

Engineering bone with mesenchymal stem cells. A need of Omics analysis to enhance their survival and repair abilities.

Hervé PETITE, Ph.D

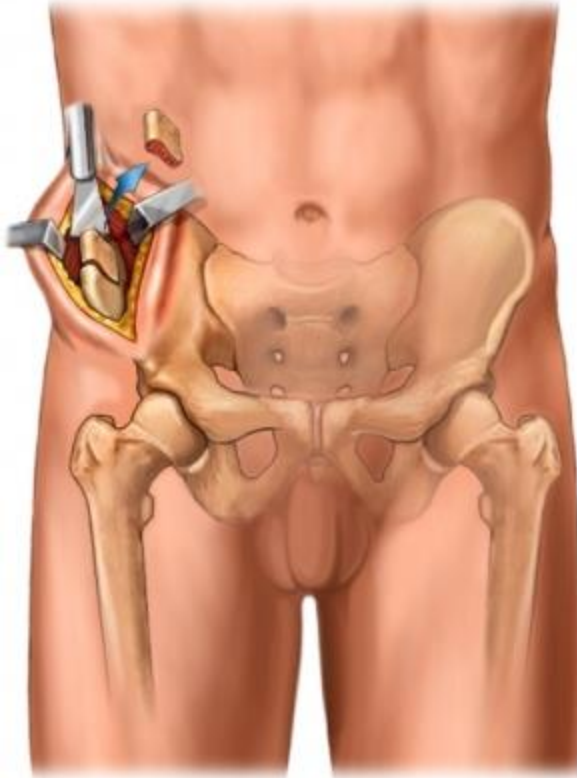
**B3OA CNRS 7052, Inserm 1271
Université de Paris,
Ecole Nationale Vétérinaire d'Alfort**



- Resection of large pieces of bone to treat benign and malignant tumours and osteomyelitis
- Traumatism
- Spinal arthrodesis
- Peri-prothhetic osteolysis
- Maxillo-facial surgery

Need for autologous bone graft

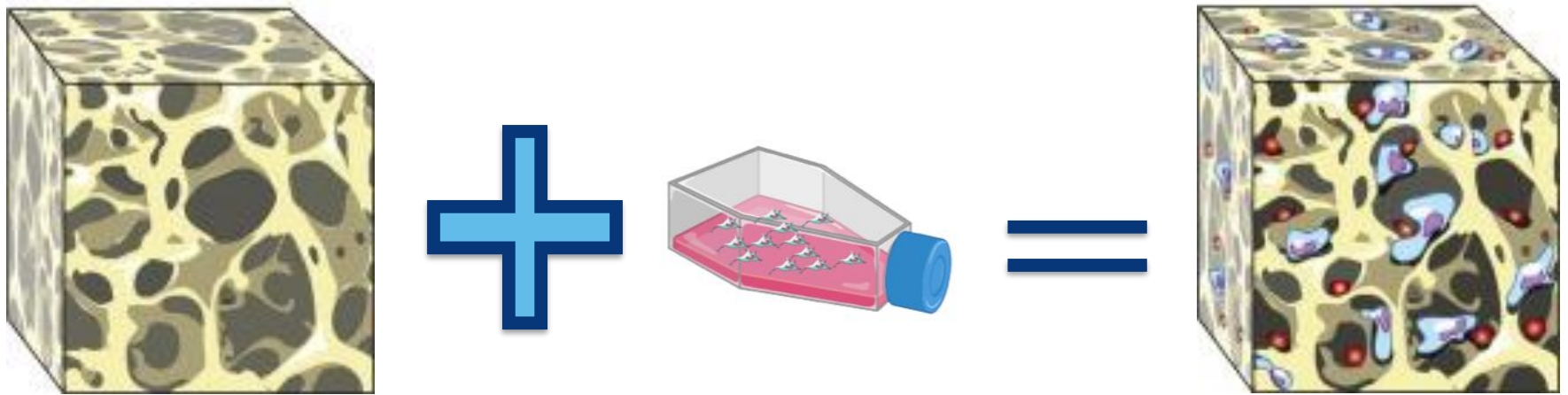
Limitations of autologous bone grafts



<http://www.itriagehealth.com>

- Morbidity at the donor site
(10-30% patients)
 - Post-operative continuous pain
(Damien 1991, , Arrington ED 1996, Cowley SP 1983, Prolo DJ 1985)
 - Pelvic instability
(Cowley SP 1983, Prolo DJ 1985, Coventry MB 1972)
 - Infection
(Lane JM 1999, Arrington ED 1996)
 - Paresthesia
- Amount of bone is limited

