In Vivo Tracking of Systemically Delivered, Iron-labeled Mesenchymal Stem Cells in Rat with Myocardial Infarction with Magnetic Resonance Imaging

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“Adult” Cardiomyocytes

No potential for regeneration after birth

- No capacity to reenter cell cycle in adult mammalian heart

Rumyantsev PP, Int Rev Cytol, 1977

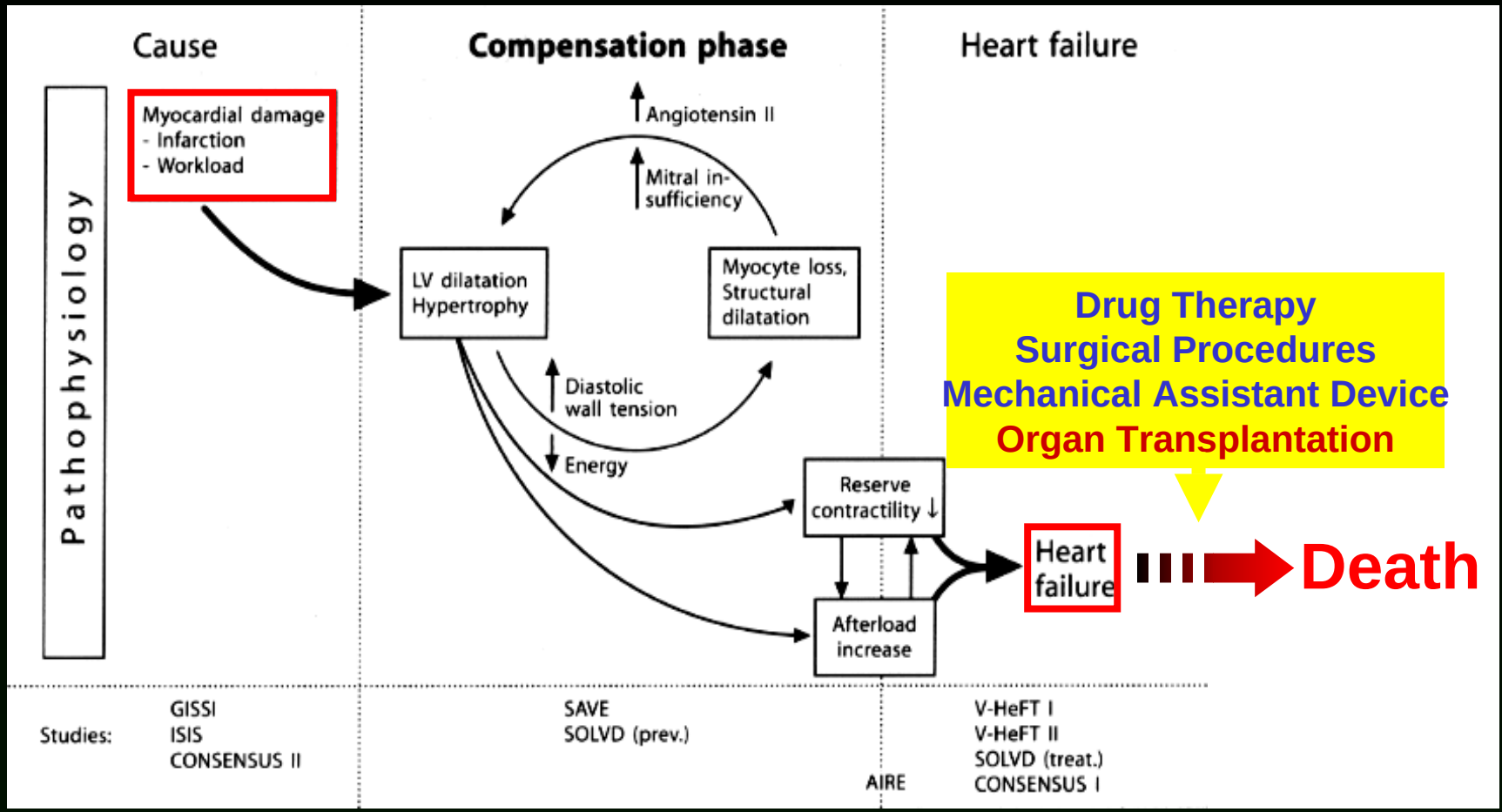
- Cardiomyocytes respond to mitotic signals by cell hypertrophy rather than by cell hyperplasia

Kodama H, et al., Circ Res, 1997
Pan J, et al., Circ Res, 1997

“Therefore”

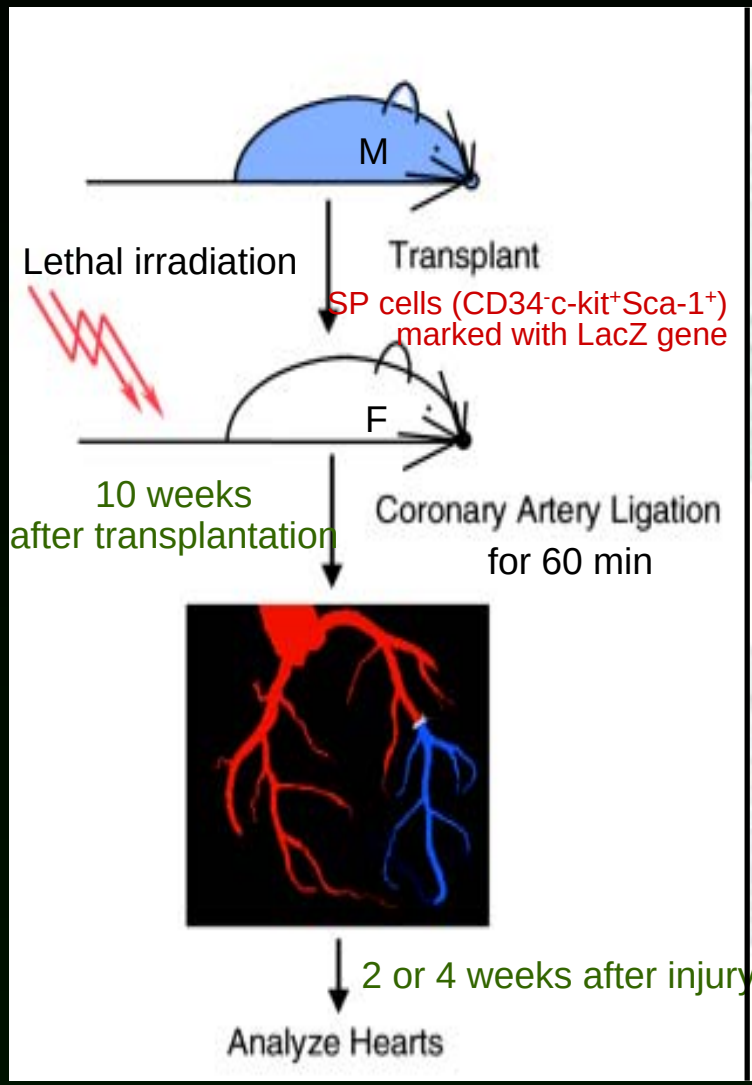
Loss of cardiomyocyte will result in permanent reduction of number of functioning units in myocardium.

