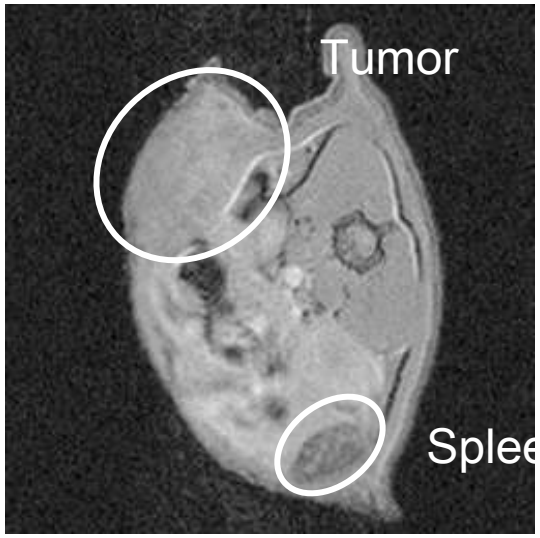
B

Cellules Hela (2h, 10mM)

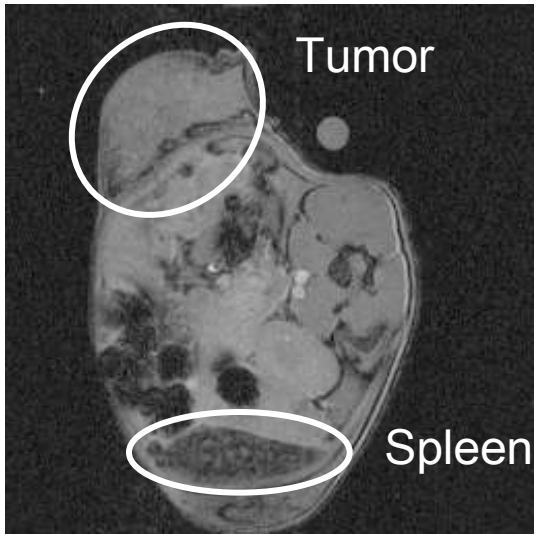




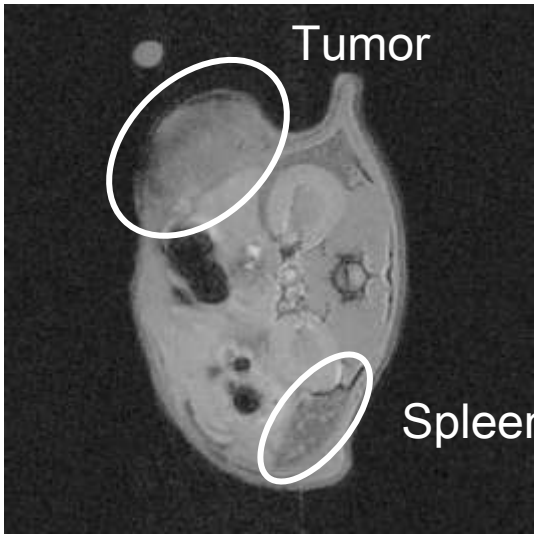
7 T in vivo MRI



Before injection

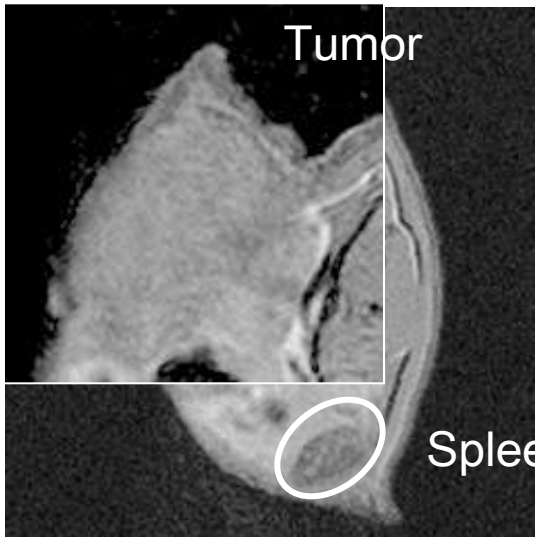


24 h post injection

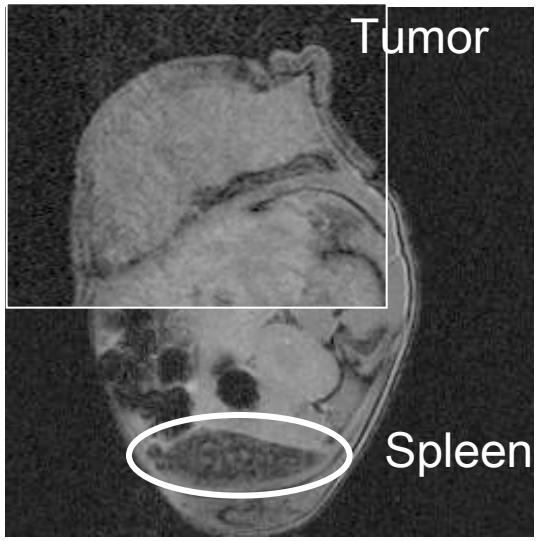


72 h post injection

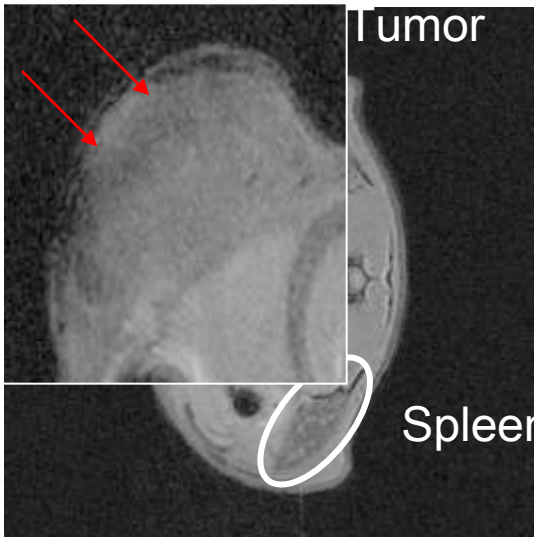
7 T in vivo MRI



Before injection



24 h post injection



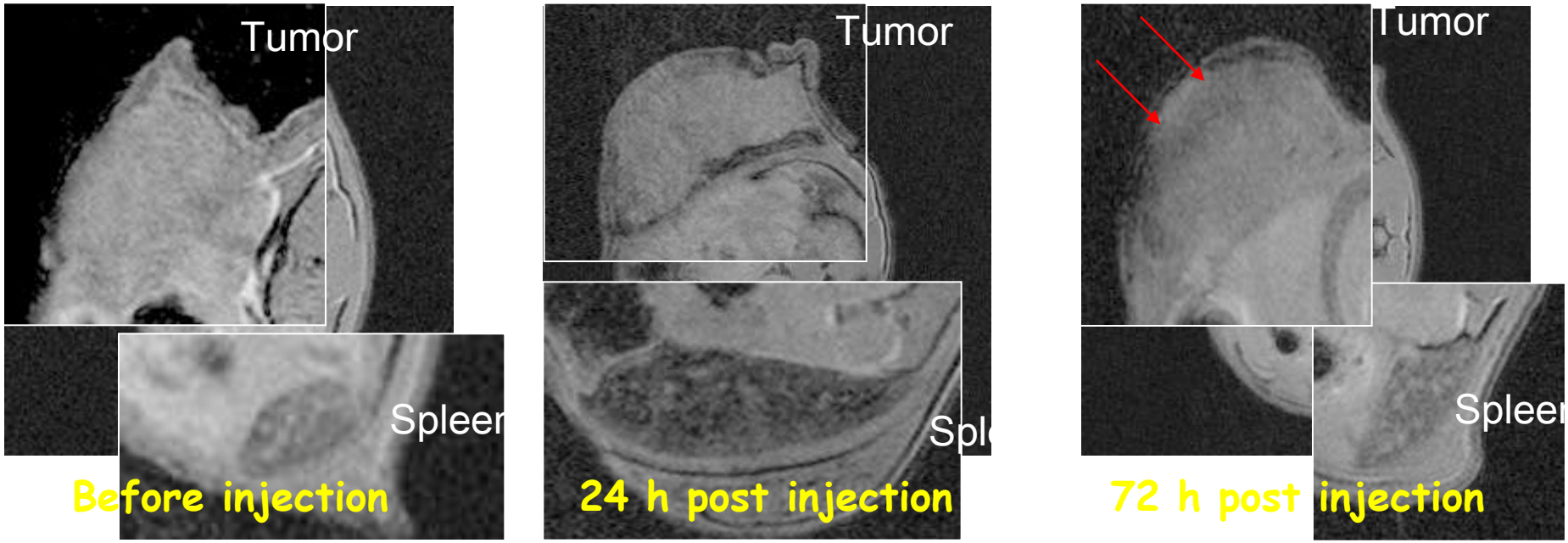
72 h post injection

Hypo-signals into **tumeurs 72 h** after magnetically labeled lymphocytes transfer

