Labelling of rabbit bone-marrow cells with radioisotope In-111 and NMR contrast agents

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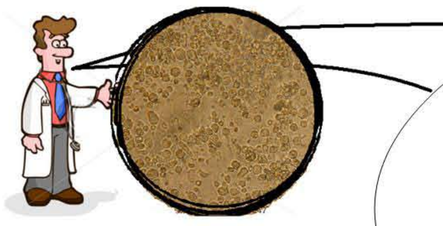

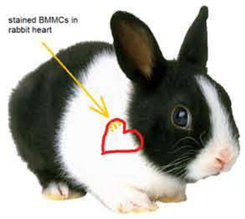

These cells are bone-marrow cells.
Bone-marrow mesenchymal cells (BMCs).
If we have an experimental animal with myocardial ischemia and BMMCs are injected into the myocardium, BMCs adhere to myocardium and induce regeneration of affected tissue. Mechanism of this regeneration process is still unsufficient (Klabusay and Skopalík 2009). Many studies looks for cellular basis of the mechanism and correlation between number of cells which adhere inside the myocardium and their precise localization. Experimentators want to know

- **where BMCs adhere**
- **how many BMCs adhere**

Tracking and quantification of injected cells in animal studies has been based on fluorescence labelling or on genetic modifications by the introduction of genes expressing fluorochromes or metabolic enzymes, however detection of fluorescence and enzyme reactions are limited by the need to kill the animals. Labelling with radionuclide and NMR contrast agents could be a noninvasive method for tracking of cells delivered into the infarcted heart. In published literature, labeling methods for human, canine, pig and rat BMMCs were evaluated. Evaluation of these methods is commonly based on determining of efficiency of labelling, surviving of cells and minimum number of detectable cells. Evaluation and comparison of these type of labelling methods for rabbit BMMCs is still lacking.

Labelling with In-111

Rabbit BMMCs were isolated and cultured for 1-3 weeks. BMMC labbeling with radioactive 111-In was based on incubating with 111-In-troplone complex (5-15 min). Details will be presented in Skopalík (2010). Labelling efficiency was determined. Surviving of BMMCs during 1 week was monitored. Different numbers of labelled cells were placed in phantom of rabbit chest and several methods of gamma-camera imaging were tested.

Results:

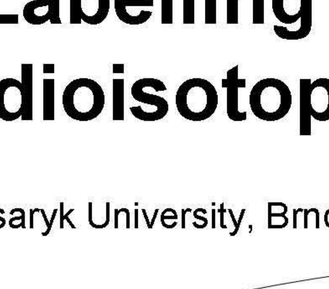

Fig 1.A (LEFT) Quantification of radioactive staining in 10 independent experiments. (RIGHT) Cells display good viability 24hours after radioactive staining.

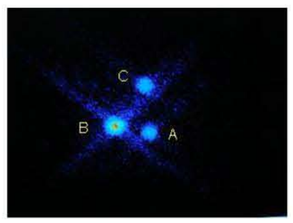

Fig 1.B Scan of phantom with BMMCs. Area A – 10 thousands of In-stained cells, area B – 200kBq control sample, area C 100kBq control sample.

Labelling with NMR contrast particles

Rabbit BMMCs were isolated and cultured for 1-3 weeks. Two types of iron oxide particles (Resovist or supermagnetic maghemite) were added to the BMCs culture (final concentration 100 ug/mL). Labelled BMCs were washed after several dals, viability was tested and different number of labelled cells was placed in phantom of rabbit chest and NMR imaging was tested

Results:

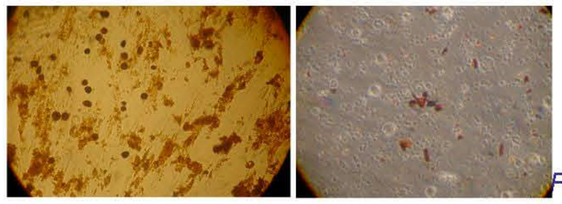

Fig.2.A *Fig.2.B*

Incorporating of Resovist into the cells was clearly visible under microscope (Fig.2.A). But cells have problems with maghemite incorporating(Fig 2.B).

10 thousand cells stained with Resovist was injected into plastic capillary (diameter 1mm) in isolated fresh rabbit hearth. MRI scans of heart was performed with 4.7 T apparature. Capillary with cells display high contrast.

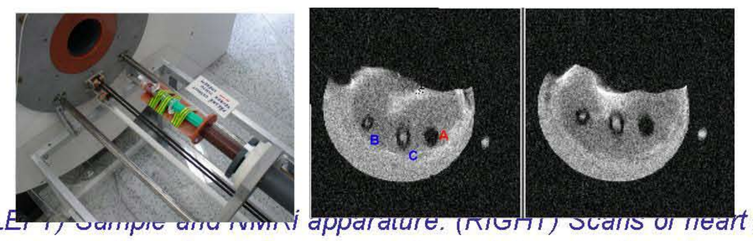

Fig 2.C (LEFT) Sample and MRI apparature. (RIGHT) Scans of heart tissue in water solution, capillary A and B contain saline solution, capillary C contain cells stained with Resovist

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Literature:

*Jin Y, Kong H, Stodůlka RZ (2005) Determining the minimum numer of detectable cardiac –transplanted 111In-tropolone-labelled bone-marrow-derived mesenchymal stem cells by SPECT. Physics in Medicině and Biology 50, 4445-4455
*Wissenberg G, Lekx K, Zabel P (2009) Cell tracking and therapy evaluation of bone marrow monocytes and stromal cells using SPECT and CMR in canine model. Journal of cardiovascular magnetic resonace, 11:11, p. 1-16,
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*Skopalik J, Pesl M, Stepan J, Starcuk Z (2010 -in preparation) Labelling of rabbit bone-marrow cells ∂with radioisotope In-111 and NMR contrast agents and bone-marrow cells localization by noninvasive methods.

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TOP Instruments

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for delivery of specific electrotechnical componets

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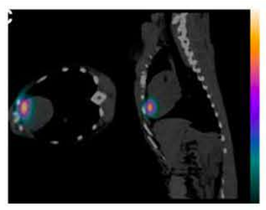
SUMMARY:

Two methods of cell staining and detection were optimised using *in vitro* culture and plastic phantom of rabbit chest. Clusters of 10 thousands of stained cells are detectable by each method. Results show that these two methods could be tested for tracking of BMCs *in vivo*.



In vivo – ideal results of future :-)