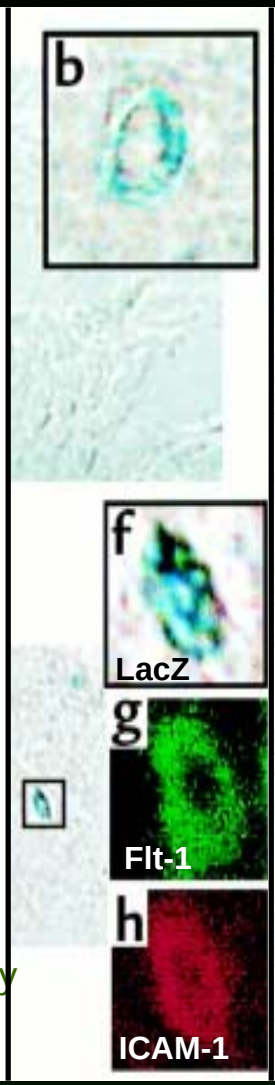
Progression to End-Stage Heart Disease

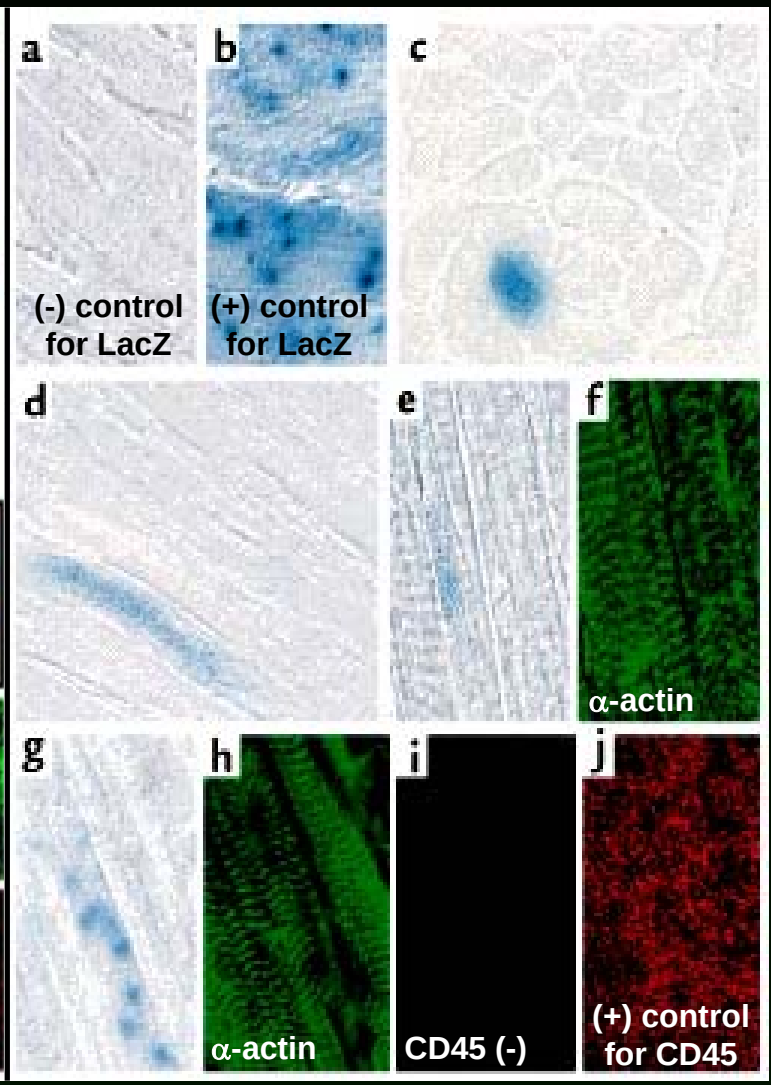


Regeneration of Myocardium & Endothelium by Transplanted BM Stem Cells

Jackson KA, et al., J Clin Invest, 2001

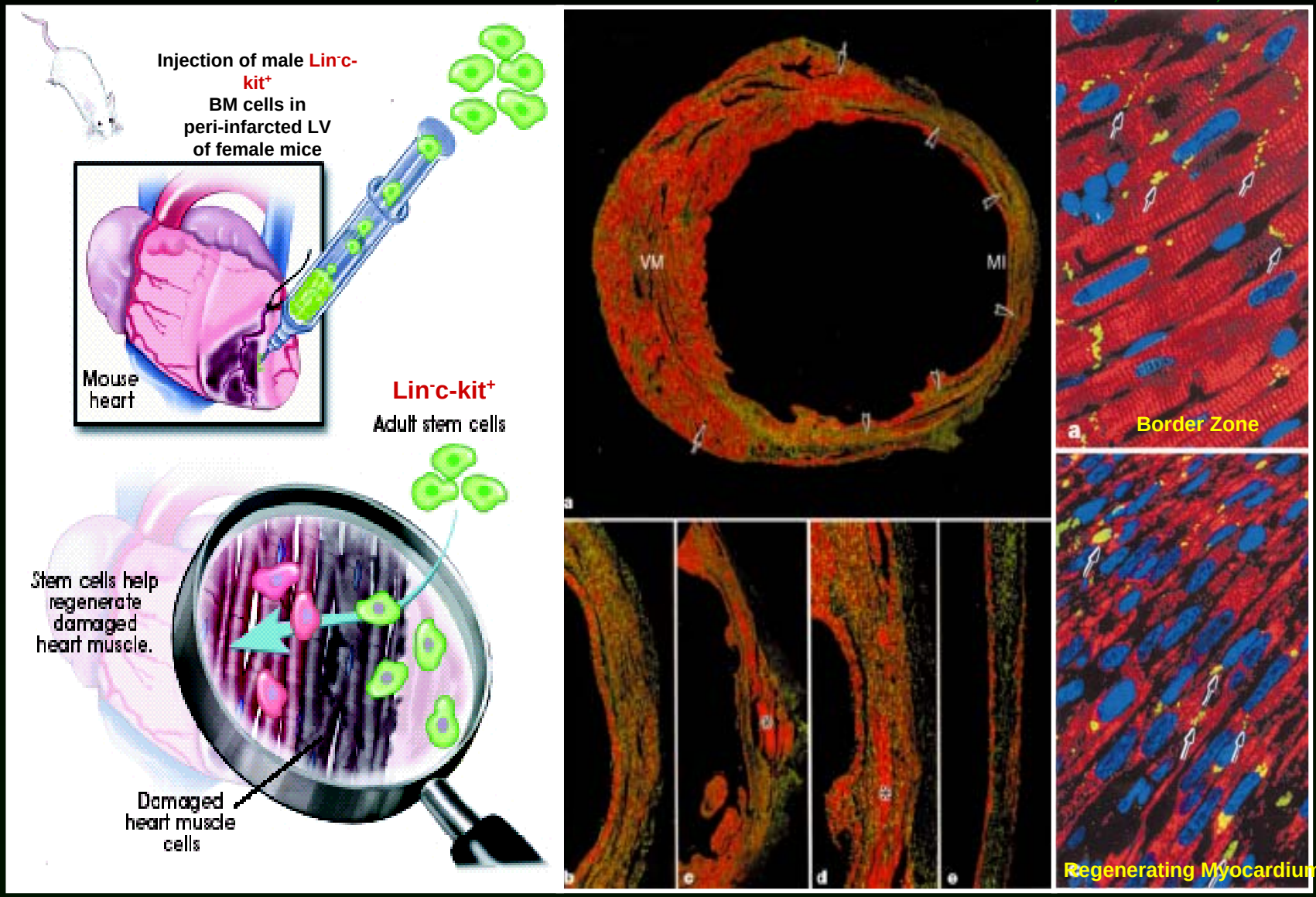






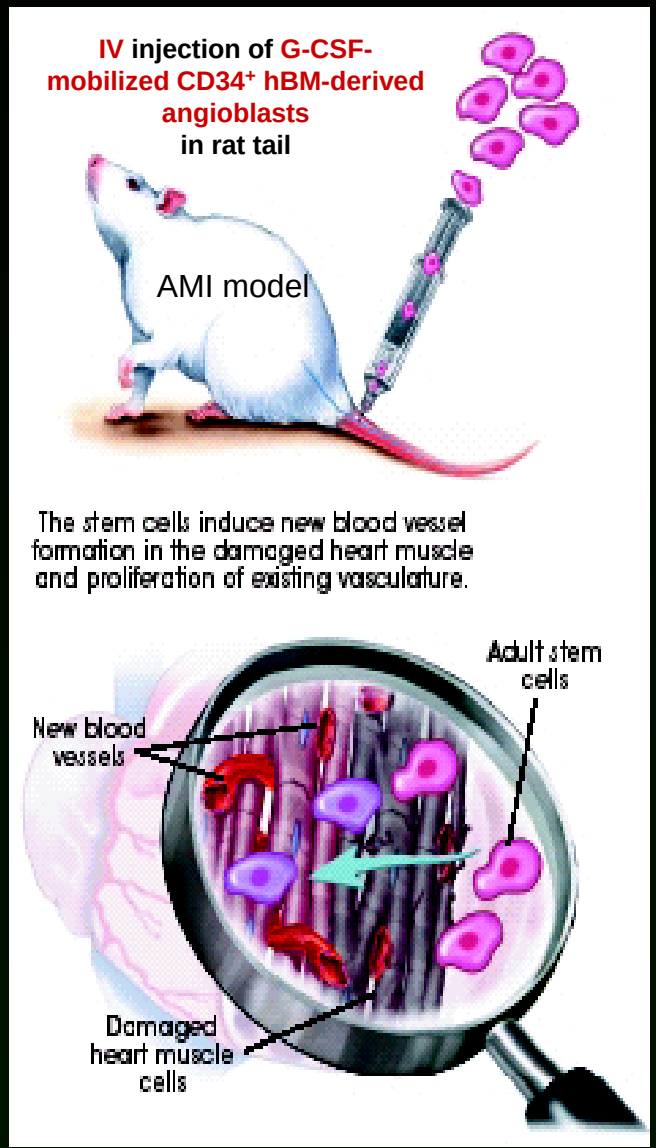
Locally Delivered BM Cells Can Regenerate *de novo* Myocardium

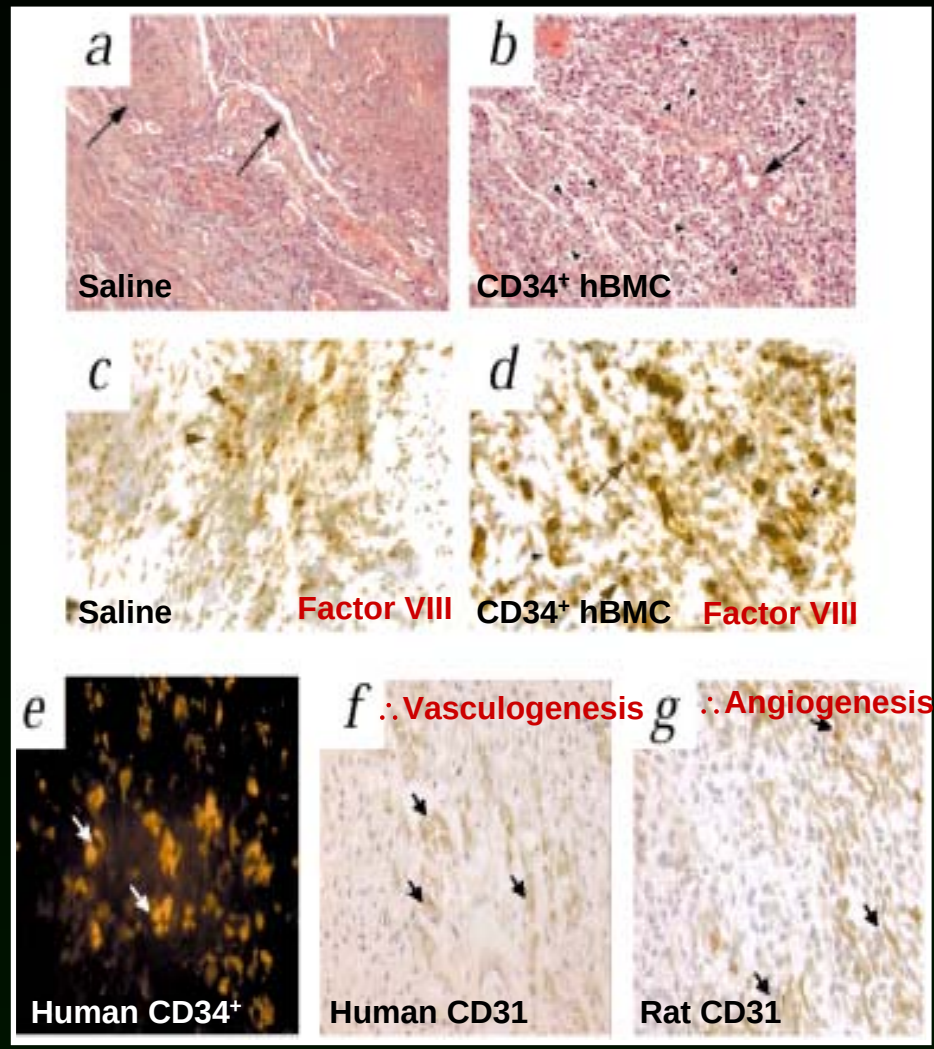
Orlic D, et al., Nature, 2001



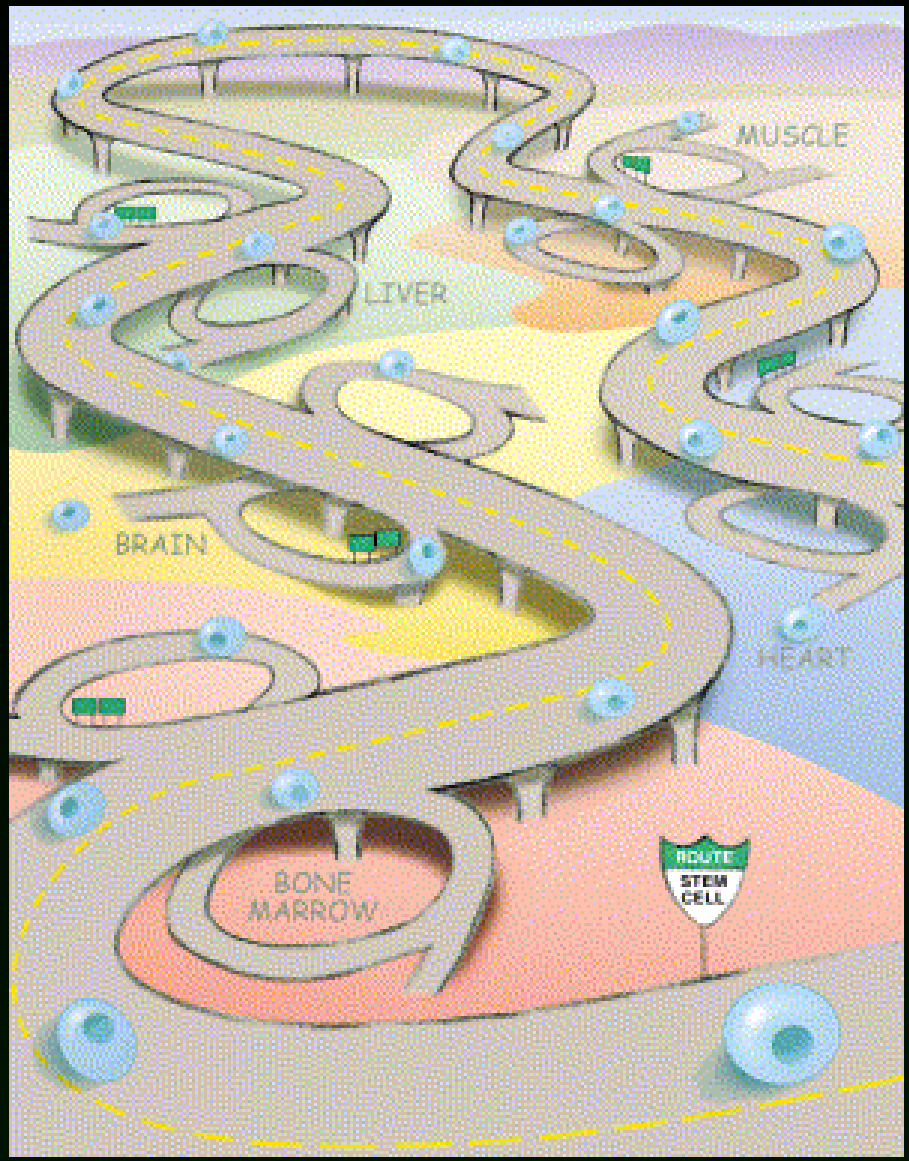
Neovascularization of Ischemic Myocardium by Systemic Injection of hBM-derived Angioblasts

Kocher AA, et al,. Nature Med 2001





Route Stem Cell



Approaches for In Vivo Tracking Transplanted SC "Prerequisite"