Tumor Enhancement : -28 ± 11 %

(TE= 3,2 ms, TR= 500 ms, α= 60°, FOV= 3,5 x 3,5 cm^2, matrice = 256^2)

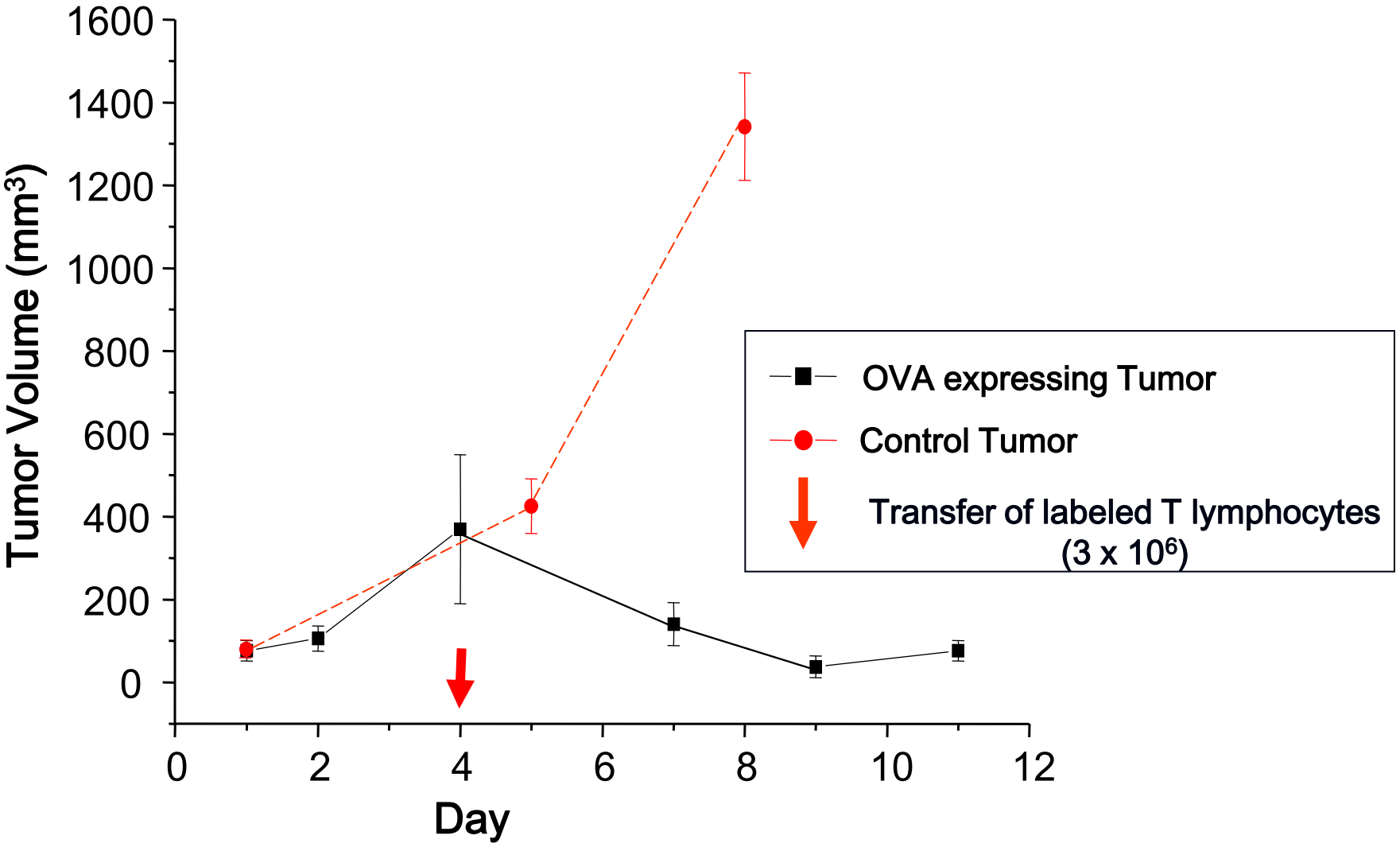
7 T in vivo MRI



Hyposignal **in spleen 24 h** after magnetically labeled lymphocytes transfer

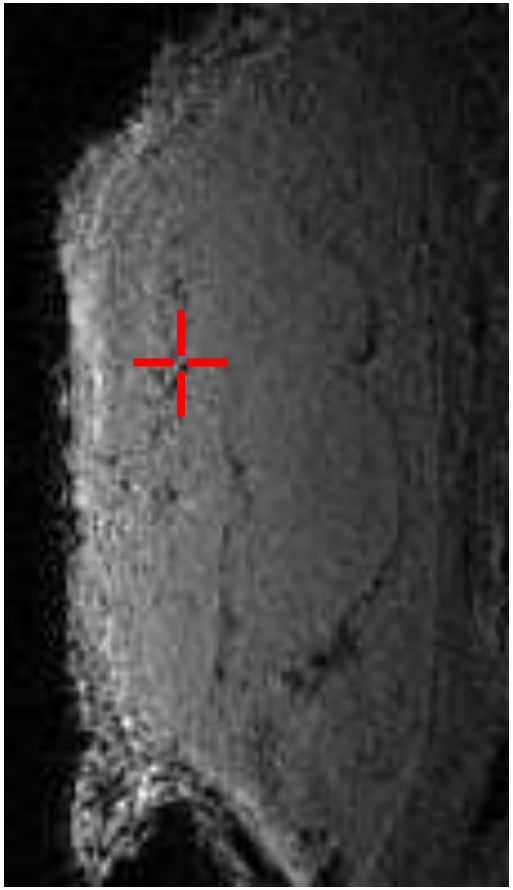
Spleen enhancement : -33 ± 10 %

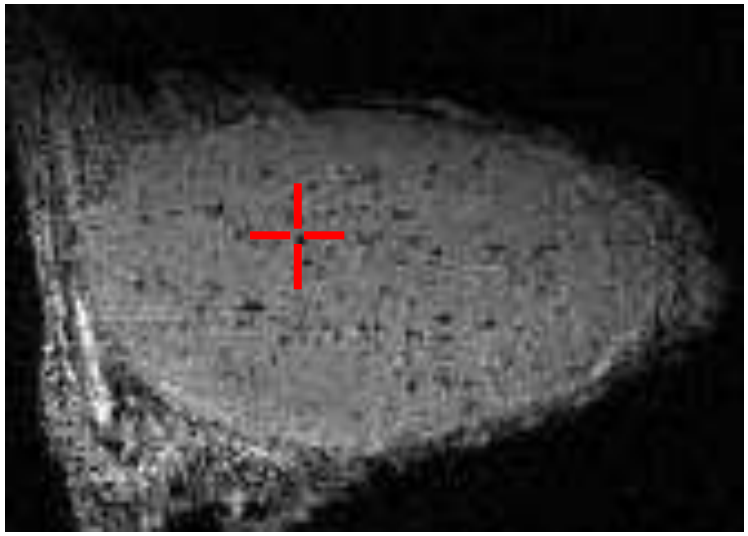
Antitumor functionnality of T lymphocytes is preserved after magnetic labeling

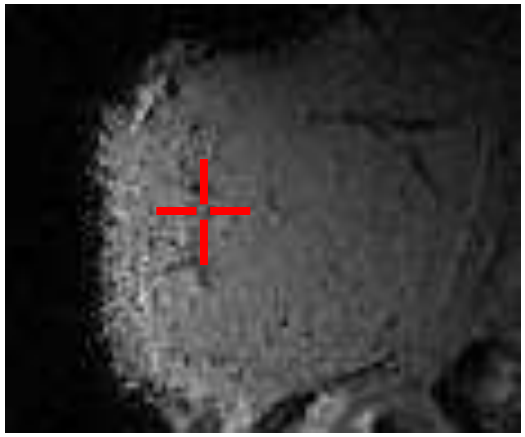


In vivo single cell detection at 1.5 T

3D punctual hyposignals ➔ labelled lymphocytes (<1 pg Fe /cell) in the tumour







Possibility of detecting cells which divide in vivo and migrate towards homing sites

(voxel size = 59 µm^3, TE = 14 ms tps. acq. = 29 min)