Stem-cell- based therapy

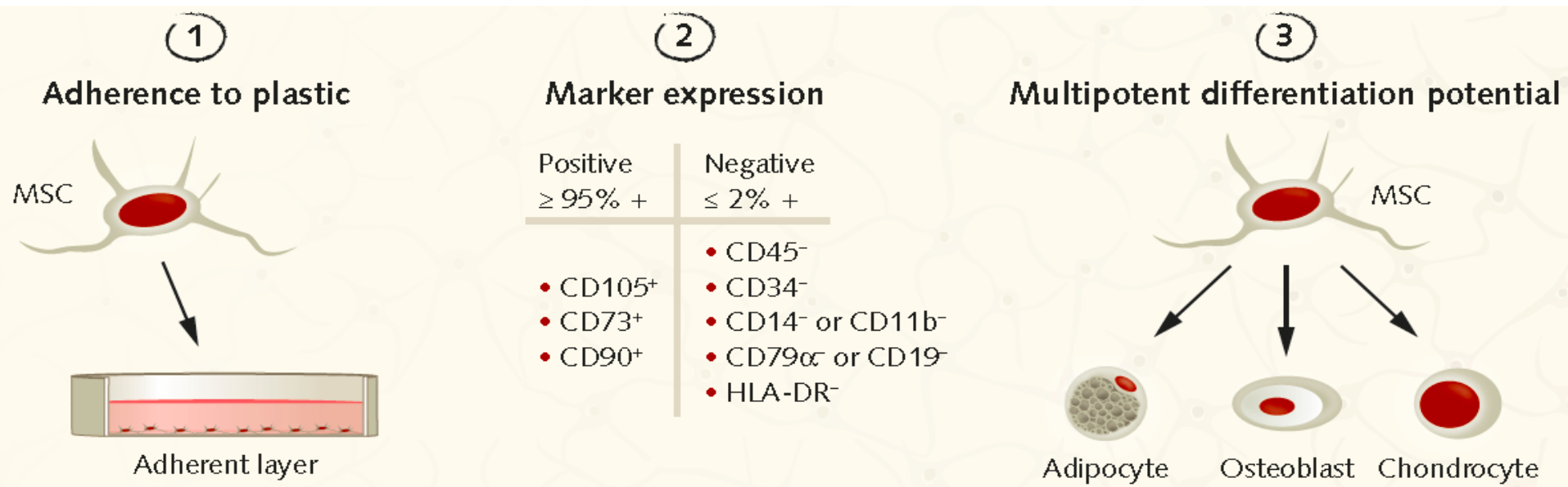


Stem-cell based tissue
construct



To decipher the role of scaffold chemical composition and internal architecture on the hMSC phenotype.