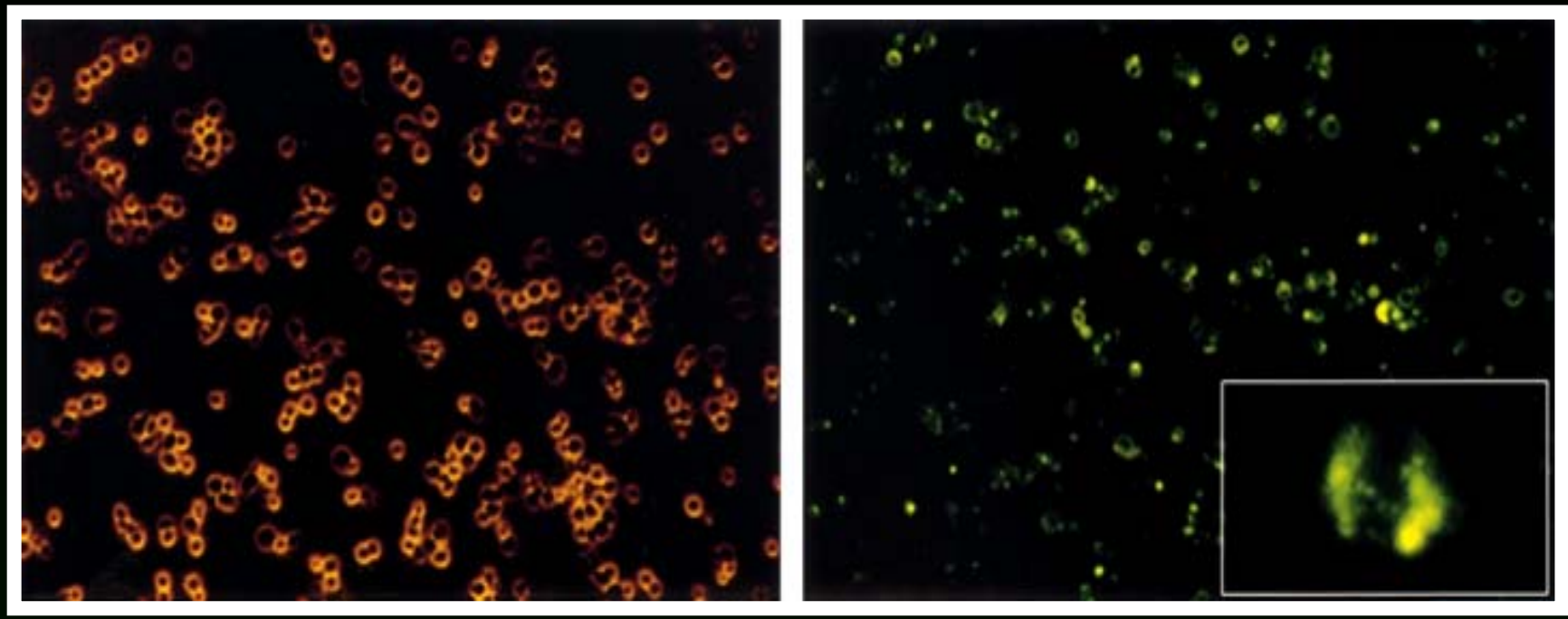
- Non-invasive
- Non-toxic to cells, esp. survival & differentiation
- Able to monitor cell migration
- Able to monitor tissue biodistribution of cells

Highly efficient endosomal labeling of progenitor and stem cells with large magnetic particles allows magnetic resonance imaging of single cells

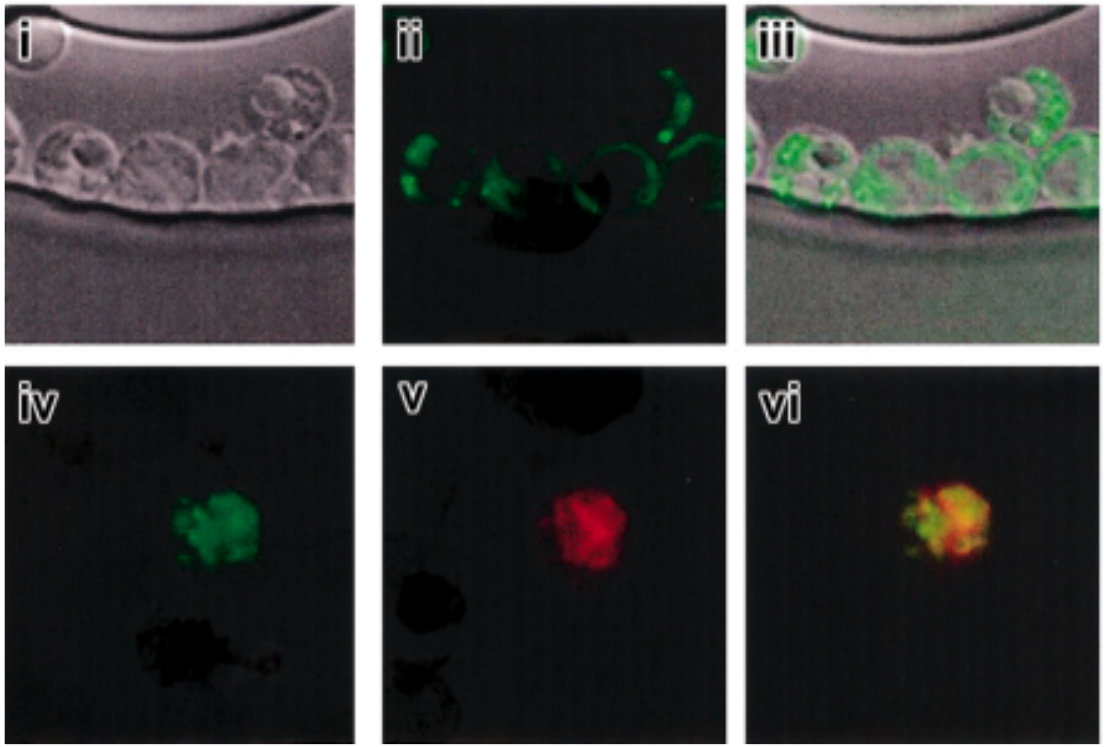
Kathleen A. Hinds, Jonathan M. Hill, Erik M. Shapiro, Mikko O. Laukkanen, Alfonso C. Silva, Christian A. Combs, Timothy R. Varney, Robert S. Balaban, Alan P. Koretsky, and Cynthia E. Dunbar

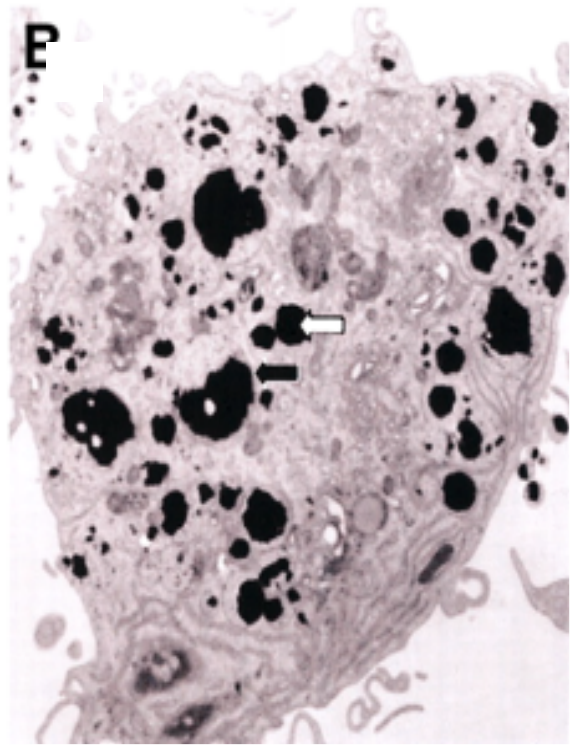
Tracking transplanted stem cells using magnetic resonance imaging (MRI) could offer biologic insight into homing and engraftment. Ultrasmall dextran-coated iron oxide particles have previously been developed for uptake into cells to allow MRI tracking. We describe a new application of much larger, micron-scale, iron oxide magnetic particles with enhanced MR susceptibility, which enables detection of single cells at resolutions that can be achieved in vivo. In addition, these larger particles possess a fluorophore for histologic confirmation of cell distribution. We demonstrate highly efficient, nontoxic, endosomal uptake of these particles into hematopoietic CD34+ cells and mesenchymal stem cells documented by confocal and electron microscopy. Labeled cells retain biologic activity with preservation of colony-forming ability and differentiation capacity. MRI studies could detect labeled CD34+ cells and mesenchymal stem cells (MSCs) at single cell resolution. This appears to be a promising tool for serial noninvasive monitoring of in vivo cell homing and localization using MRI. (Blood. 2003;102:867-872)