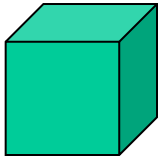
Smirnov et al, Magn. Res. Med, 2008

Cryoprobe

Cooled with Helium
S/B * 8

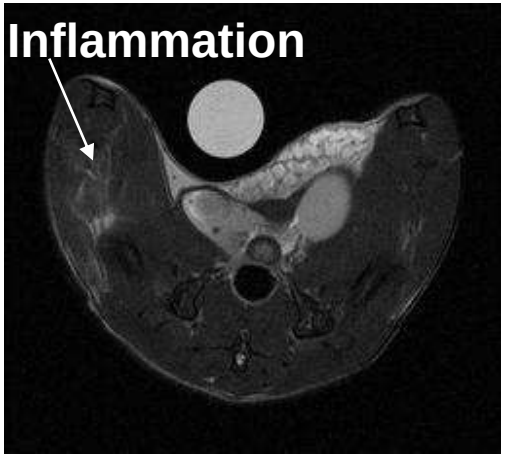
Surface cool emit/transmit

Zone of interest 1 cm^3



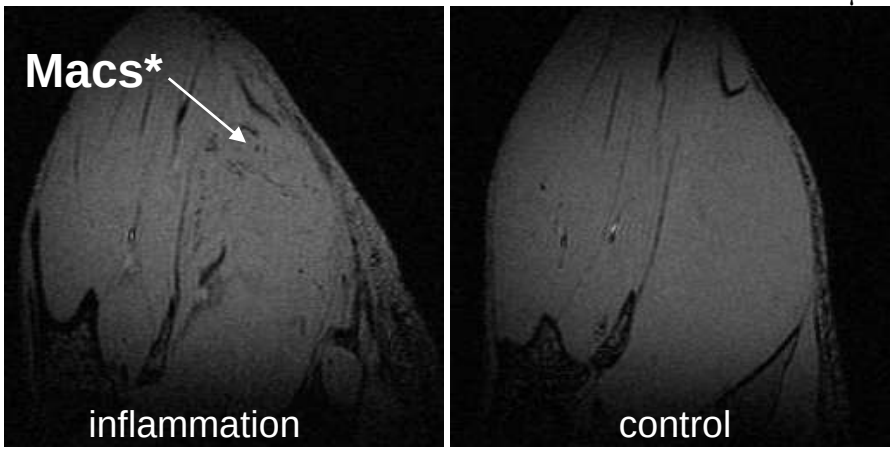
For mice only in vivo
or in vitro

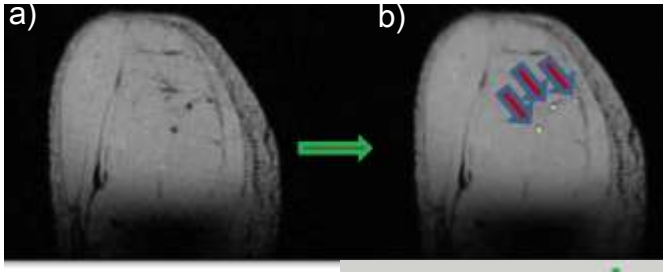
Labeled macrophages in calf inflammation