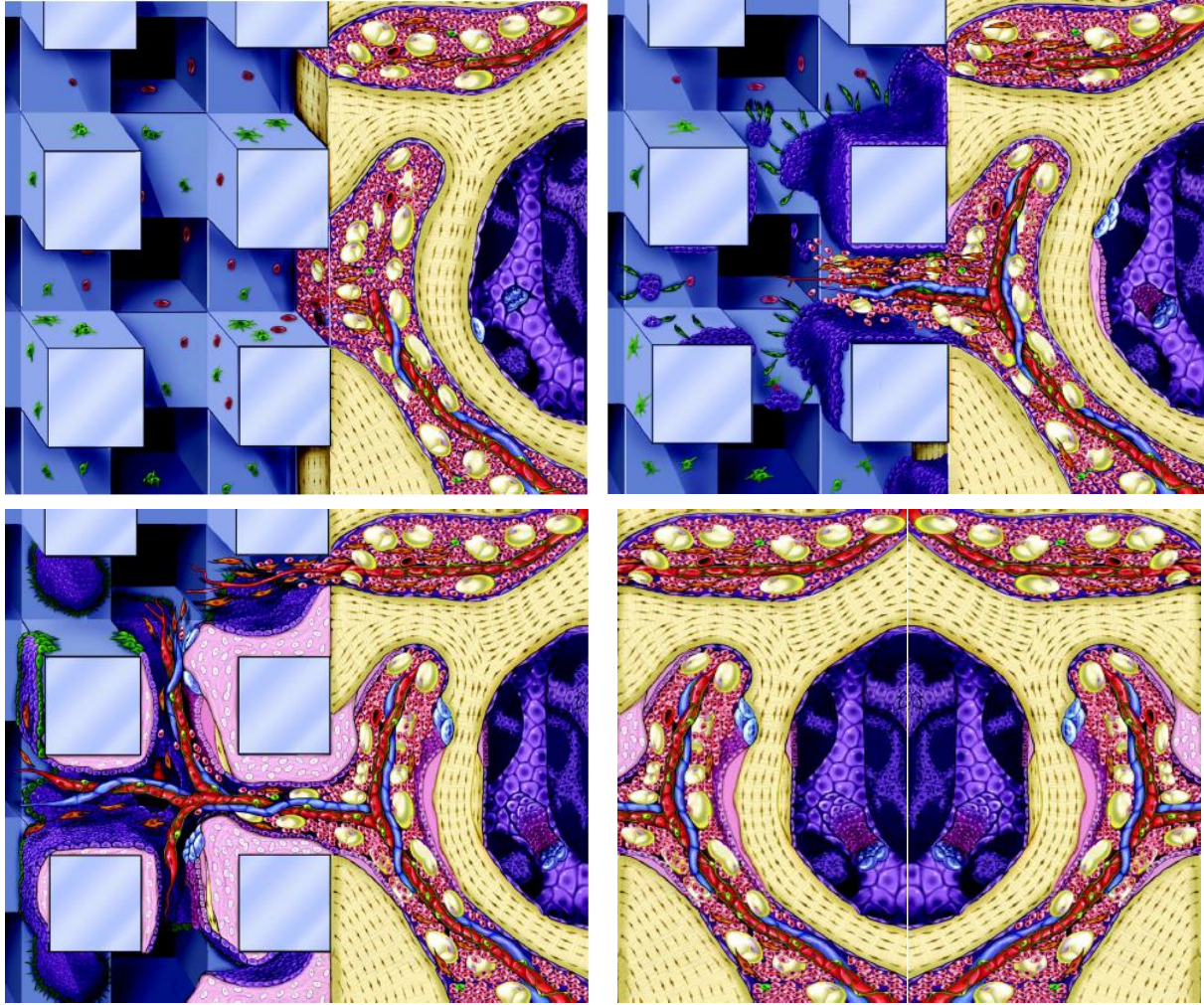
Bone marrow derived MSCs



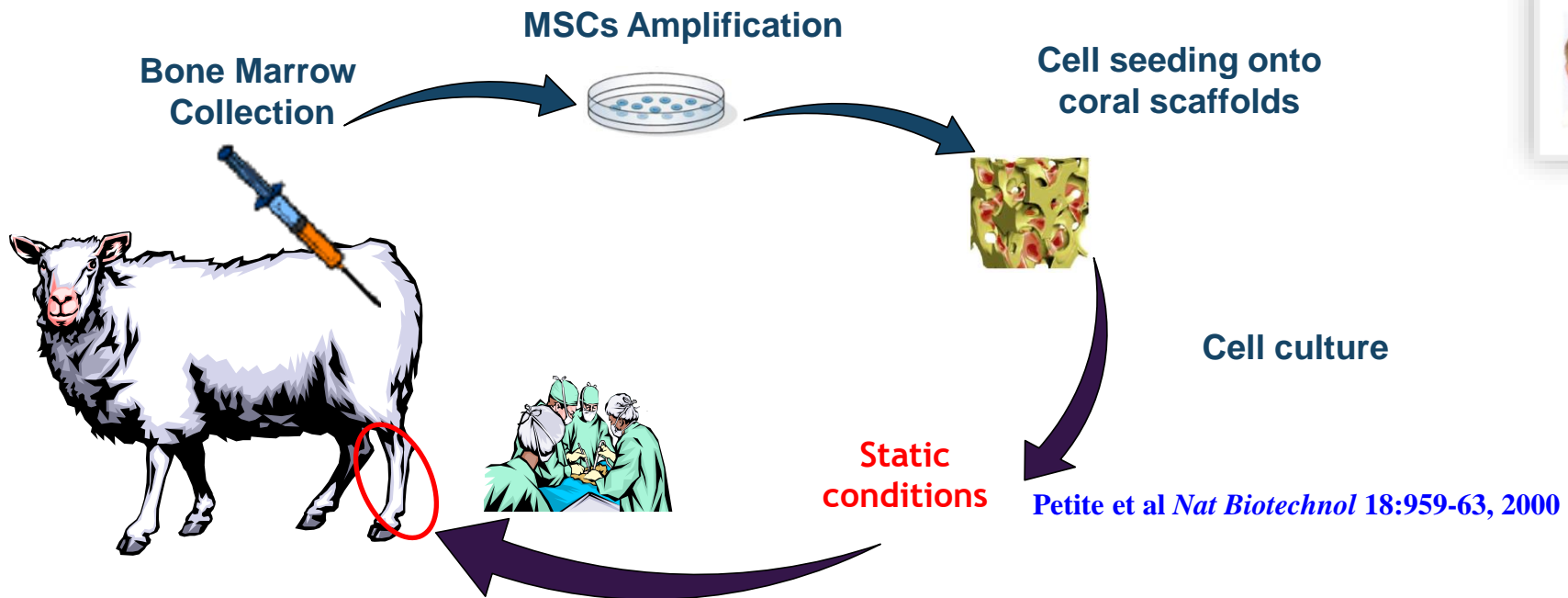
• 900 clinical trials are officially registered with MSCs.