Iron-Labeling of Human Peripheral Blood $CD34^{+}$

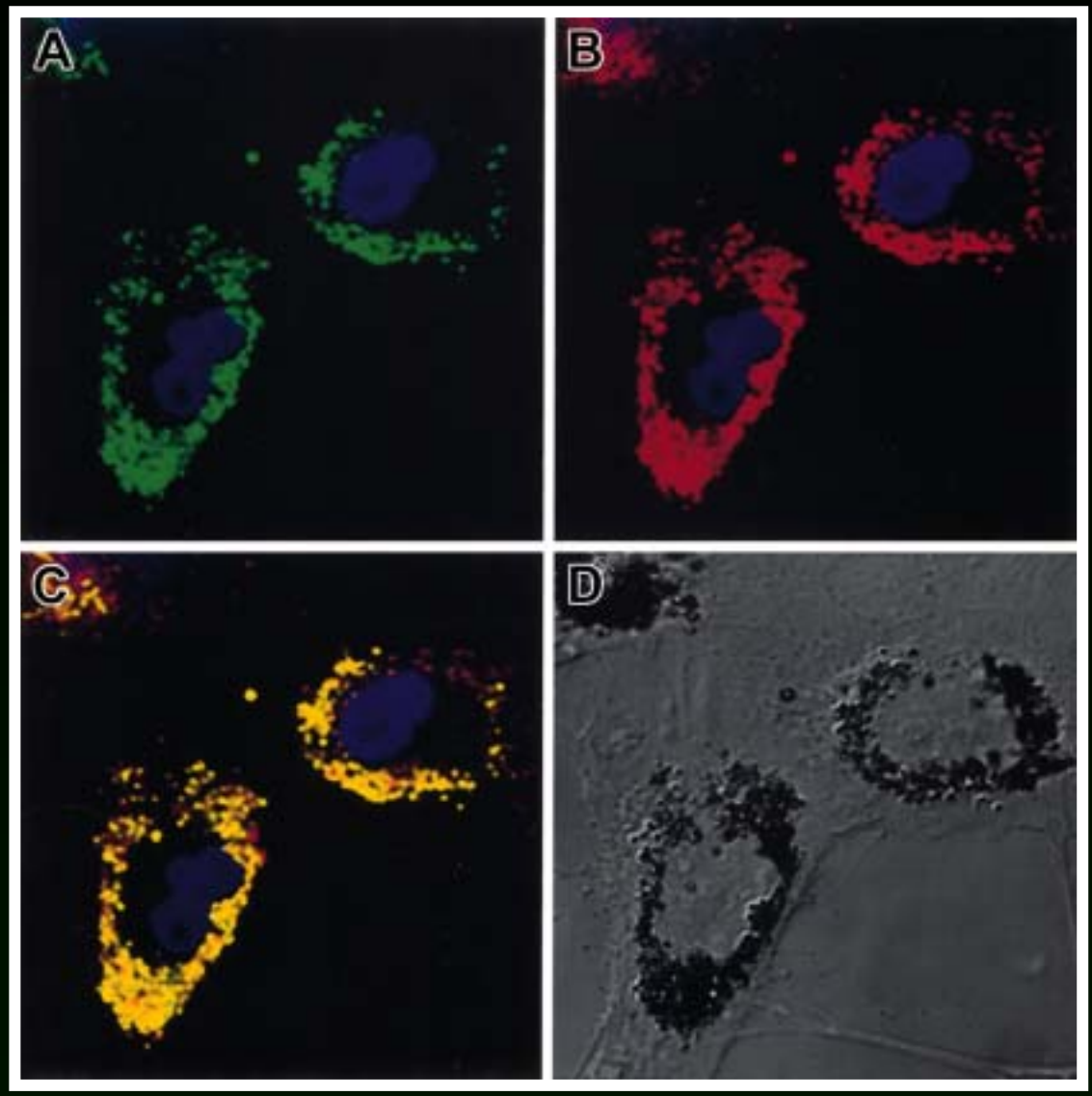


Confocal Fluorescent & EM Images of CD34^{+} cells

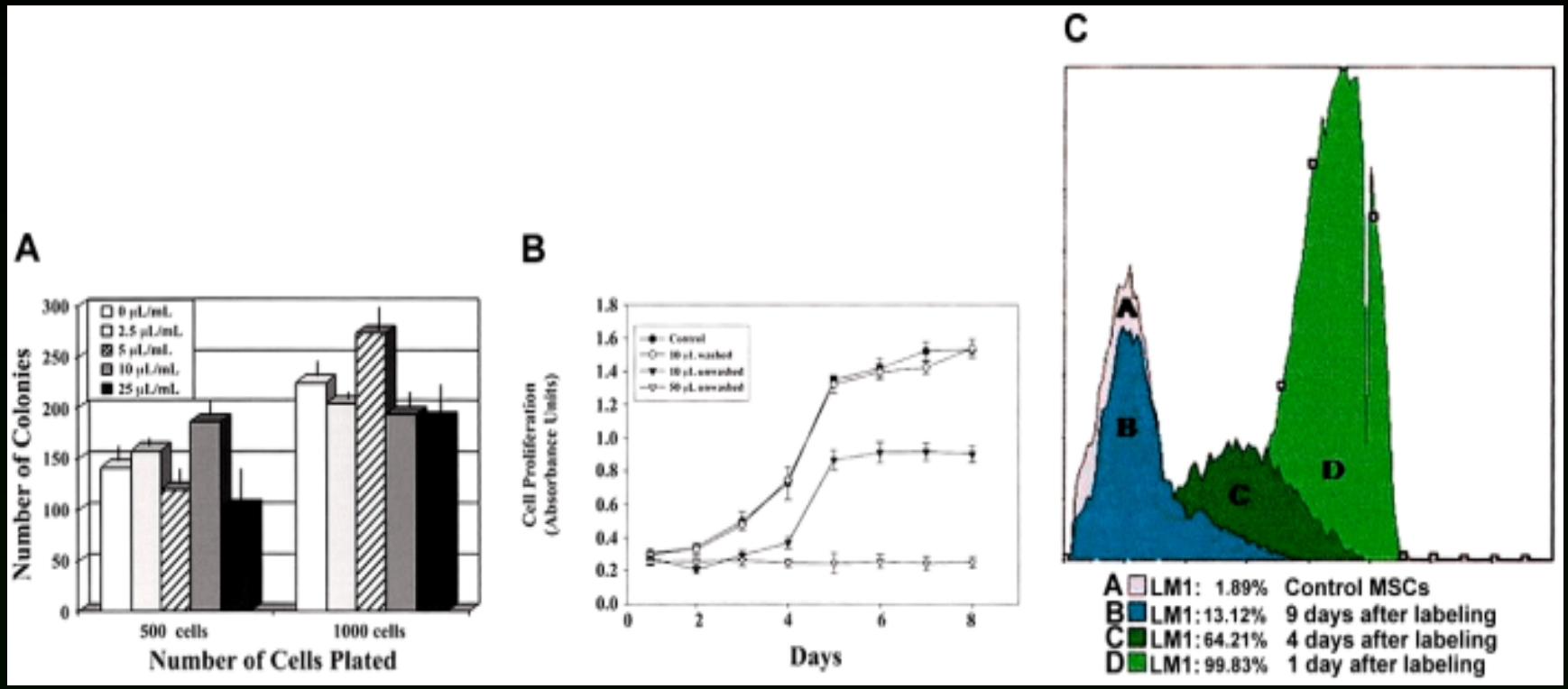




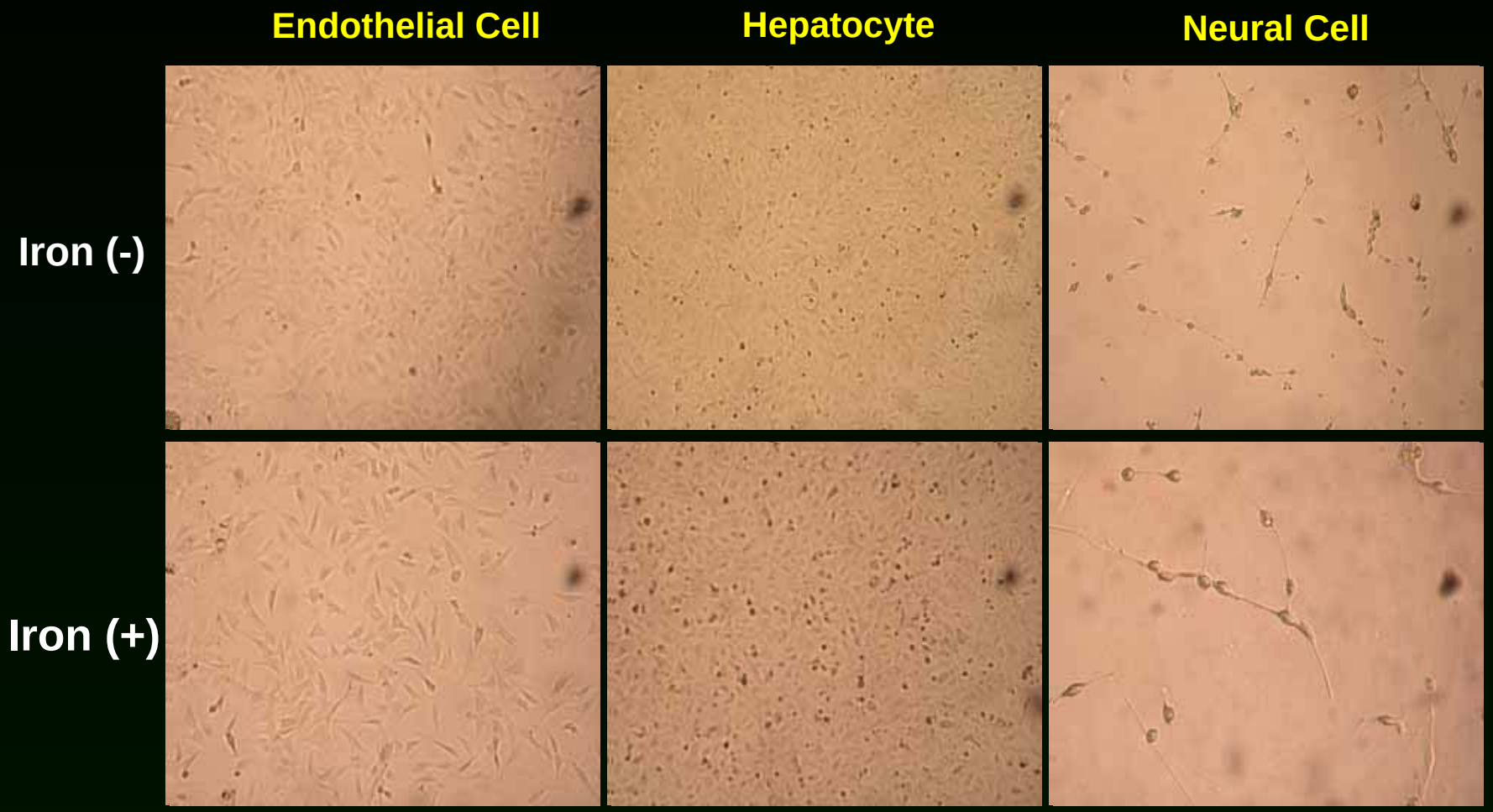
Confocal Images of Iron-Labeled MSCs



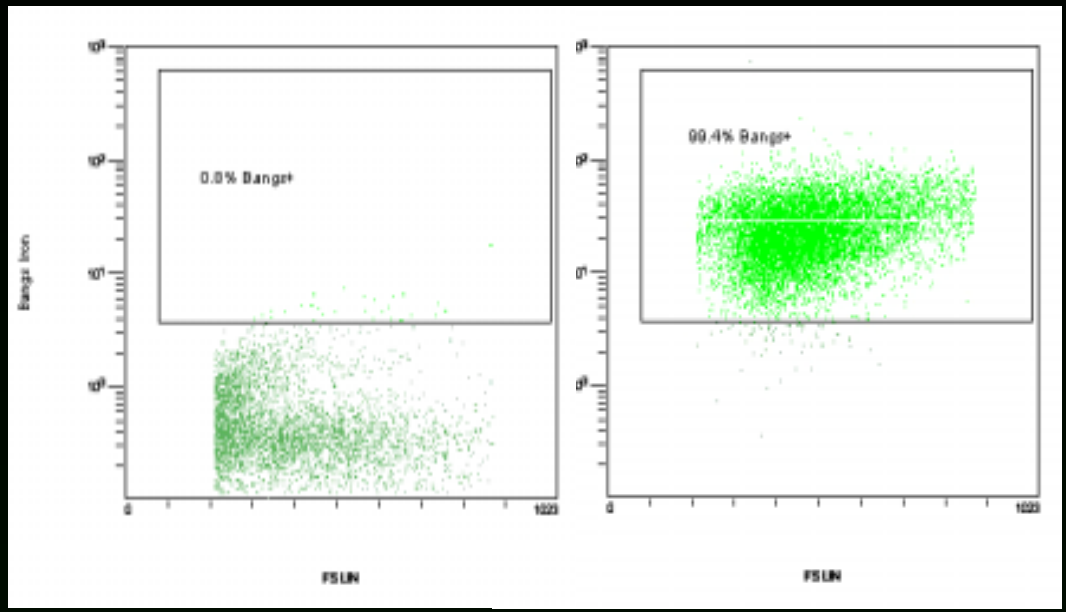
Impact of Iron Particle Exposure on Hematopoietic Progenitor & MSC Proliferation

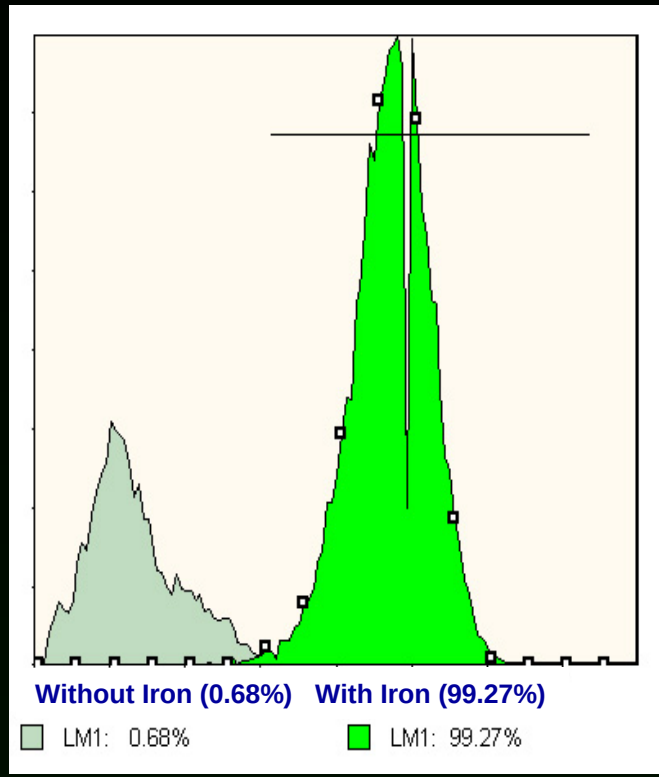


Differentiation of Iron-labeled MSCs is O.K.?

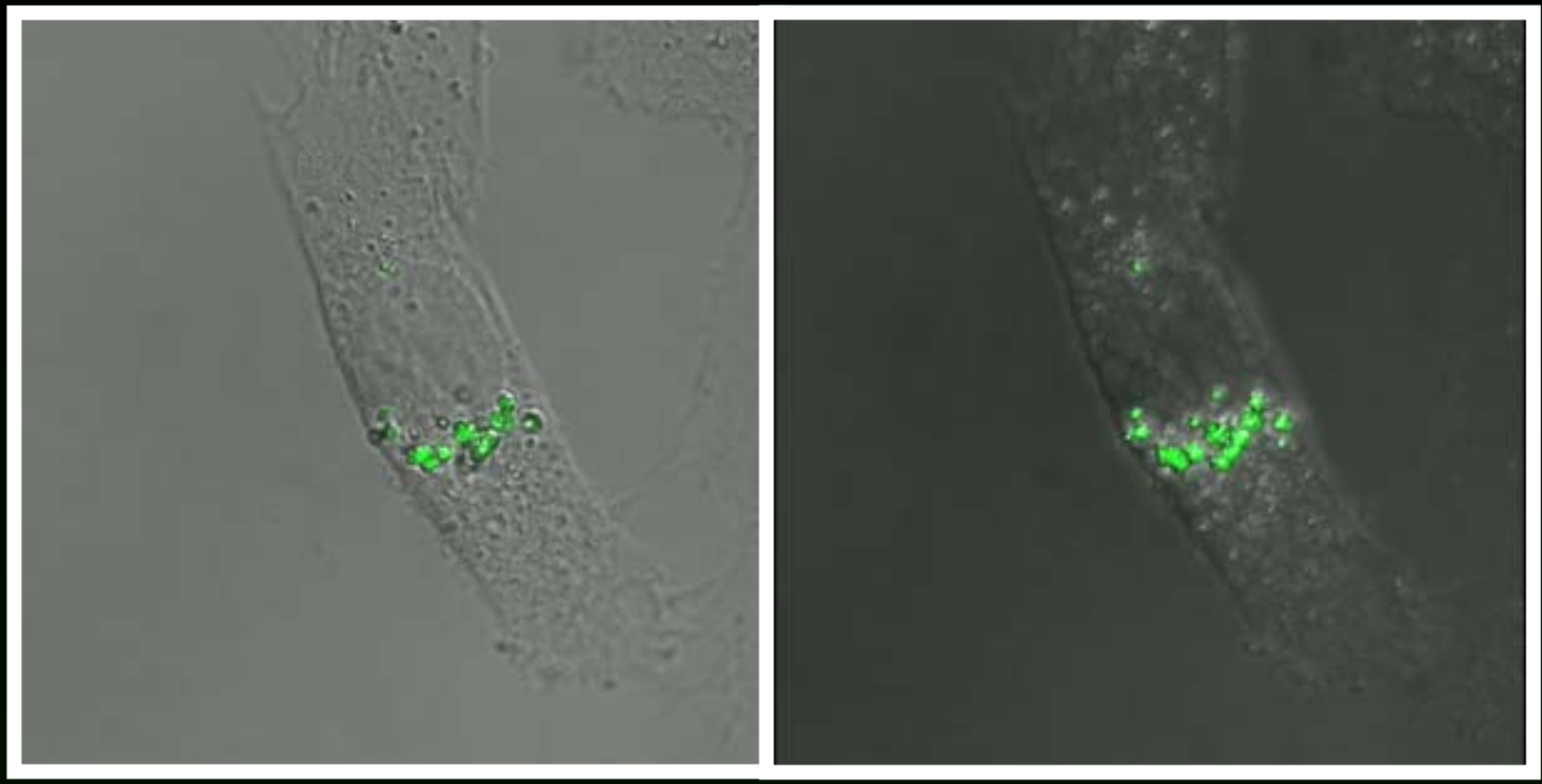


Iron-Labeling Rate





Are Iron Particles within MSCs ?