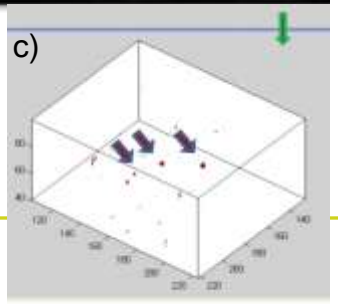


Cryoprobe FISP 3D FID

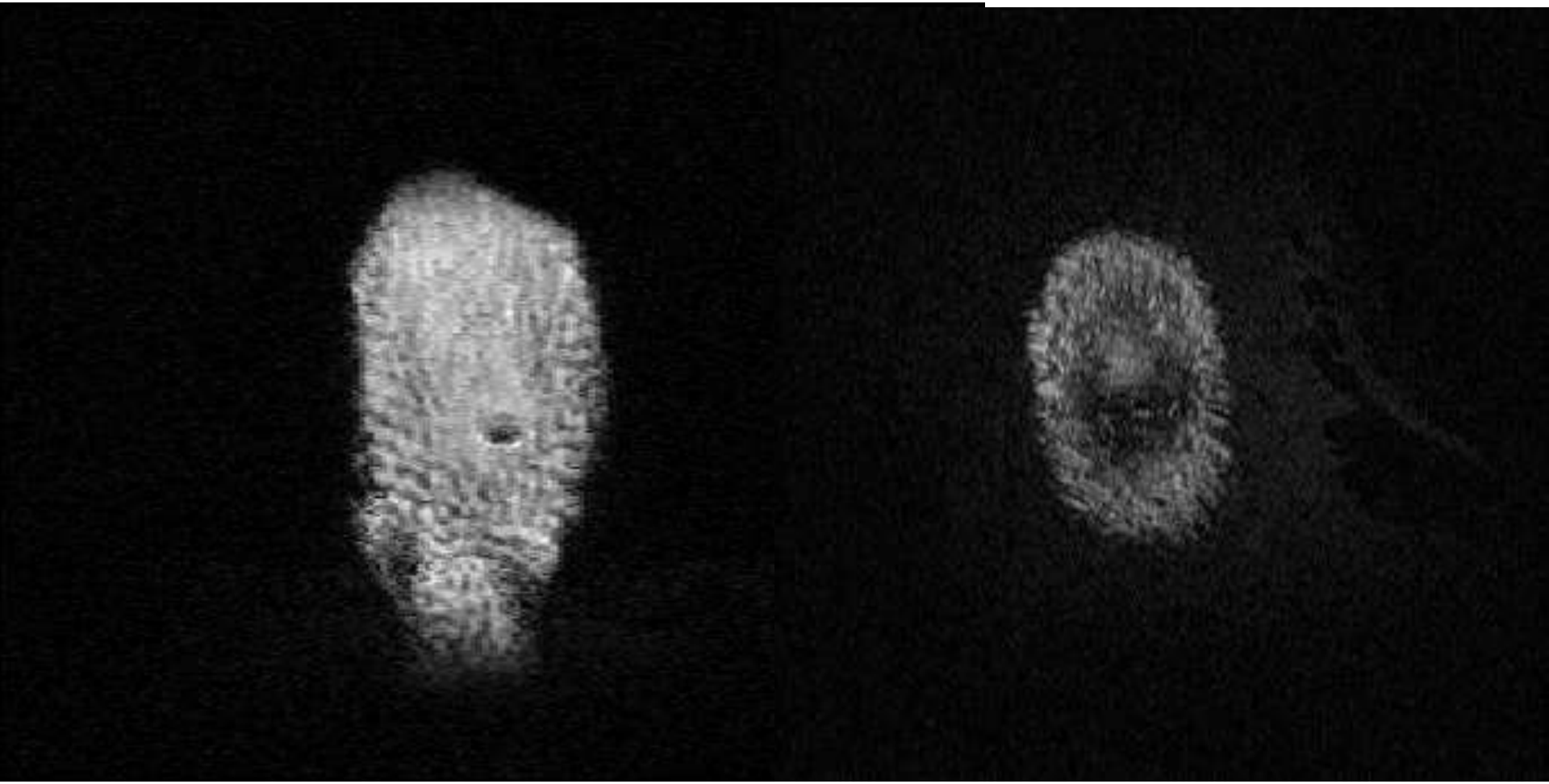
R = 50 × 50 × 50 μm 10’43







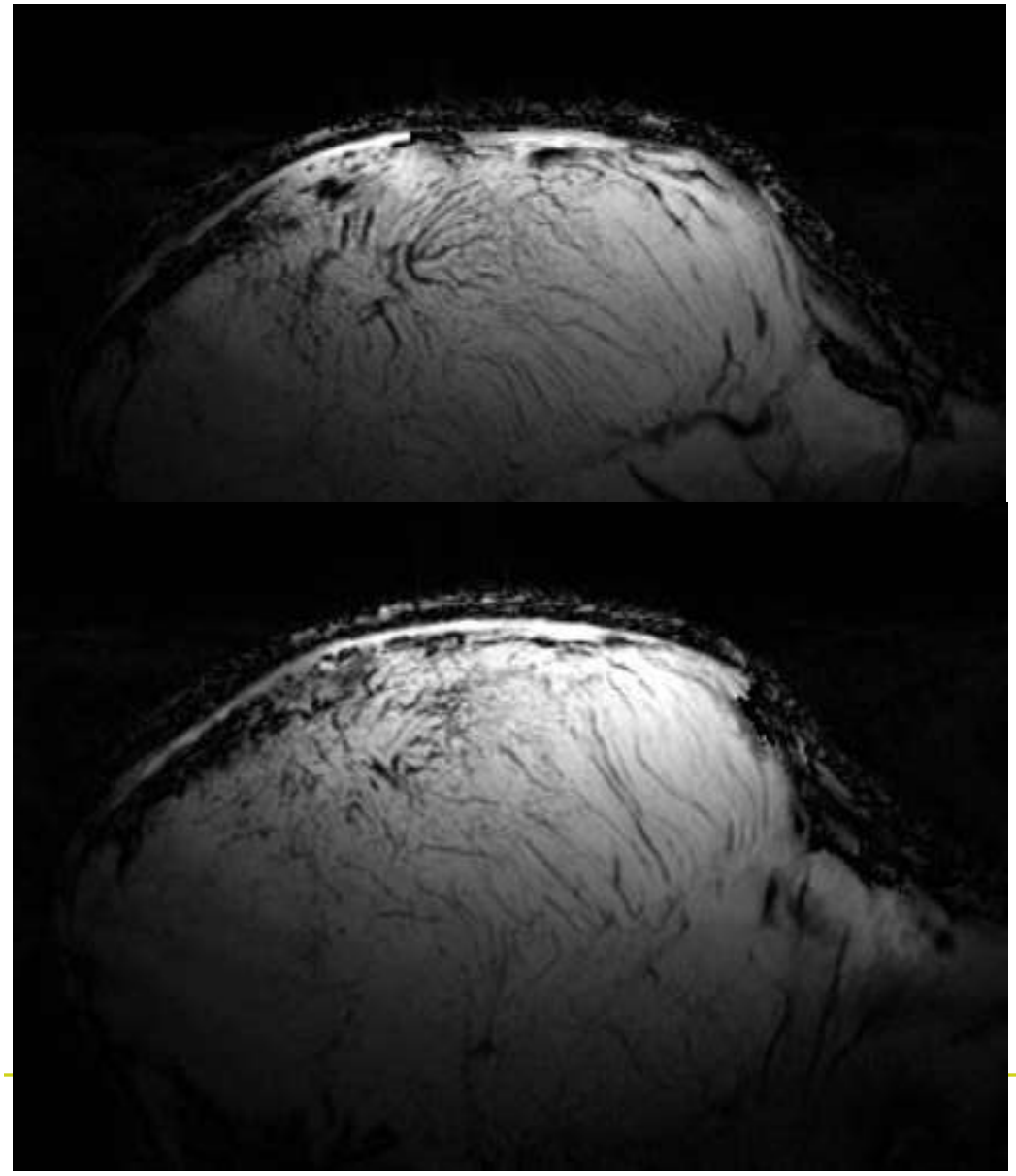
a) After filtering
b) Segmentation



Labeled Mural progenitor cells (pericytes) in tumor angiogenesis (N Faye)

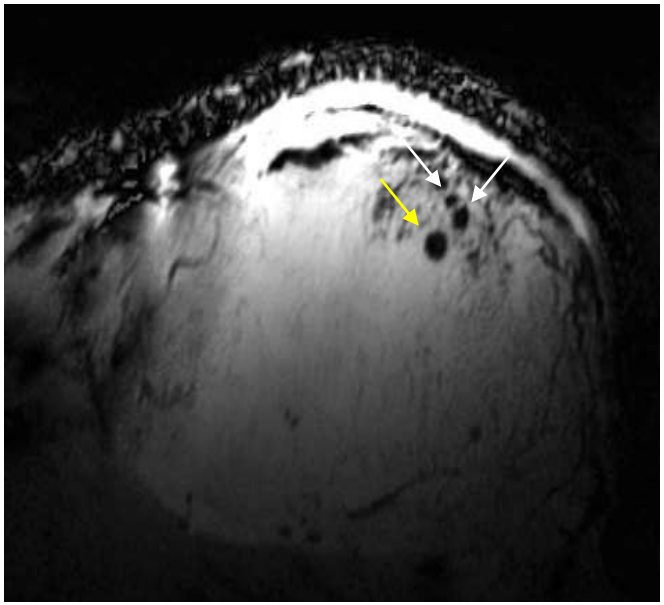
- Human progenitor cells
- Labeling with ionic particles
- Human implanted head and neck tumor
- MRI 21 days post implantation
- and 7 post pericytes injection in the tumor periphery

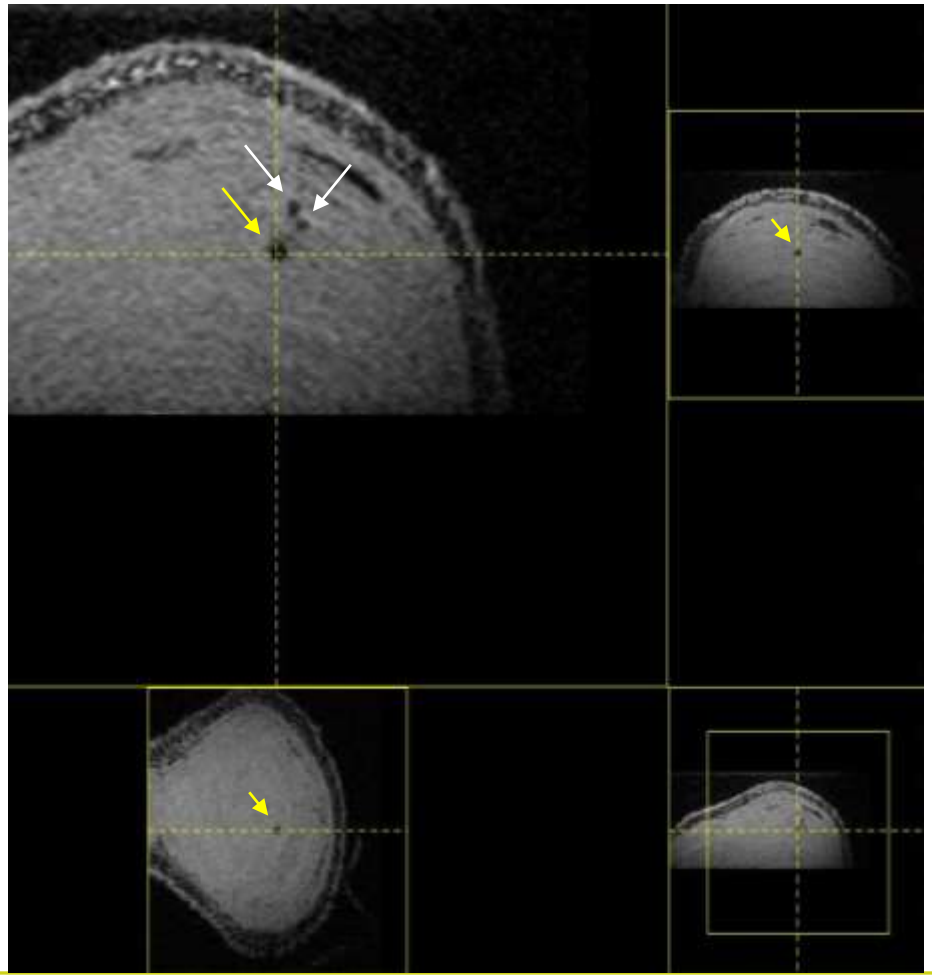
Labeled Mural progenitor cells (pericytes) in tumor angiogenesis (WIP)



Sequence 2D SWI

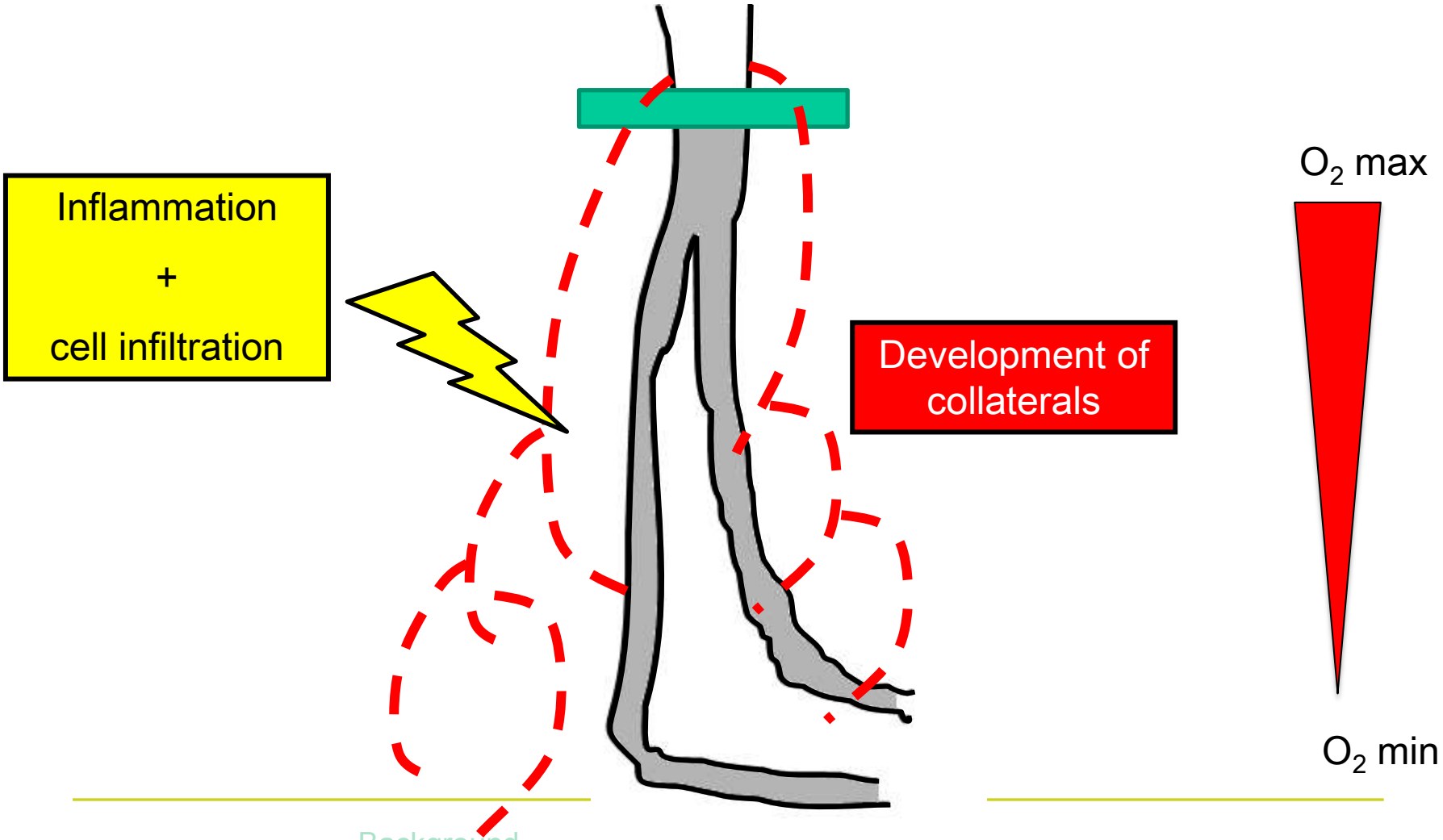
Labeled Mural progenitor cells (pericytes) in tumor angiogenesis