* Mesenchymal stem cells, Multipotent stromal cells, Marrow stromal cells

The rationale for using MSCs



Proof of concept in a clinically relevant model



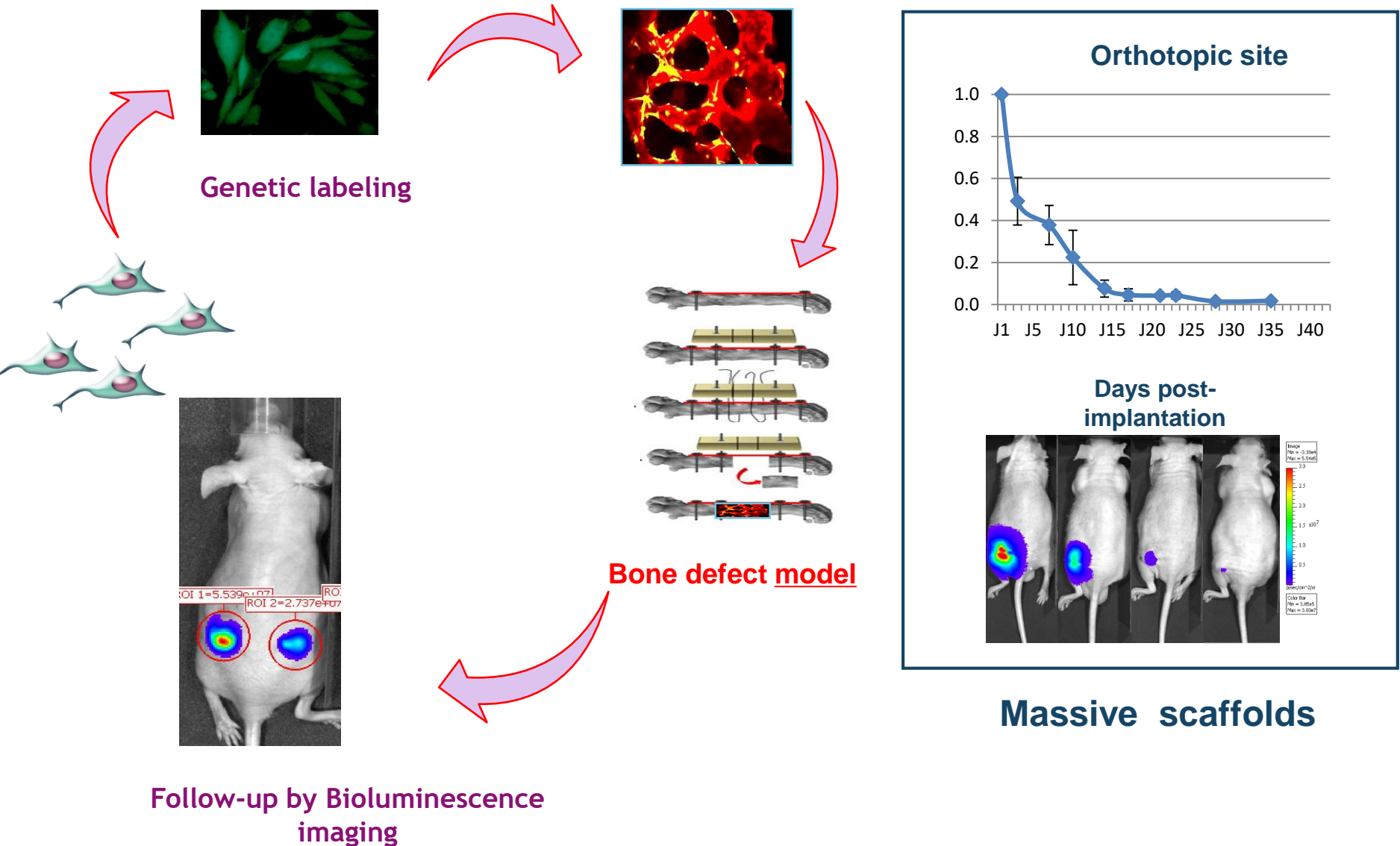
Proof of concept in large animals

- ✓ Bruder et al *J Bone Joint Surg Am* 80:985-96, 1998
- ✓ Marcacci et al *Calcif Tissue Int* 64:83-90, 1999
- ✓ Kon et al *J Biomed Mater Res* 49:328-37, 2000
- ✓ Petite et al *Nat Biotechnol* 18:959-63, 2000
- ✓ Bensaid et al *Tissue Eng* 11:814-24, 2005
- ✓ Viateau et al *J.Orthop.Research* 2007

Bone forming ability

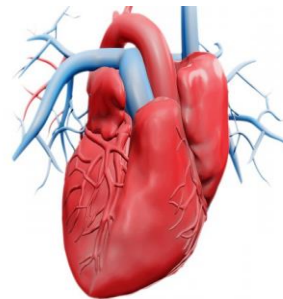
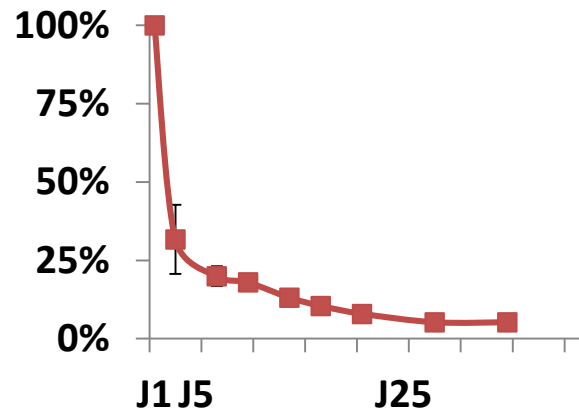
MSCs + scaffold > scaffold
MSC + scaffold < autograft

Massive hMSCs death in a femoral bone defect

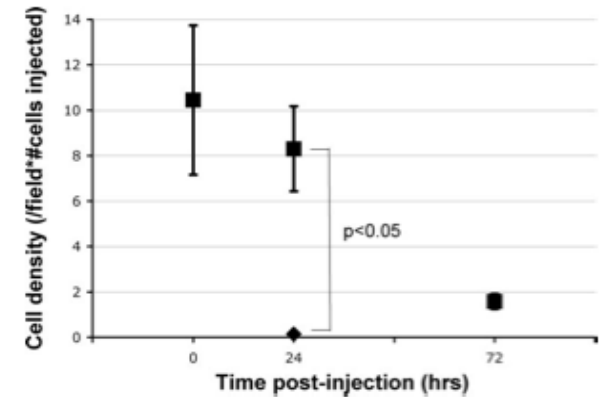


Massive MSCs death in thick engineered tissues

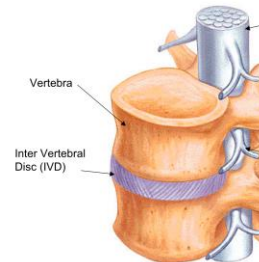
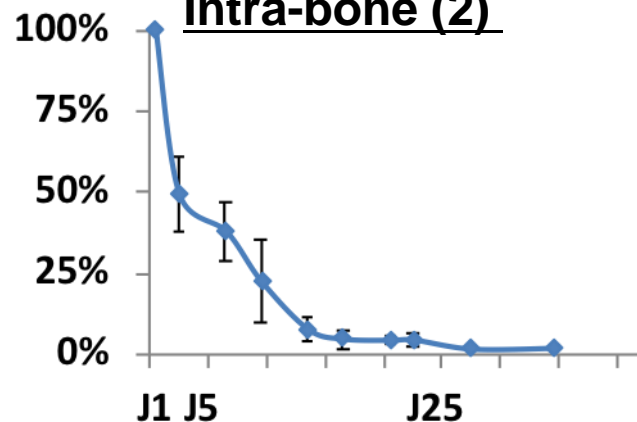
Subcutaneous (1)