Aims of Study

Testify if iron-labeled MSCs, when injected systemically, home to infarcted myocardium and cells can be in vivo tracked by MRI

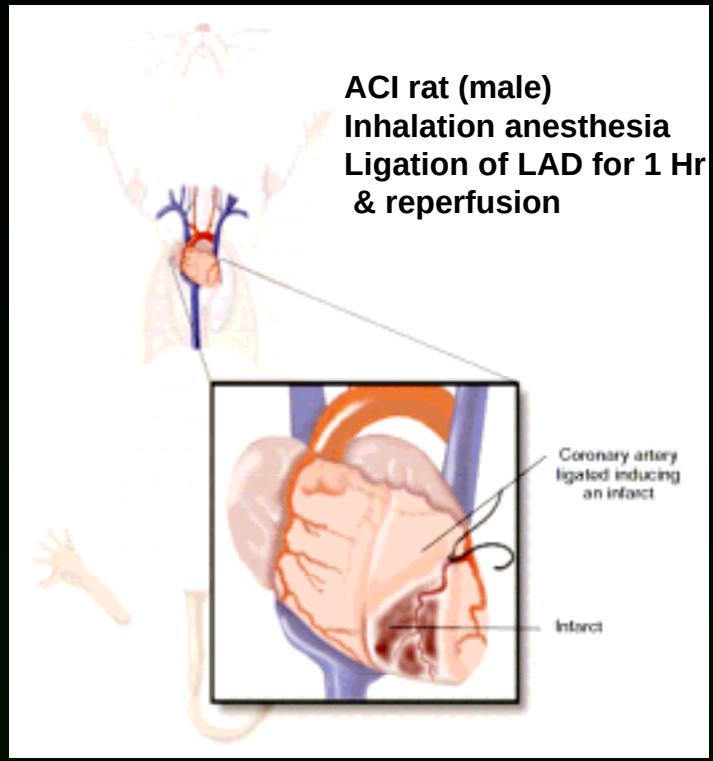
And…

- Determine the time course of homing to infarcted heart
- Observe whether MSCs can differentiate
- Assess whether these contribute to functional competence

Materials & Methods

Preparation of iron-labeled MSCs

Harvesting BM
Expansion of attached cells
Confirmation of multipotentiality
Incubation with iron particles for at 37°C



Make AMI
Inject Iron-labeled MSC (ACI) I.V. over 20 min
(8-10×10^6 Cells/kg/1mL of PBS)

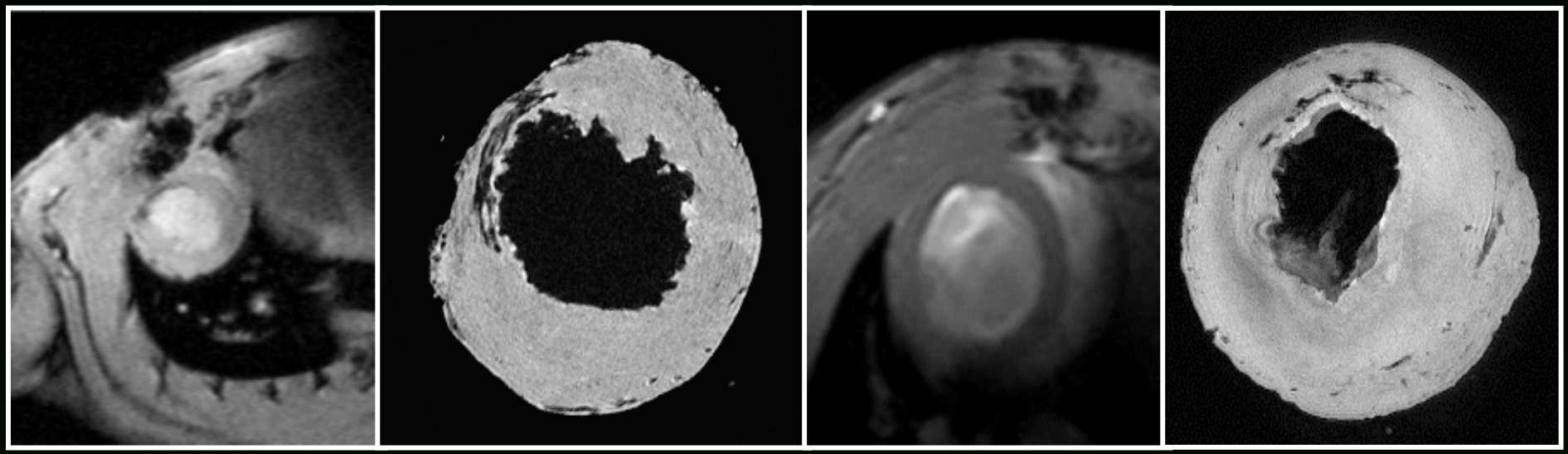
MI group : Iron-labeled MSC (n=8)
Control group : Iron only (n=8)

MRI & Histological Analysis

- **MRI analysis**
  - In vivo analysis with 1.5T (Oxford Instrument, UK)
  - Ex vivo analysis (3DR) with 7T (Magnex Scientific, UK)
  - Left ventricular ejection fraction (modified Simpson)
- **Histopathological analysis**
  - Prussian blue stain
  - Anti-desmin / Anti-connexin 43