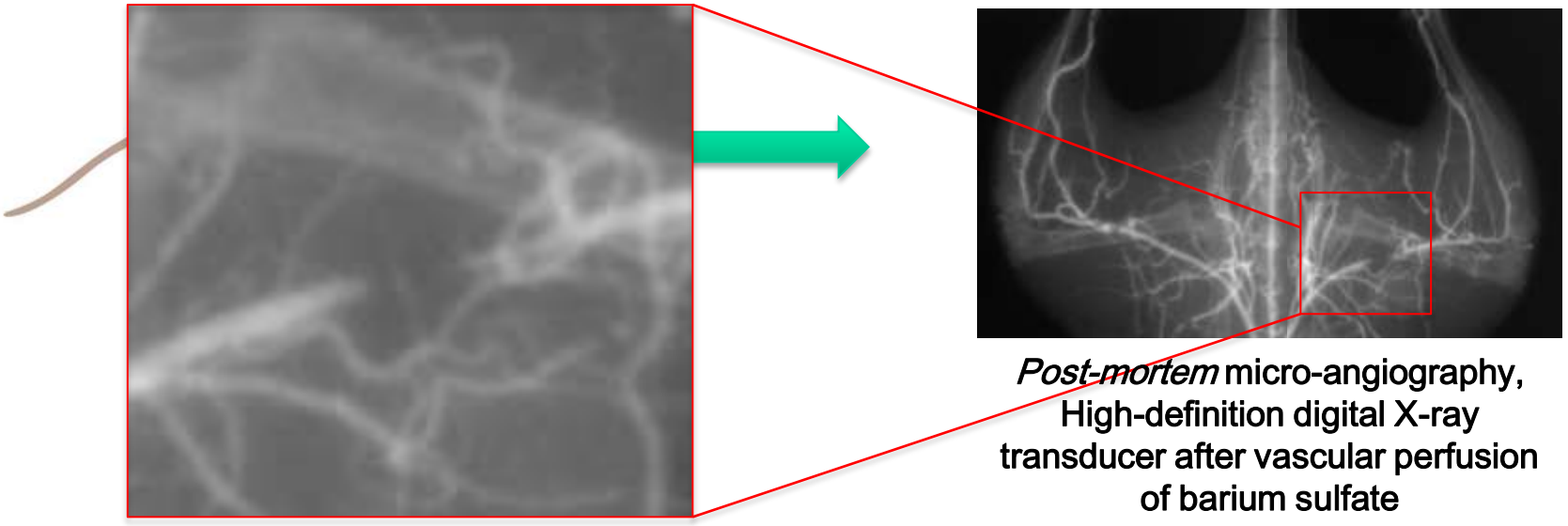
Sequence 3D FISP

resolution 50*50*50μm


Inflammation
+
cell infiltration
Development of collaterals
O_2 max
O_2 min

Pro-angiogenic role of monocytes
(collaboration with J Vilar and JS Silvestre)

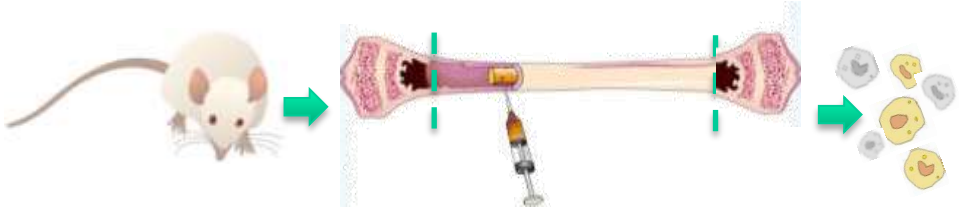
Pro-angiogenic and tissue remodeling activity in our model*



Post-mortem micro-angiography, High-definition digital X-ray transducer after vascular perfusion of barium sulfate

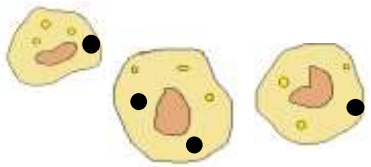
- Can we track the monocytes and their effect **non-invasively in real-time by HR-MRI?**

1. Extraction of bone marrow mononuclear cells



2. Monocyte isolation after gradient density centrifugation

3. Monocyte labeling with citrate coated iron oxide nanoparticles

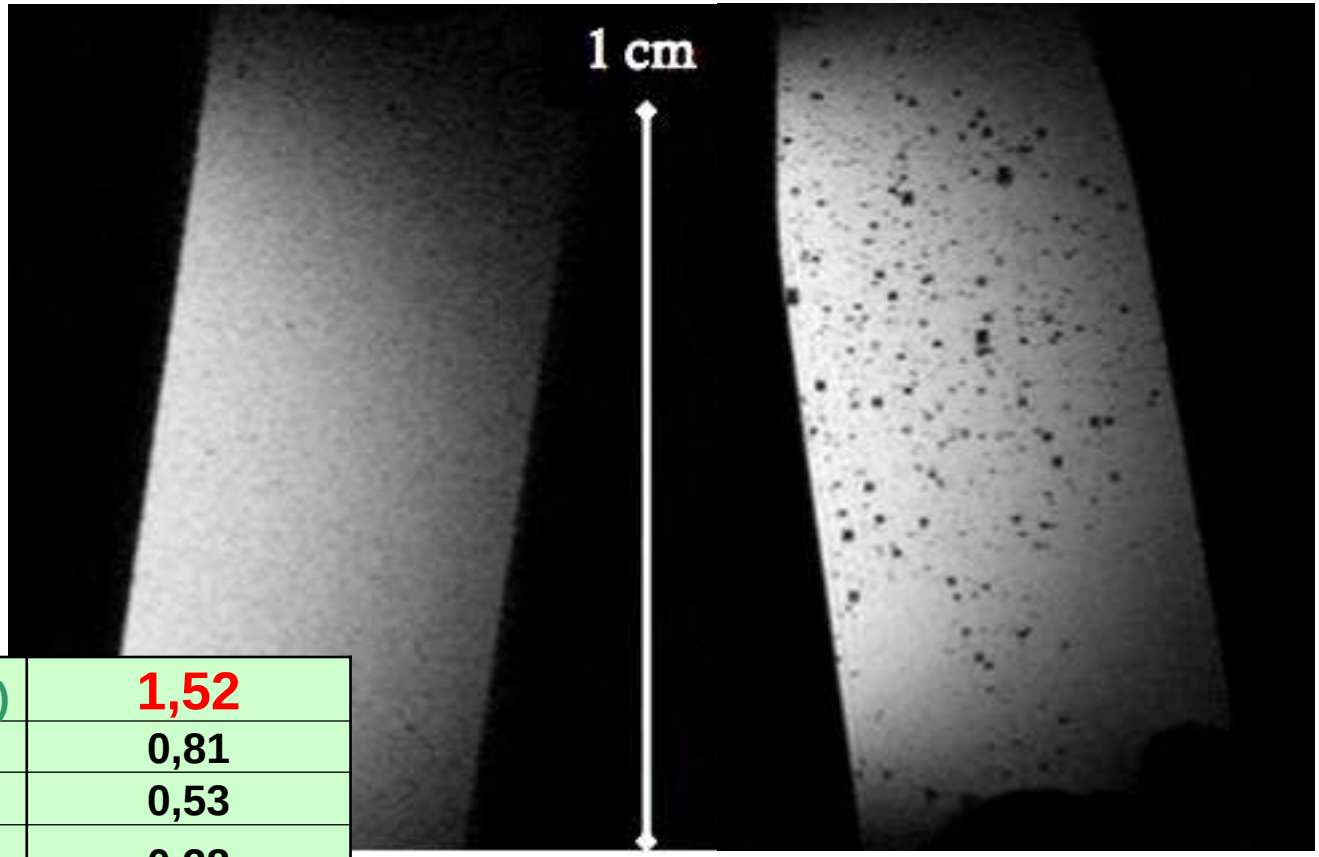


4. I.V. injection to mice with ischemic paw

- HR-MRI (D1,D2),
- microangiography (D14)

