Imagerie Cellulaire

O Clément

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Laboratoire Matières et Systèmes Complexes, CNRS UMR 7057
Tissue Regeneration

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

Potential uses of Stem cells

- Stroke
- Traumatic brain injury
- Learning defects
- Alzheimer's disease
- Parkinson's disease
- Baldness
- Blindness
- Deafness
- Amyotrophic lateral sclerosis
- Wound healing
- Bone marrow transplantation (currently established)
- Spinal cord injury
- Osteoarthritis
- Rheumatoid arthritis
- Myocardial infarction
- Muscular dystrophy
- Diabetes
- Crohn's disease
Imaging human cell repair *In vivo*

- **Cell Labeling**
- **Implantation**
- **Nucl Med**
- **Microscopy Optics**
- **MRI**
Approved drugs: Feridex®, Resovist® for liver MRI
Chemically synthesized nanomagnets (5-20 nm)

- Magnetic field source ($B_0$)
- Remote magnetic forces (Grad $B$)
- Nanoheaters ($B(\omega)$)

Detection Imaging
Manipulation and Targeting
Thermally activated therapy
• 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

Ferrofluid: particles suspension
- [Fe] 50 to 100 mM
- Maghemite Fe$_2$O$_3$ 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

Iron mass per cell

Label any cell type

- Predictible cell iron load
- High labeling efficiency
- Short incubation time (10 min – 2 hrs)
Biocompatibility: Cell Proliferation is not affected

Labelled cells share their magnetic load when dividing

Cellules Hela (2h, 10mM)
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, $\alpha= 60^\circ$, 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.

Transfer of labeled T lymphocytes (3 x 10^6)
In vivo single cell detection at 1.5 T

3D punctual hyposignals $\rightarrow$ 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 $\mu$m³, TE = 14 ms tps. acq. = 29 min)

Cooled with Helium
S/B * 8

Surface cool emit/transmit

Zone of interest 1 cm³

For mice only in vivo or in vitro
Labeled macrophages in calf inflammation

Cryoprobe FISP 3D FID

<table>
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<tr>
<th>R = 50 \times 50 \times 50 , \mu m</th>
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<td>10'43</td>
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Inflammation

Macs*

inflammation
c

control

a) After filtering

b) Segmentation

c) Labeled macrophages in calf inflammation
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
Development of collaterals

Inflammation + cell infiltration

Background

O₂ max

O₂ min
Pro-angiogenic role of monocytes (collaboration with J Vilar and JS Silvestre)

Pro-angiogenic and tissue remodeling activity in our model*

Background

- 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)

Materials and methods
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)
Results

MinIP 25 slices (d =1.25 mm)

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<tr>
<td>mean mFe(pg)</td>
<td>1.52</td>
</tr>
<tr>
<td>SD</td>
<td>0.81</td>
</tr>
<tr>
<td>Δm/m</td>
<td>0.53</td>
</tr>
<tr>
<td>min mFe(pg)</td>
<td>0.38</td>
</tr>
<tr>
<td>max mFe(pg)</td>
<td>3.71</td>
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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)

Control

Monocytes

NPs@Monocytes
Evaluation of the pro-angiogenic effect: 
Quantification of neovascularization (D14) 
by Primedangio software

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<th>Surf I/NI ± SD</th>
<th>Length I/NI ± SD</th>
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<tr>
<td>PBS (N=8)</td>
<td>0.890 ± 0.069</td>
<td>0.930 ± 0.063</td>
</tr>
<tr>
<td>Mono (N=8)</td>
<td>1.221 ± 0.070</td>
<td>1.275 ± 0.086</td>
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<tr>
<td>Mono-NPs (N=8)</td>
<td>1.187 ± 0.039</td>
<td>1.296 ± 0.060</td>
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Results
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

Magnetic targeting ... in a tumor

Magnetic liposomes

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 $\rightarrow$ restoring lost function
- Several delivery systems

Sekine, Shimizu et al. 2008; Itabashi, Miyoshi et al. 2005

E. Blondiaux et al. Stem cells cardiac patches, ECR
Conclusion: cellular imaging

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