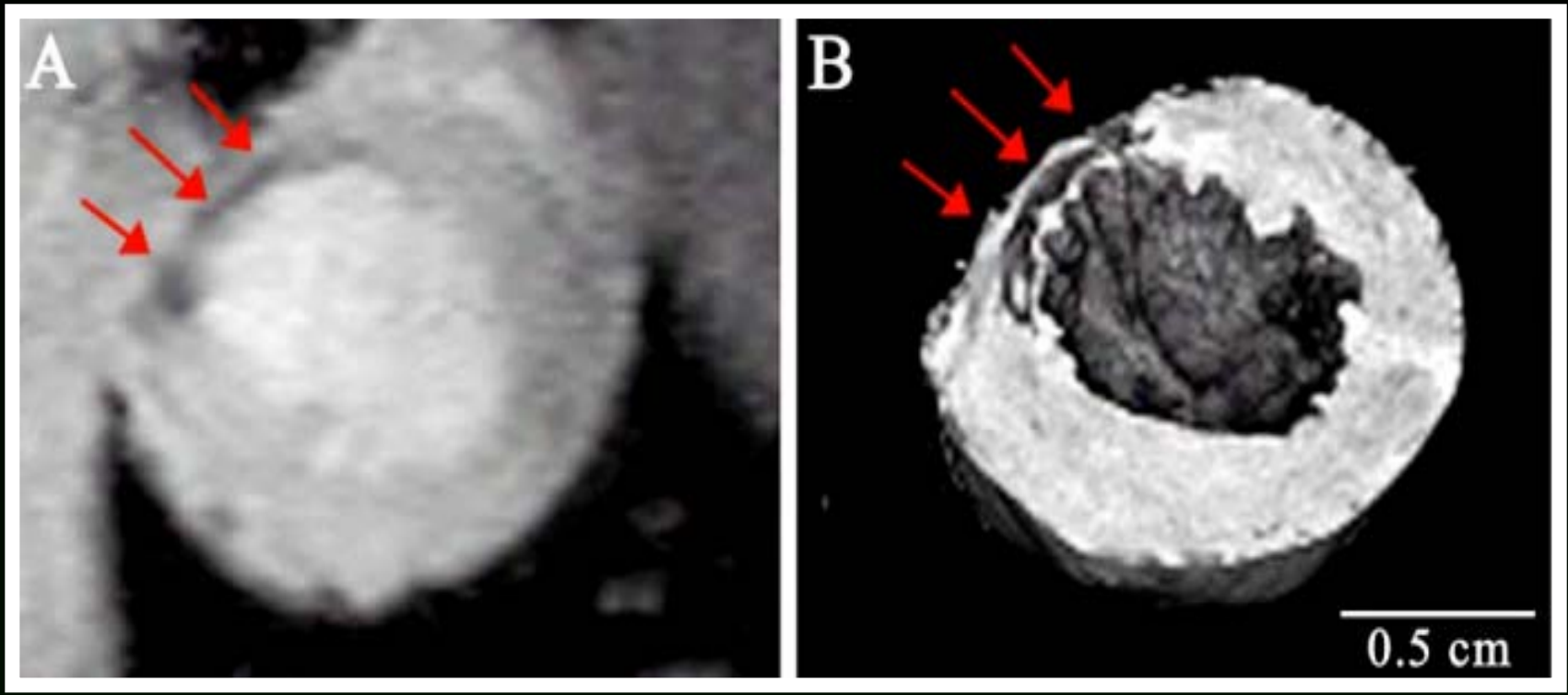
MRI Imaging : In vivo & Ex vivo

5 Days after surgery **6 Weeks after surgery**

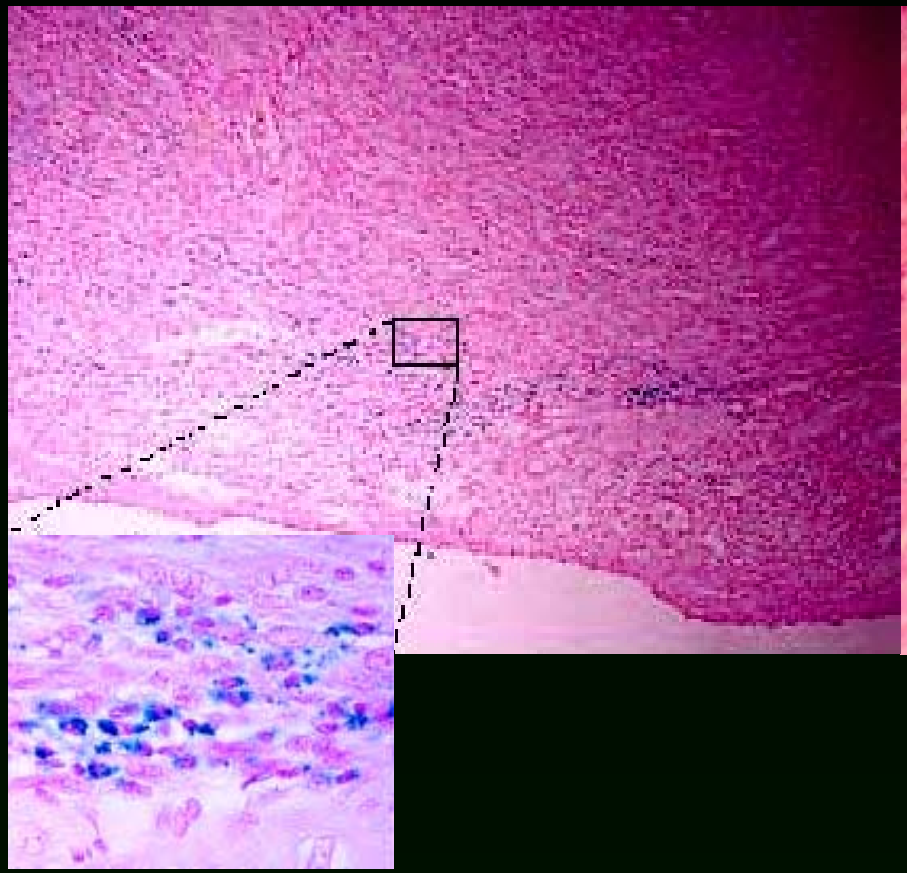


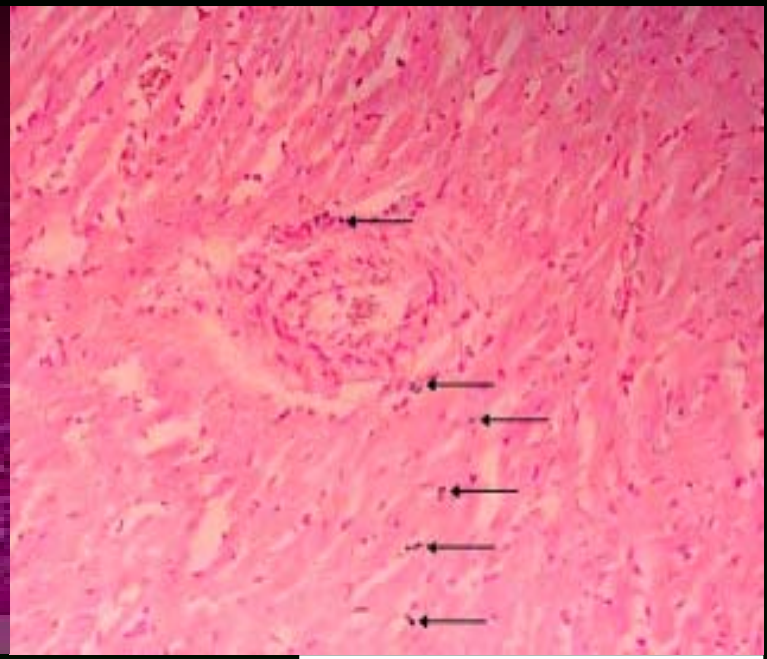
In vivo, 240 mm^2 resolution Ex vivo, 60 mm^2 resolution In vivo, 240 mm^2 resolution Ex vivo, 60 mm^2 resolution

Comparison between In Vivo & Ex Vivo MR Images

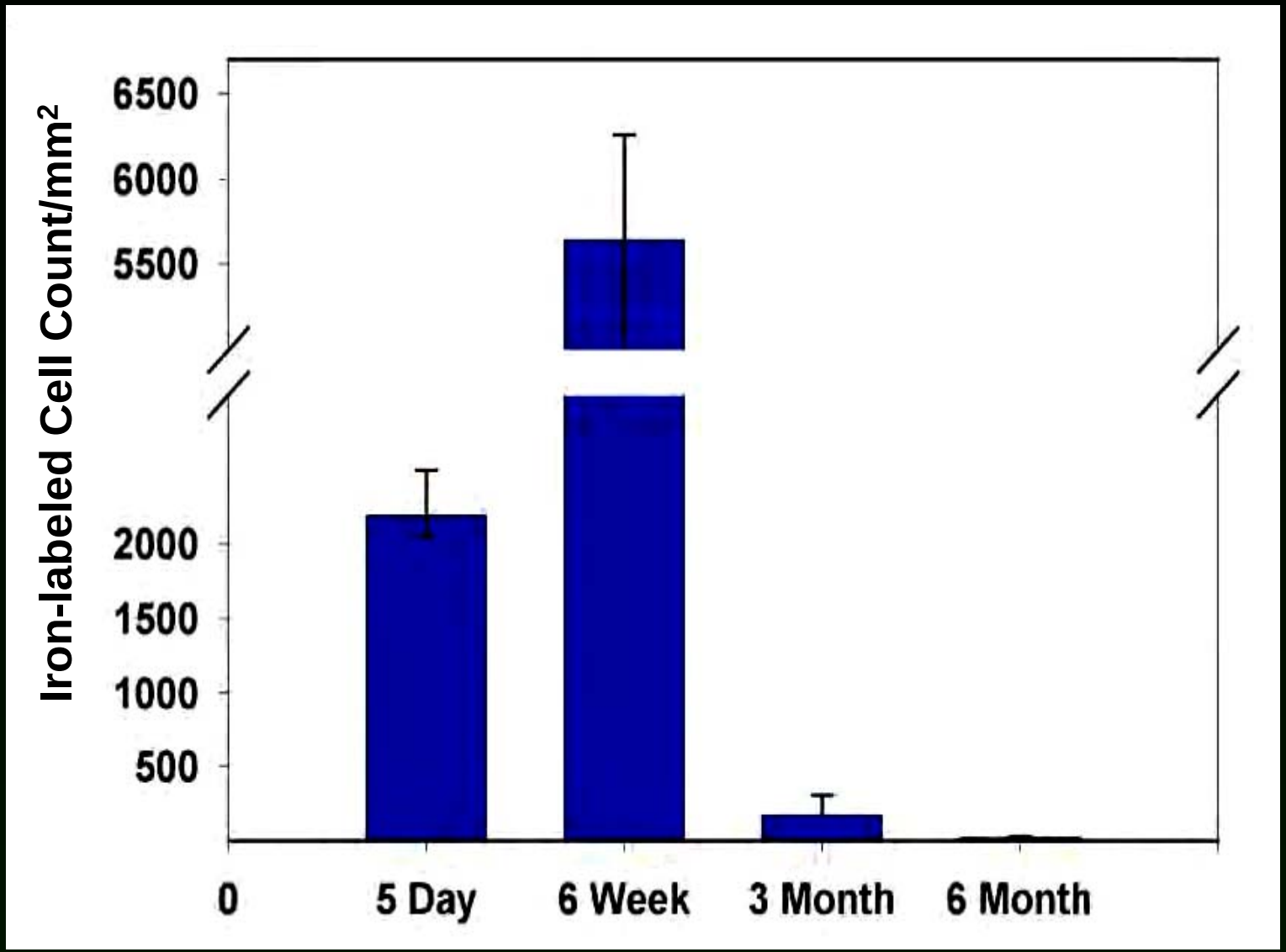


Histological Identification : Prussian Blue Stain

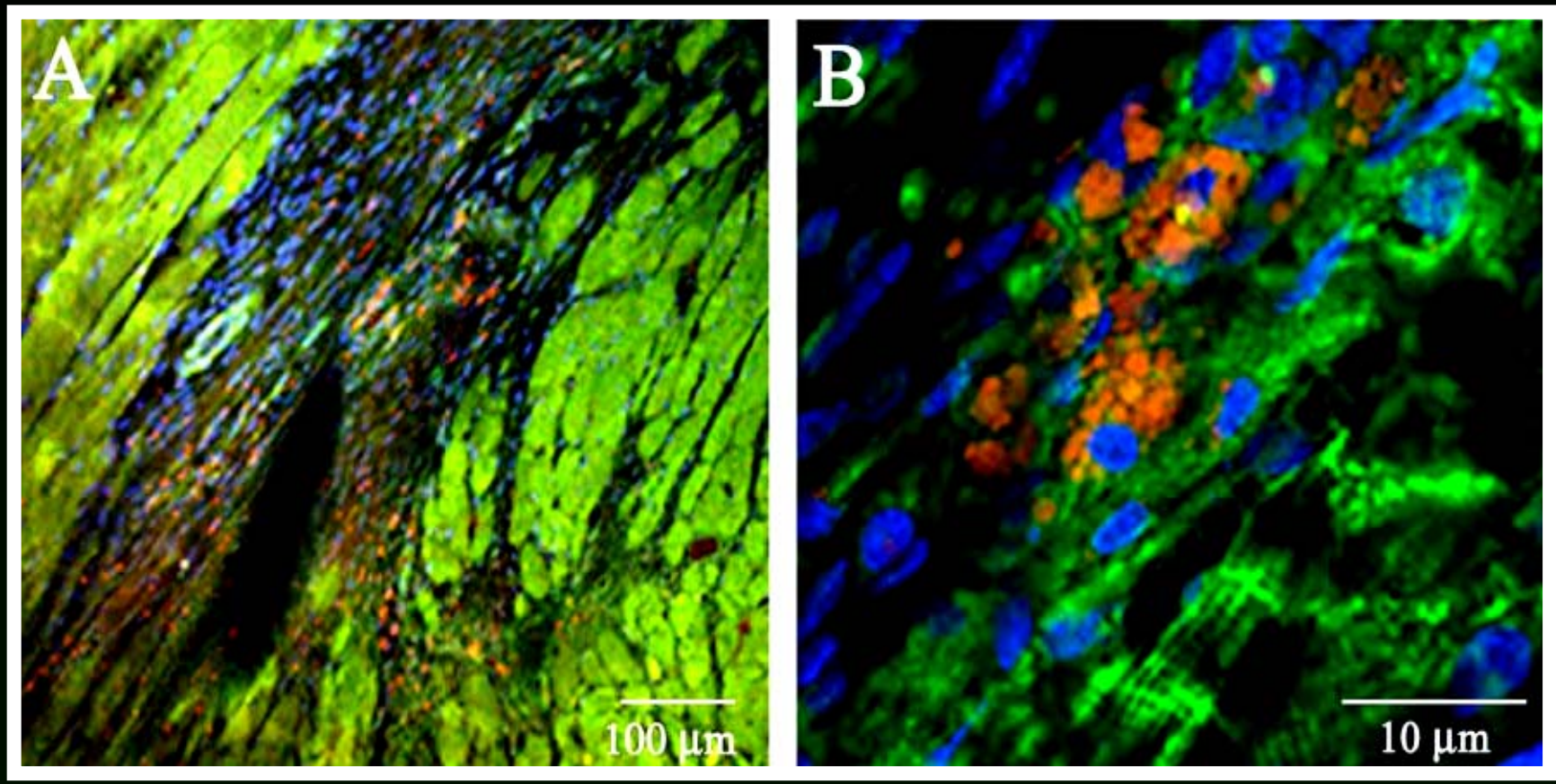




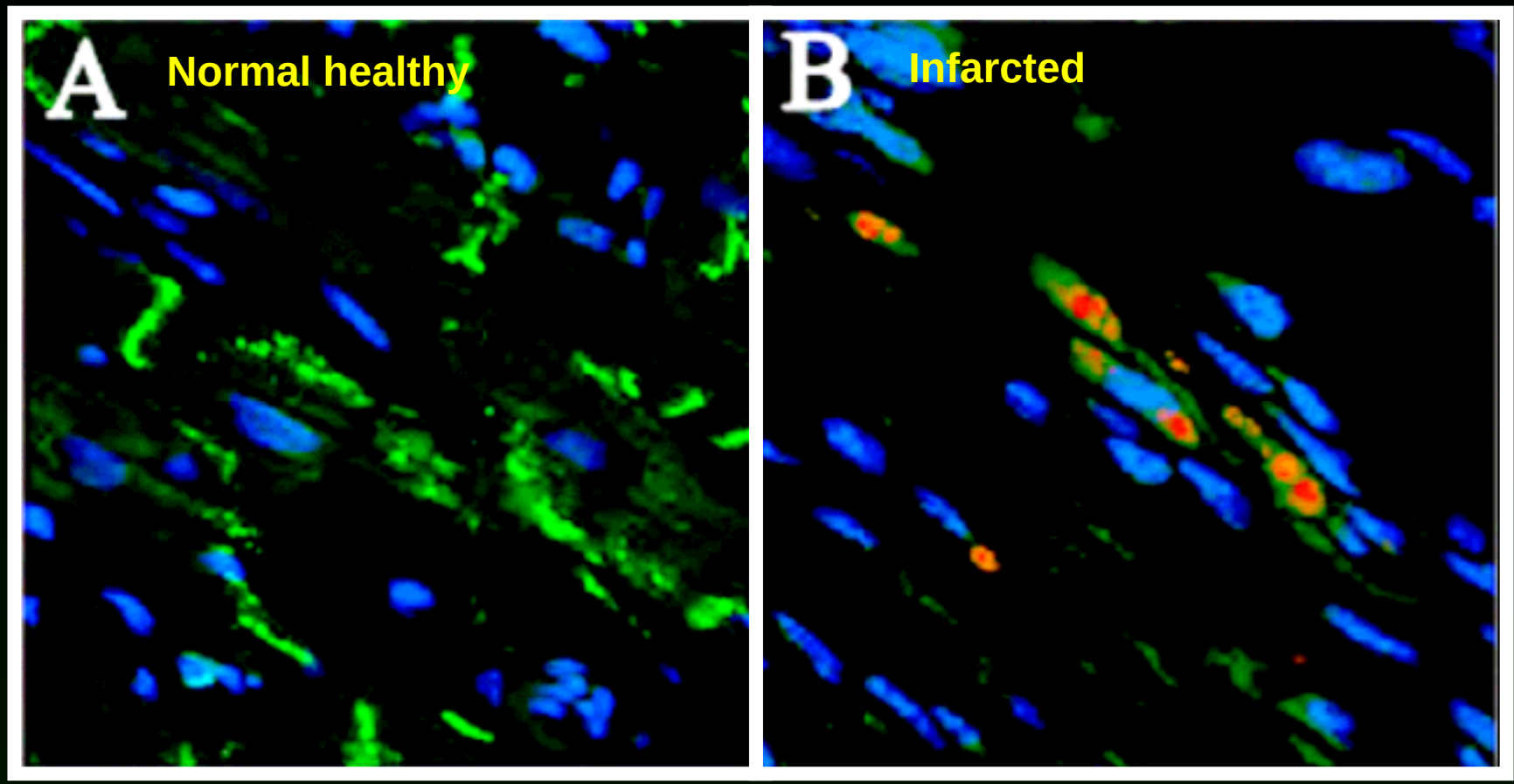
Quantification of Cell Engraftment Over Time