Objectives

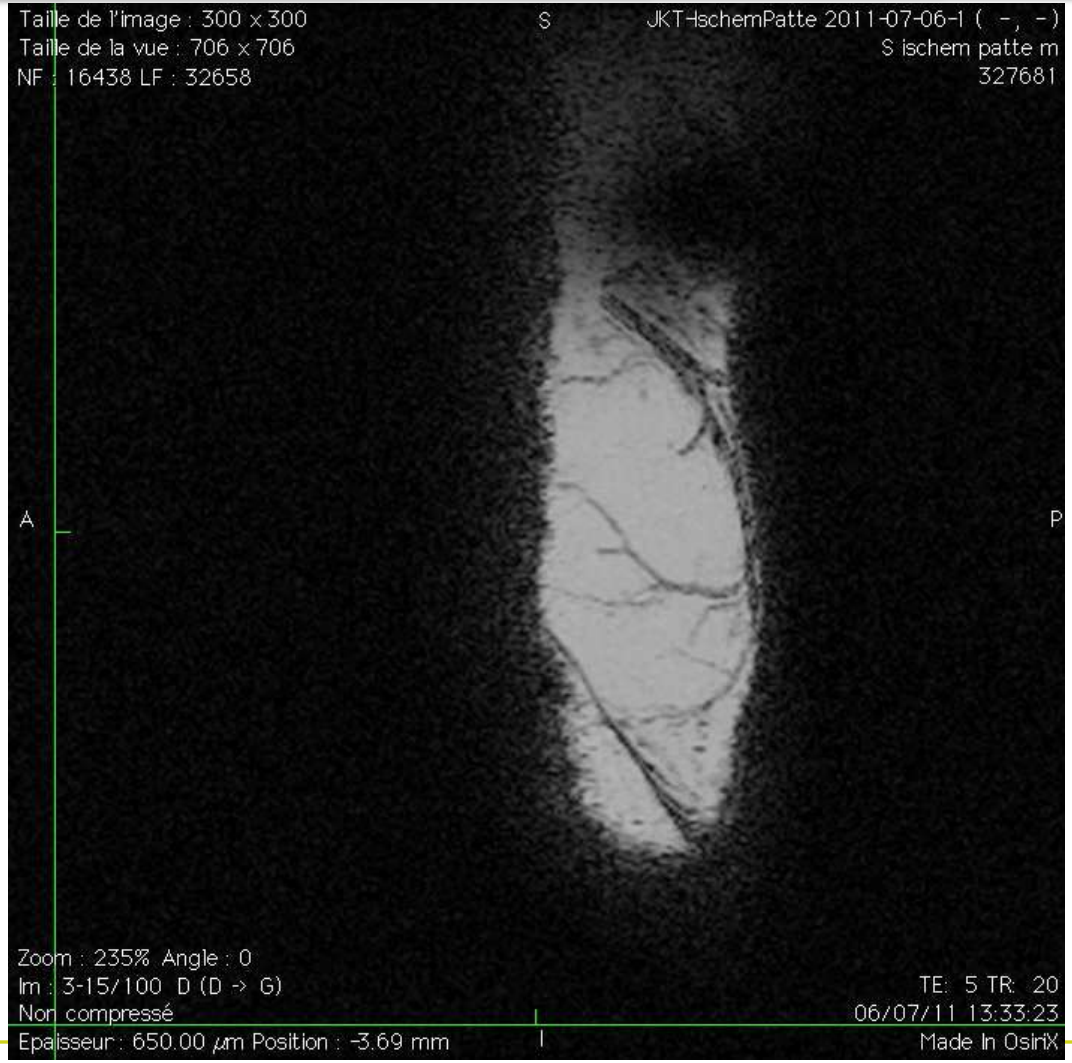
- Evaluation of **cell labeling** with anionic maghemite nanoparticles (D-1)
- Evaluation of **kinetics of infiltration** to the site of action by HR-MRI (D1, D2)
- Evaluation of the **pro-angiogenic effect** of labeled cells (D14)



mean mFe(pg)	1,52
SD	0,81
Δm/m	0,53
min mFe(pg)	0,38
max mFe(pg)	3,71

MinIP 25 slices (d =1.25 mm)

Evaluation of kinetics of infiltration:
In vivo follow-up (D1)



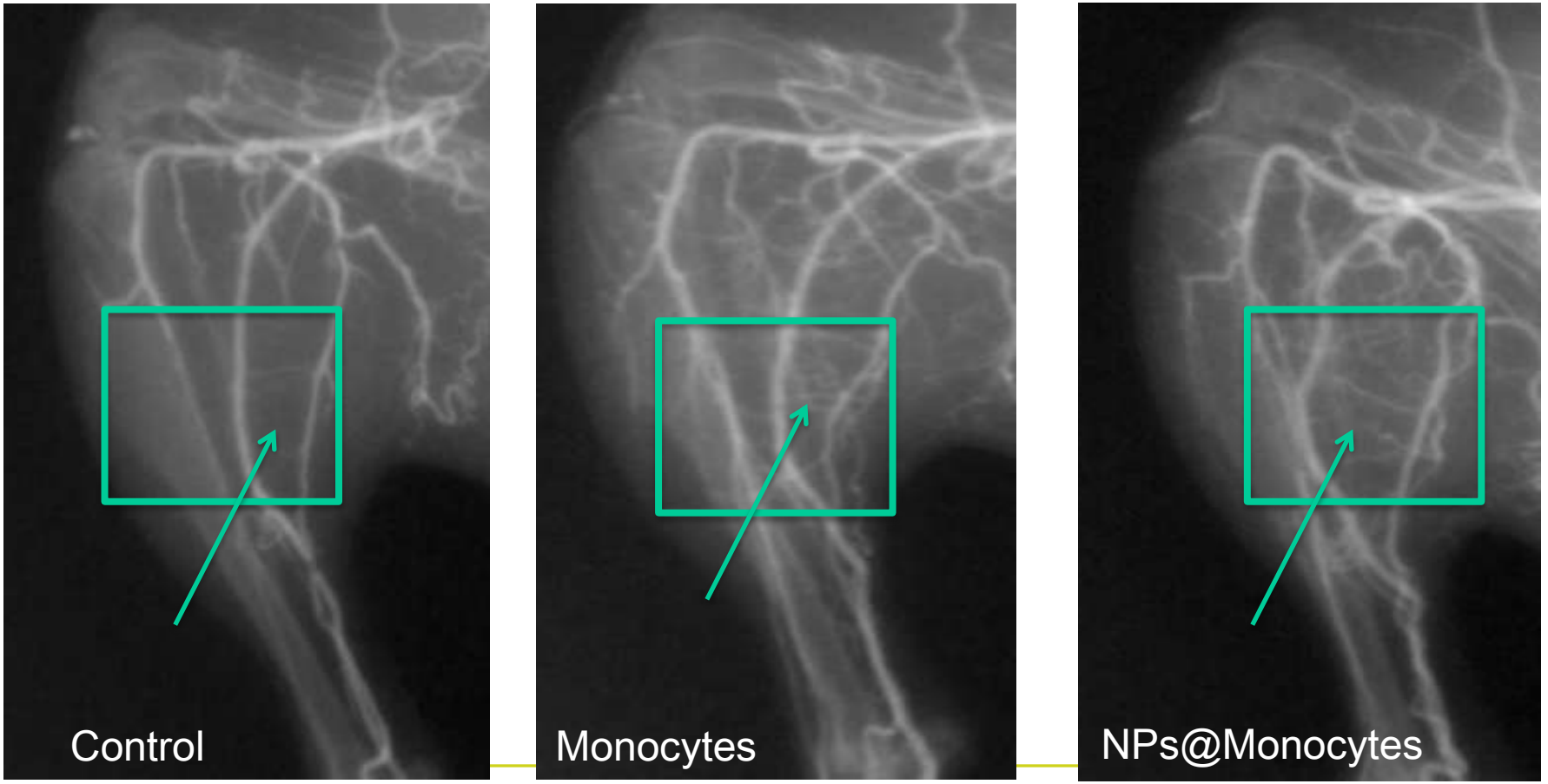
FOV = 1.5 cm

MinIP 13 slices

Evaluation of the **pro-angiogenic effect:**

Post-mortem angiogenesis evaluation (D14)