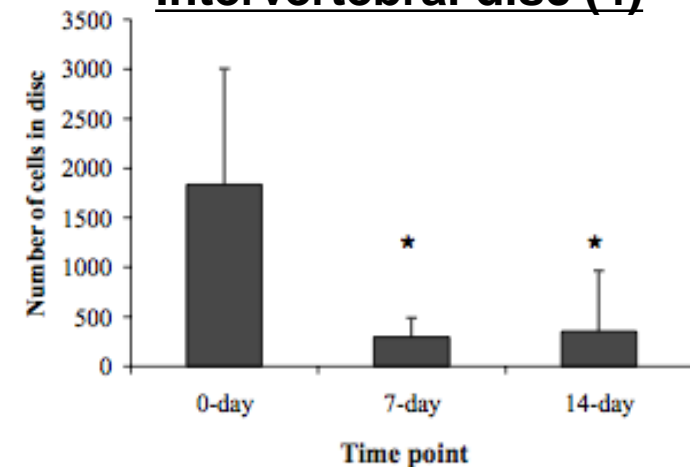
Intra-cardiac (3)



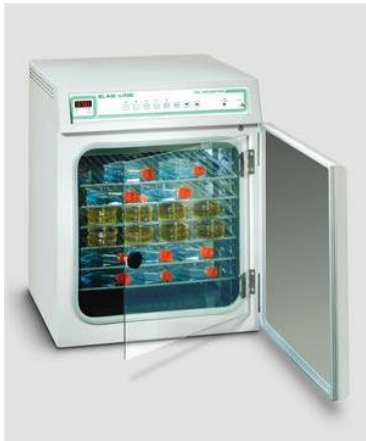
Intra-bone (2)



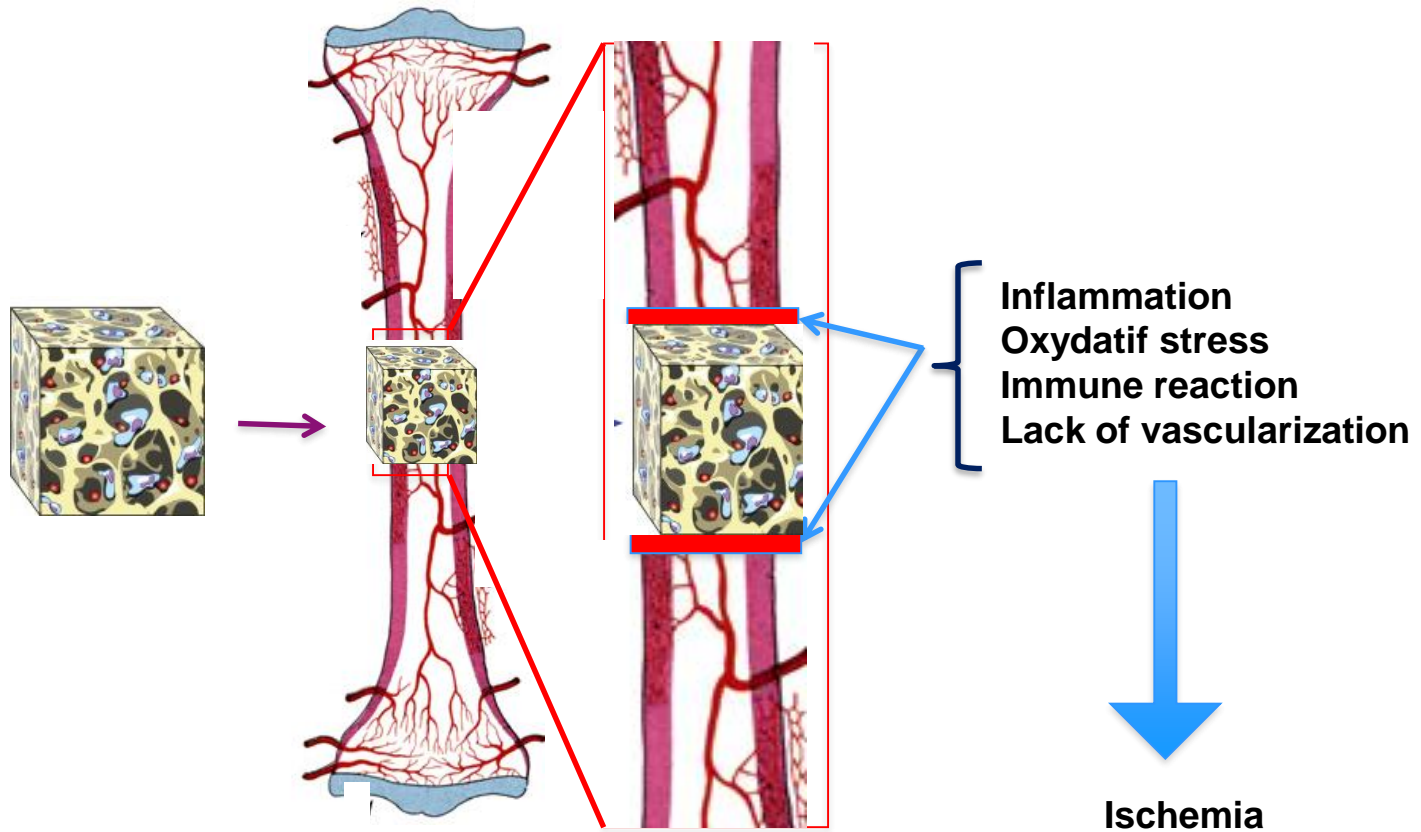
Intervertebral disc (4)



Why?