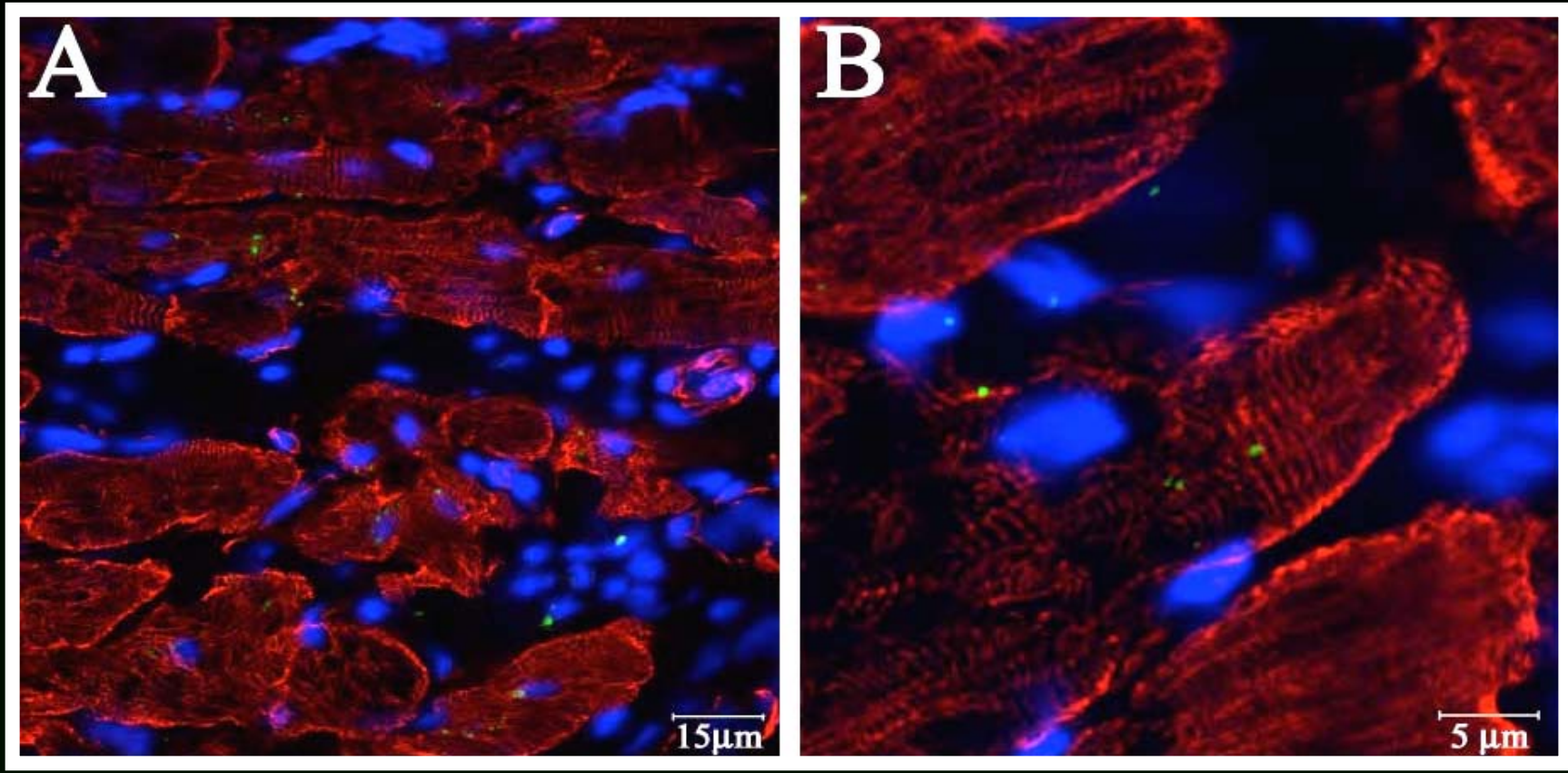
Early Desmin Expression of Iron-labeled Cells in Infarcted Myocardium (postop. 6 weeks)



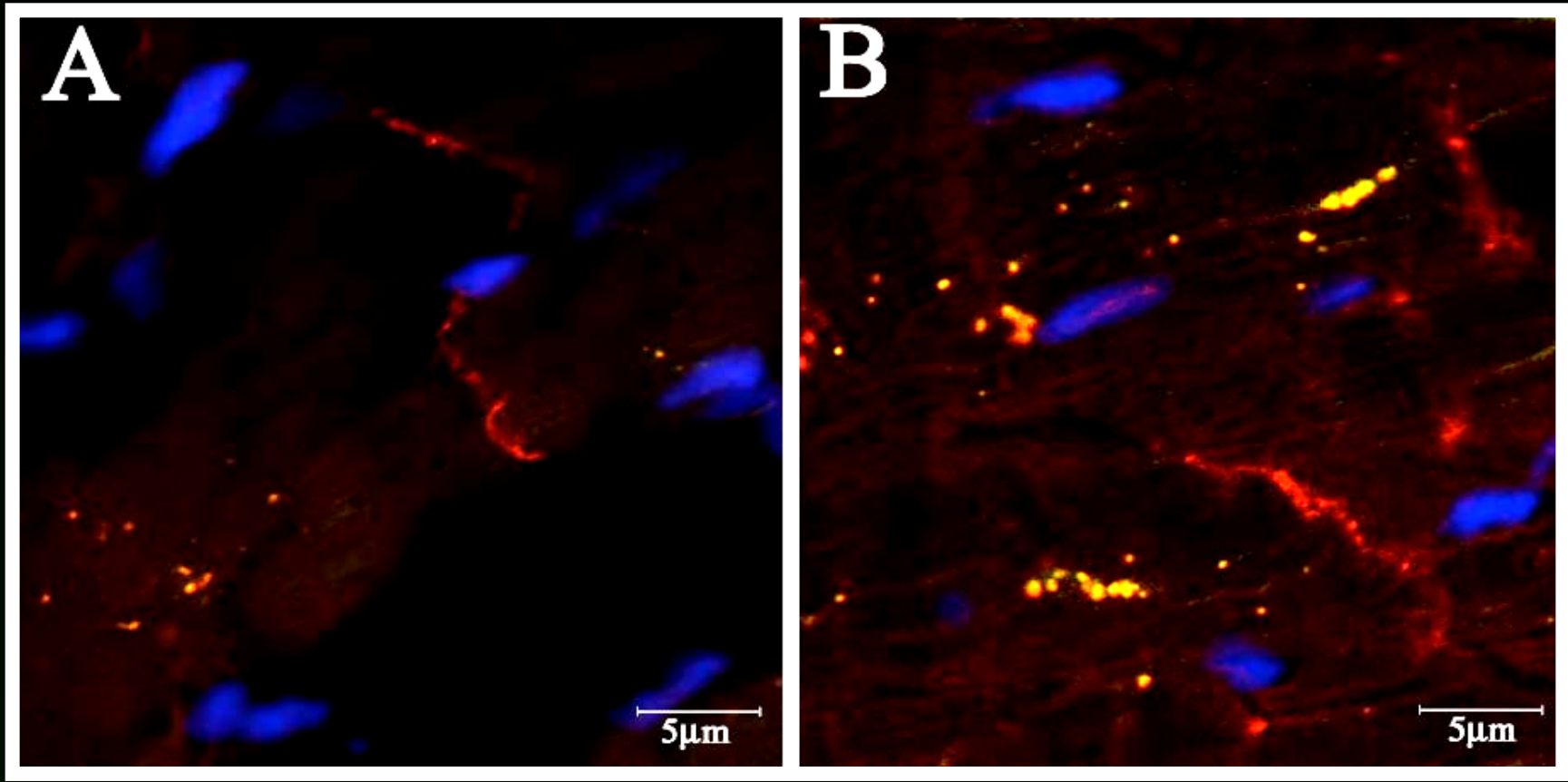
Early Connexin-43 Expression of Iron-labeled Cells in Normal & Infarcted Myocardium (postop. 6 weeks)



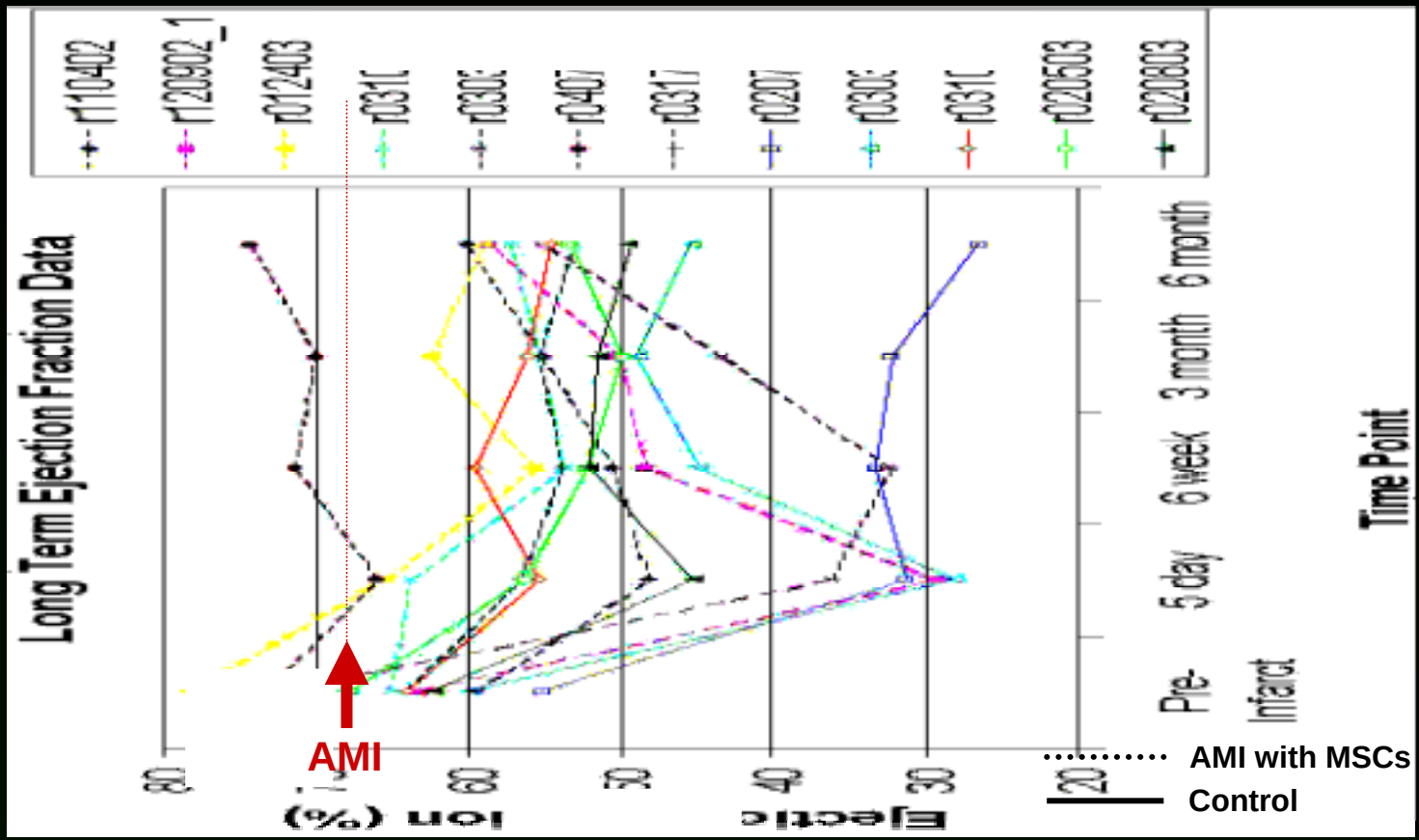
Late Desmin Expression of Iron-labeled Cells in Infarcted Myocardium (postop. 3 months)