(High-definition X-ray after vascular perfusion of barium sulfate**)**

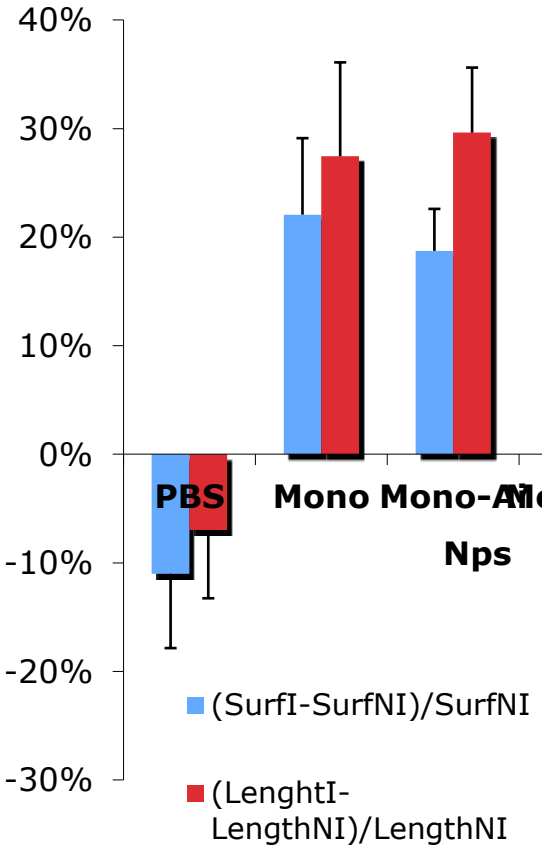


Evaluation of the pro-angiogenic effect:

Quantification of neovascularization (D14)

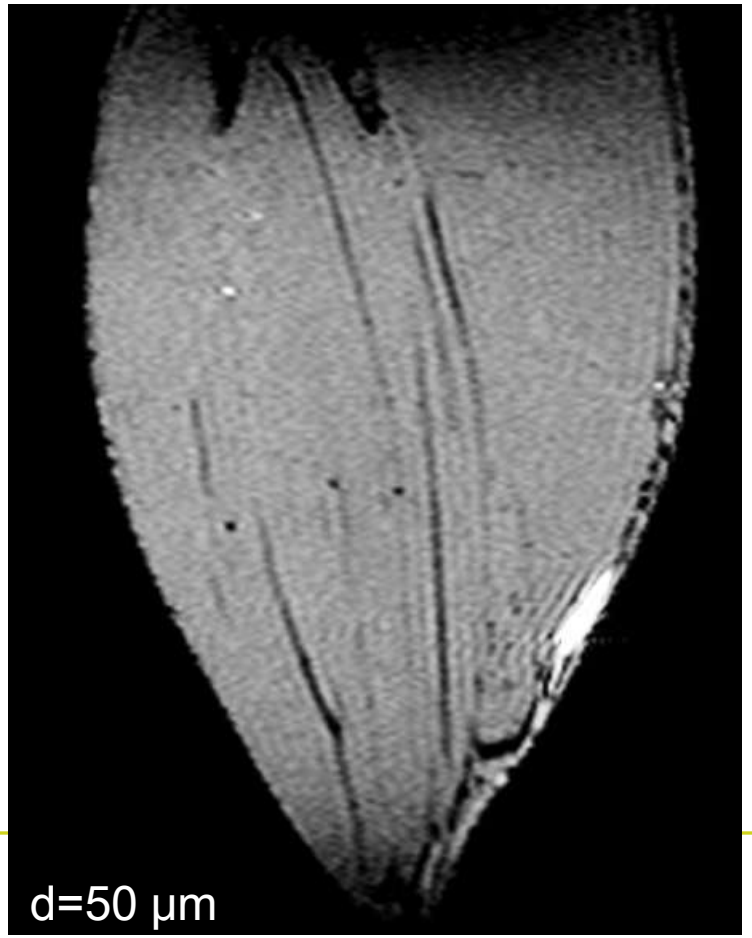
by Primedangio software

	Surf I/NI	± SD	Length I/NI	± SD
PBS (N=8)	0,890	0,069	0,930	0,063
Mono (N=8)	1,221	0,070	1,275	0,086
Mono-NPs(N=8)	1,187	0,039	1,296	0,060



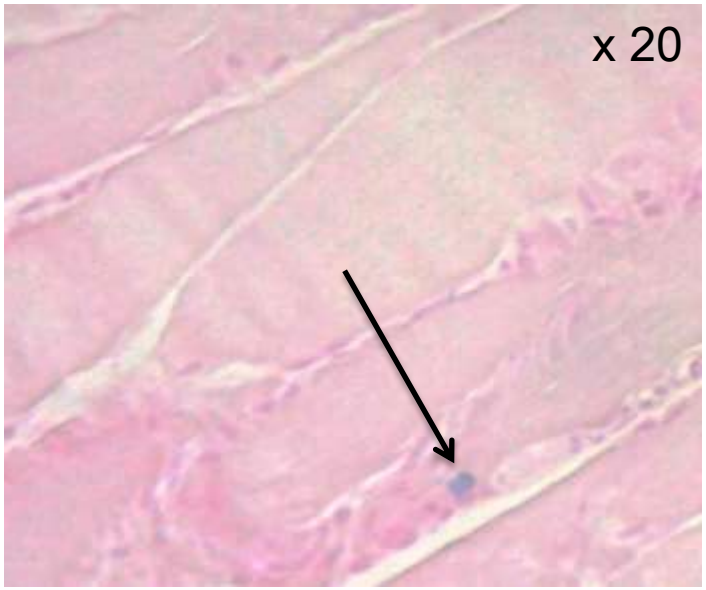
New study for histological correlation

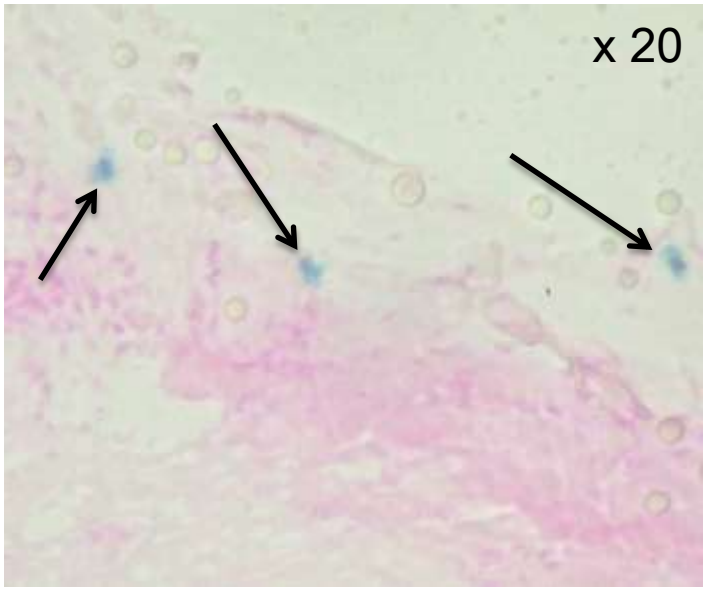
- injection of 5 mio cells/mouse (no ischemia) + magnet, N=5, (Control, N=1, Donor, N=2)
  - HR-MRI (left and right lower hindlimb)