- ✚ High pO₂ (21%)
- ✚ Excess of nutrients



To characterize the liquid of the ischemic site to which MSCs are exposed.

Critical determinant(s) for *in vivo* hMSCs death?

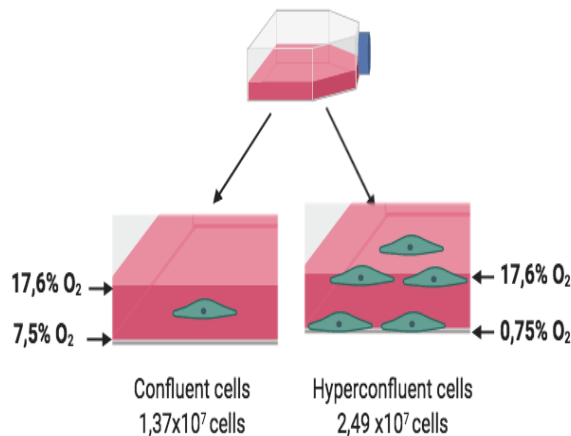
Oxygen?

Glucose?

3 reasons to suspect O₂

1- O₂ is poorly diffusive

Culture in normoxia
25cm² / 5 ml Medium



Pettersen 2005

2- Literature

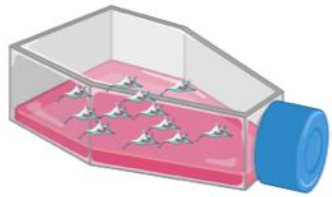
- « [...] hypoxia is a critical factor for MSC survival.” (Amini and Nukavarapu, 2014)
- “[...] importance of providing oxygen supply to preserve stromal cell viability.” (Davis et al., 2007)
- “Oxygenated environment enhances stem cell survival” (Benjamin et al., 2013)
- “ [...] oxygen limitation is believed to be one of the main causes of cell dysfunction and death in the post-transplantation period.” (Lazard et al., 2012)
- « [...] cells were competing for limited survival factors, e.g. oxygen delivery.” (Zhang et al., 2001)
- “[...] hMSCs exhibited a reduced rate under reduced oxygen tension compared with that under 21% oxygen.” (Holzwarth et al., 2010)

3- pO₂ = 0,1% in bone fracture

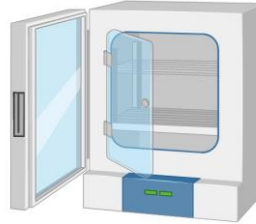
Current Paradigm: The lack of oxygen is hold responsible for cell death and numerous oxygen carriers are developped.

Our Hypothesis: Glucose (but not oxygen) is accountable for hMSCs death post-implantation.

hMSC survival relied on glucose in near anoxia



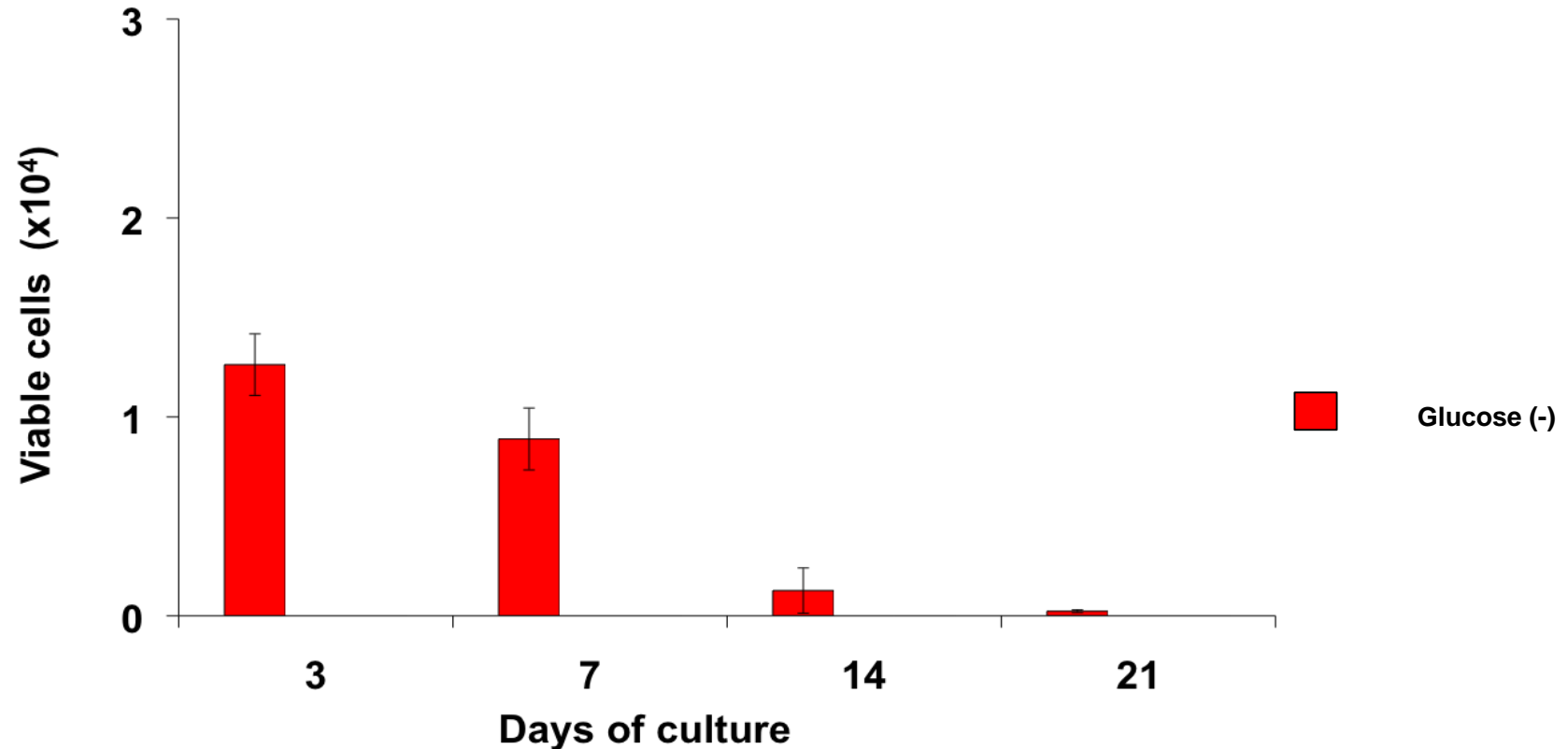
hMSCs cultured in MEM with or without glucose (no serum)