Late Connexin-43 Expression of Iron-labeled Cells in Infarcted Myocardium (postop. 3 months)



Comparison of LV Ejection Fraction

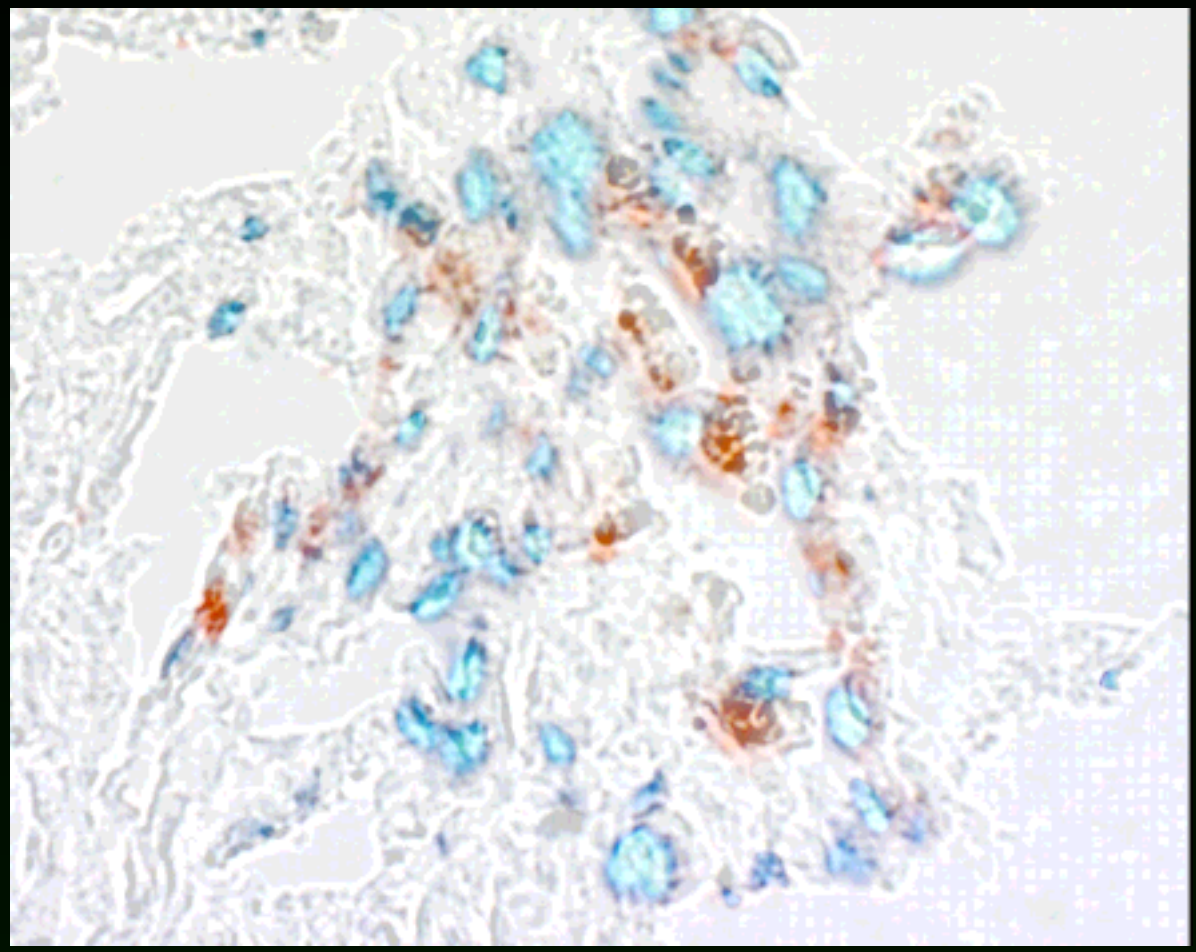


Summary & Conclusion

- Mesenchymal stem cells, when administered systemically to MI rat, preferentially home to the area of injury, and such cells, if labeled with fluorescent iron oxide microparticles, can be in vivo tracked with MRI.
- Maximal engraftment takes place at six weeks, after which there is a decrease in cell number.
- After homing, cells appear to have undergone differentiation.
- While treated animals exhibited a trend towards improved cardiac function, a larger sample size is needed to resolve differences.

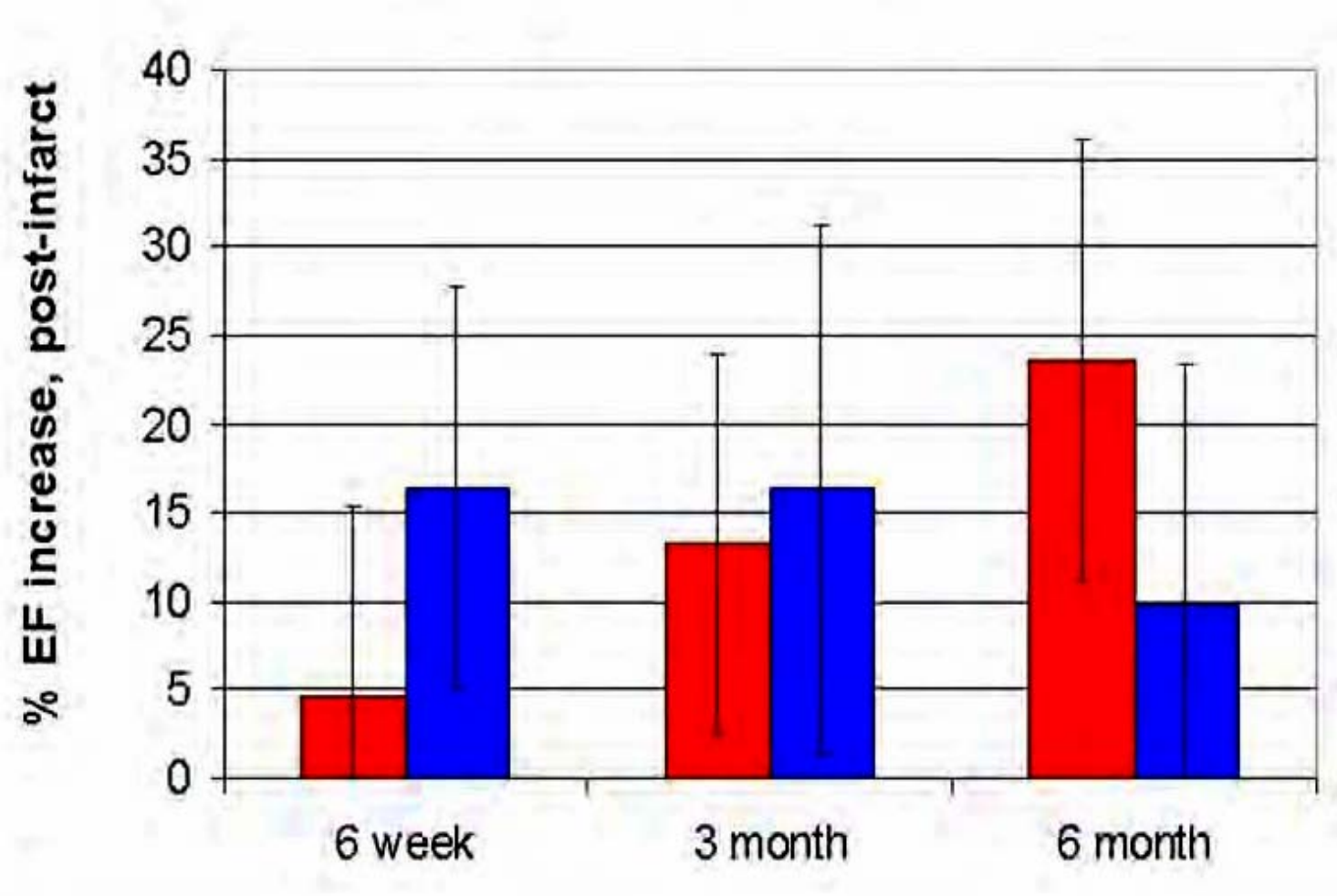
Acknowledgement

Pulmonary Pathology of Dead Rat

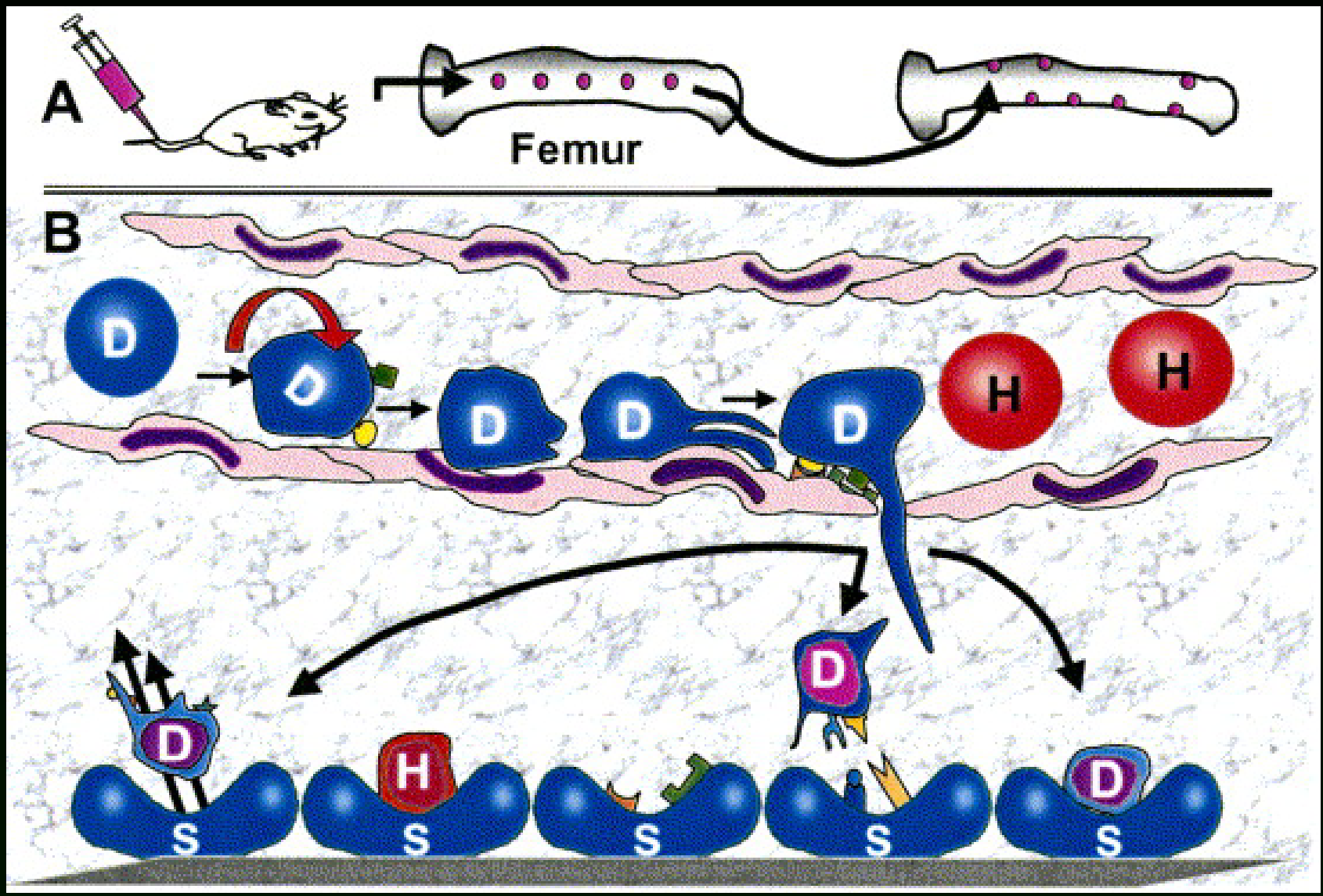


Pulmonary embolism !!!

Comparison of LV Ejection Fraction



Stem Cell Homing after IV Injection



Quesenberry PJ, et al. Exp Hematol. 2005;33:9-19