Cryosections of 7 µm

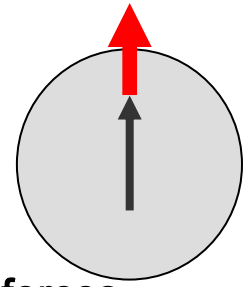
Nuclear Red and Pearls staining



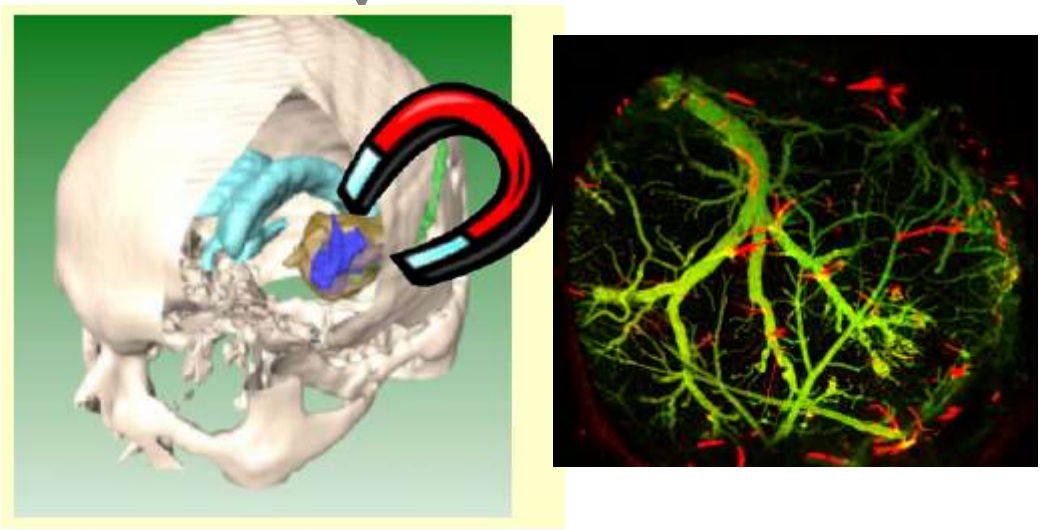


Cell manipulations

Grad B

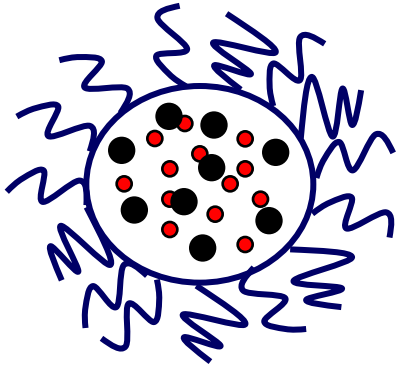


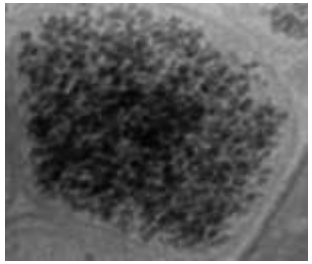
Remote magnetic forces



Manipulation and Targeting

MAGNETIC DRUG VECTORS

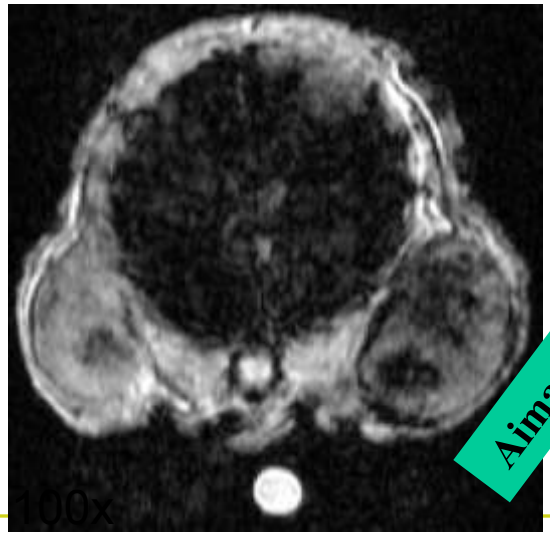


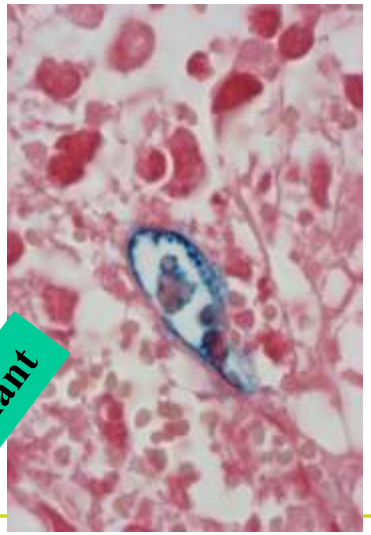


200 nm

Magnetic liposomes

Magnetic targeting … in a tumor



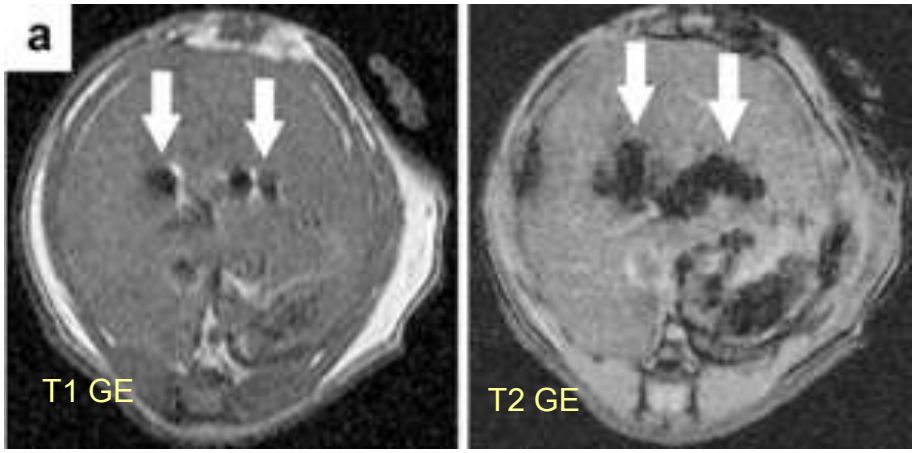


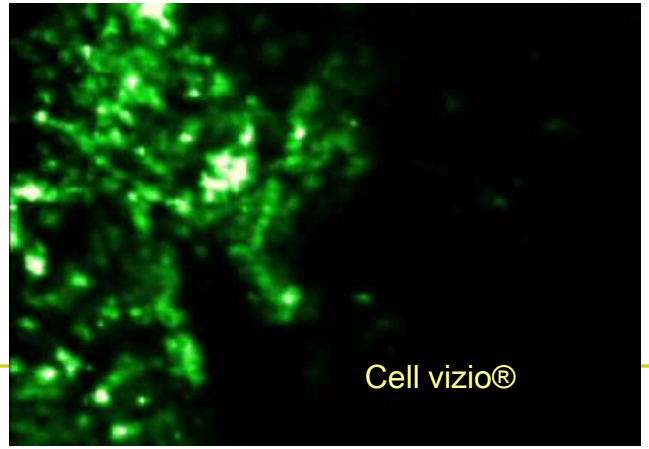
Fortin et al, Radiology (2006)

MAGNETIC TARGETING IN LIVER IMAGING

Hepatic cell transplantation : optimize cell delivery in the liver using magnetic forces

- HUH7 hepatoma cells injected in the spleen
- Dual labeling

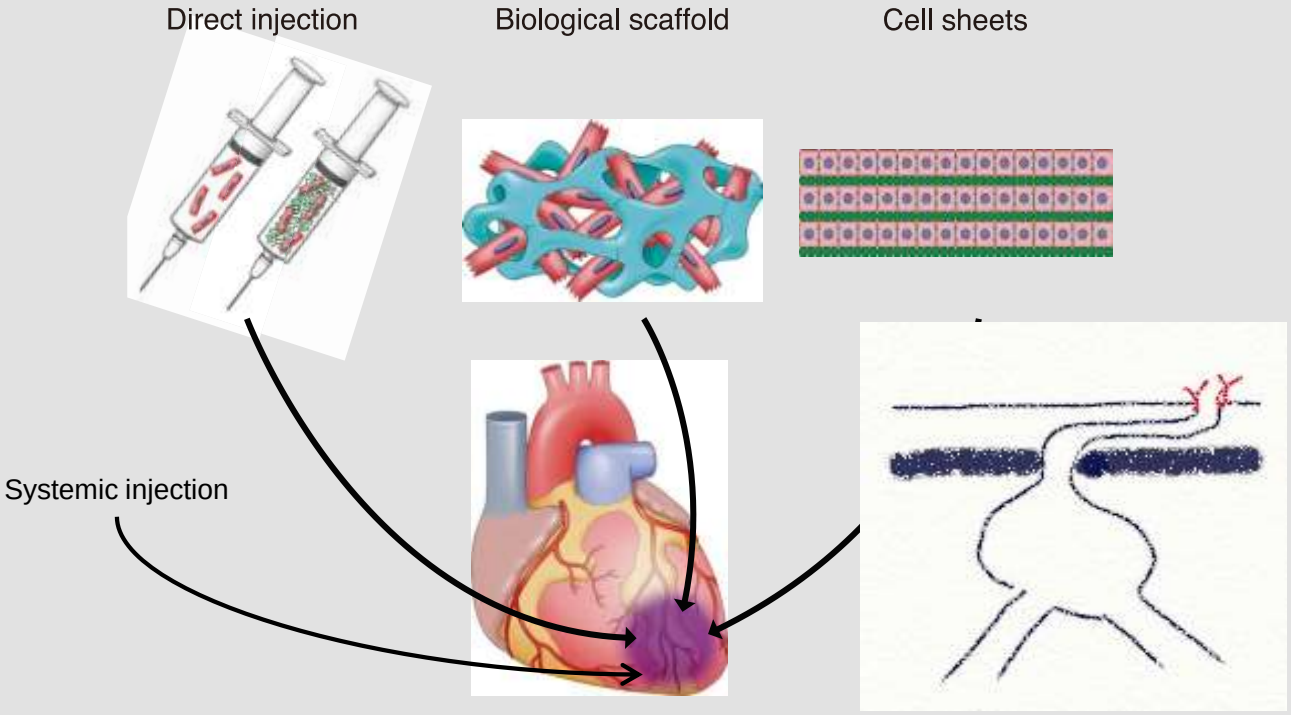




Luciani et al, European Radiology, 2009

Cellular and regenerative therapies : from cells to tissue engineering

- Regenerative therapy → restoring lost function
- Several delivery systems



Fujita et al. Am Jour Physiol Heart Circ Physiol 2012
Sekine, Shimizu et al. 2008; Itabashi, Miyoshi et al. 2005

Conclusion : cellular imaging

- Optimization of injection protocols for preclinical studies
- Follow up of therapy
- Enhanced homing with external magnets