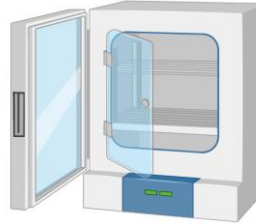
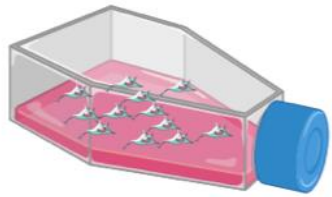
Hypoxic station at $pO_2 = 0,1\%$



Viability assessment



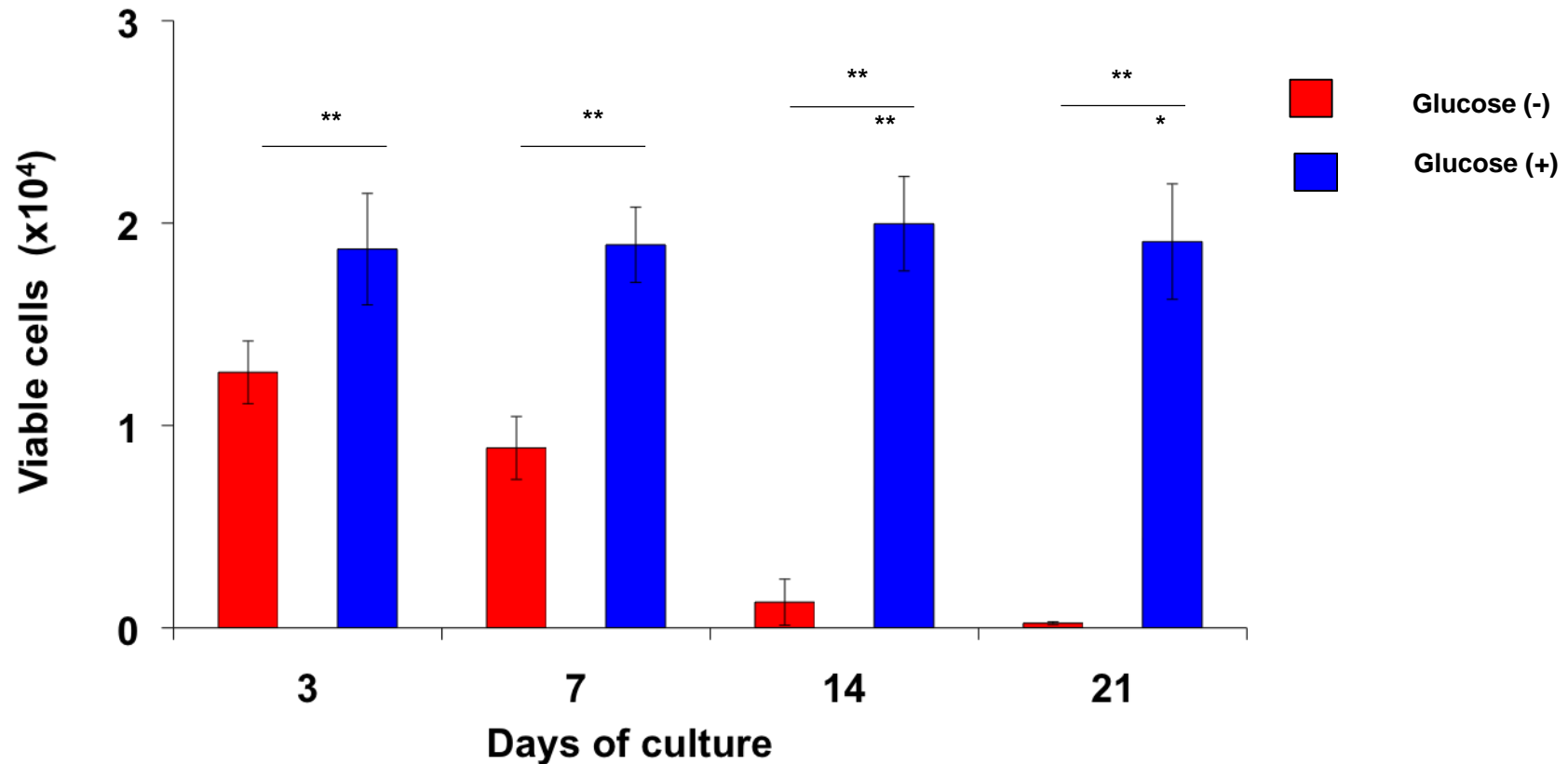
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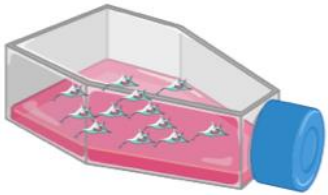
Viability assessment



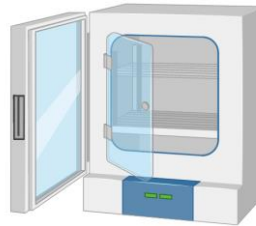
Recherches partenariales



- To decipher the role of glucose on cell death/cell survival-related pathways and secretory pathways.
- To further characterize the metabolism of MSCs in near anoxia.



hMSCs cultured in MEM with or without glucose (no serum)



Hypoxic station at $pO_2 = 0,1\%$

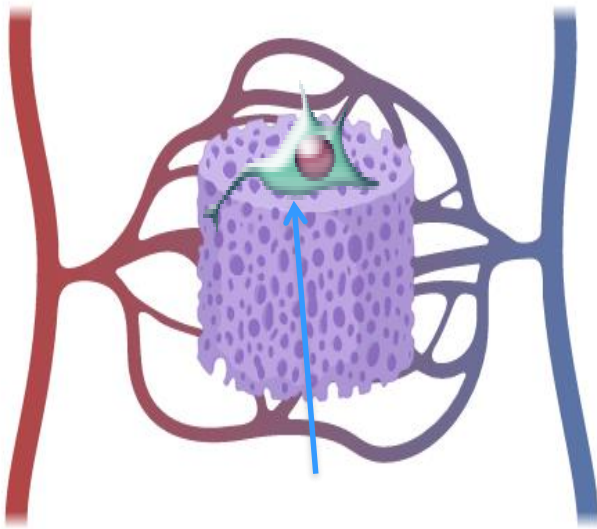


At day 1, 3, 7 and 14,

- Viability assessment
- Targeted proteomics
- Untargeted metabolomics

Recherches partenariales

Ultimate objective: To develop a biomaterial that provides nutrients to MSCs to ensure their survival and paracrine functions until the incoming vascular network takes over.



Viable and fonctionnal MSCs



To characterize the micro-environment faced by MSCs upon implantation and compare it to in vitro conditions in near anoxia.

- **Untargeted proteomics?**
- **Untargeted metabolomics?**

THANK YOU FOR YOUR ATTENTION

My contact:

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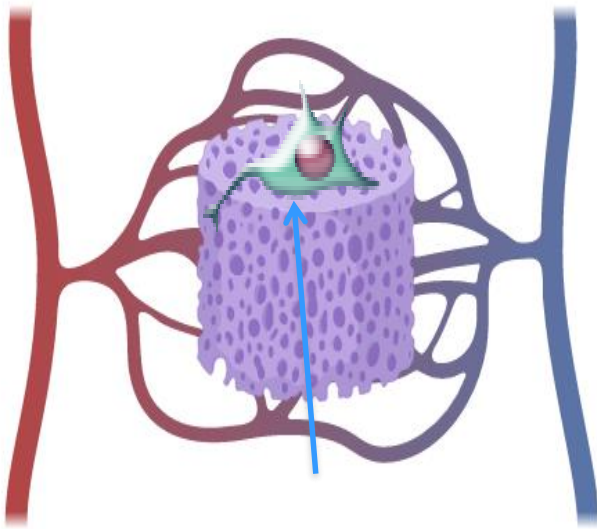
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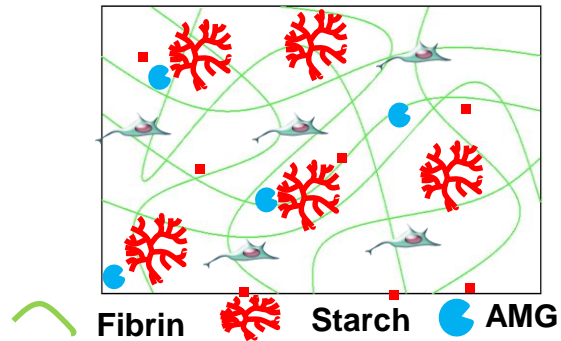
Skype :herve.petite |

Recherches partenariales

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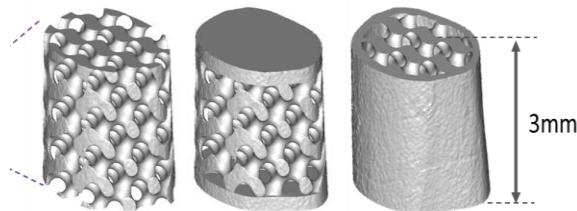
Viable and functional MSCs



Starch/AMG hydrogels

Collaboration

E. Pauthe, V. Laretta-Garde ERRMECe EA 1391



Ceramics prepared by additive manufacturing

Collaboration

D. Marchat, CIS, Sainbiose, INSERM U1059



Diffusion chambers