Labelling of rabbit bone-marrow mesenchymal cell with In-111 and NMR contrast particles

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BACKGROUND AND OBJECTIVES

Myocardial infarction and ischemic damage are irreversible. Adult heart do not have significant potential for repair ischemic affected area in hearth, but other cell sources, such as stem cells, are currently under intense investigation as a source for transplantable cardiomyocytes. Regenerative cardiac medicine has many potential cell sources. One of the main tasks is optimizing of administration of cells and monitoring of their homing.

Monitoring of cell distribution and homing after cell delivery is very important issue. Until these days most of the studies were conducted by microscopic analysis and necesary termination of the animal model in certain time after cell delivery. As a noninvasive methods were described bone-marrow mesenchymal cells (BMMCs) labeling and detection methods for canine experimental model (Blackwood 2009 - SPECT, Wissenberg 2009 - SPECT and NMR), pig model (He 2007) and rat model (Chapon 2008). Basic geometry of the cell homing and cumulation were described. Quantification of the amount of the cells and its time developing during days or weeks after transplantation is great advantage of these noninvasive methods. Results show that only 2 – 5 % of transplanted cells remain in heart after several days .

Evaluation and comparison of labelling and noninvasive tracking methods for rabbit BMMC is still lacking. In this work we test and optimize labelling of rabbit BMMC by In-111 and NMR contrast particles and BMMC detection in fantoms and isolated heart.

IN-111 LABELLING AND CELL DETECTION

Methods:

•Precultivation:
Rabbit BMMCs were isolated and cultured for one to three weeks, in cultivation media RPMI + 10% FBS. BMMCs adhere to plastic bottom of the flasks, deadhering proces were conducted with standart tripsinization for 8-12 minutes

• Radiolabelling and long-time cell development
Labelling was based on incubation of cells with complex 111In – tropolone for 5 minutes. Labeling efficiency was determined. Surviving of BMMCs during 1 week was monitored (apparature for continual cell mionitoring and viability detection Fig 1-A). Different amount of labeled cells was placed in phantom of rabbit chest and underwent basic gamma camera imaging (Fig. 1-C).

Fig. 1. *(A)* *(B)*

Results:
Radioactive labeling efficiency was 40 % (60 % of In-111 remained in the labeling solution), which resulted in BMMCs displaying about 3 Bq/cell (Fig 2-A, details in Skopalik 2010). Viability was not significantly decreased by this procedure, if labelling solution with cca 10 kBq/uL was used. BMMCs numbers as low as 50 x 10^3 could be easily localized and imaged using gamma camera (Fig 2-B). Radioactivity levels per cell in time are going down - by side of natural radioactive decay of In-111, by side of so called "wash out" of radioctivitve In-111 from the cell, which is very importnant aspeckt for future quantitative detection of cell in vivo. During first 24hours is total decline in radioactivity per cell smaller about 25 %. Quantification of wash out will be main topic of our new project

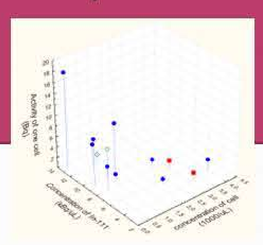


Fig. 2. *(A)*

(B)

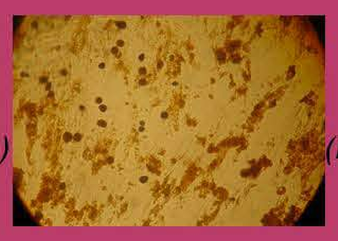
NMR CONTRAST LABELLING AND CELL DETECTION

Methods:

•Precultivation:
Rabbit BMMCs were isolated and cultured for one to three weeks, same way as cell for In-111 labelling.

•Labelling with iron particles
Two different Iron oxide nanoparticles were used for the labeling: Resovist (Schering, DE) and experimental supermagnetic syntetized particles of Maghemit (NanoCentre, CZ). Both types of particles were added in to cultivation media (concentration up to 100 ug/ml), after 3 days BMMCs were washed and placed in freshly extracted rabbit heart. In additon to NMR imaging of areas with various BMMCs concentrations, we evaluated also viability of BMMCs.

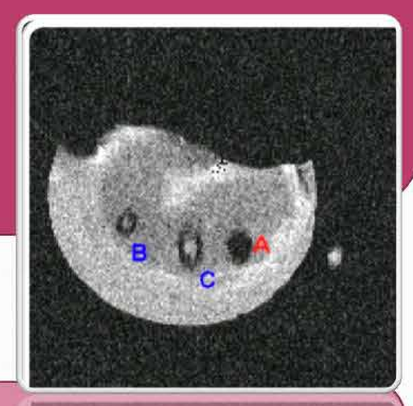
Fig. 3. *(A)*



(B)

Results:
v BMMCs prospered in either Resovist or maghemite rich media. Viability of BMMCs was not affected, in compare with control group of cells cultivated in same media without iron nanoparticles. Not less then 80% cells were surviving after wash out of iron nanopraticles and trypsinization. Visible orangebrown marks remained after wash out of Resovist (Fig. 3-A) . Maghemite labelled cells were not coloured with any visible dye (Fig. 3-B) and the cells were not detectable in MRi.
Clusters of 100 000 , 50 000 and 5000 cells with Resovist were clearly trackable, size of such cluster was around one square millimeter. Cluster of 10 000 BMMCs in heart tissue (mimicking island of BMMCs remained in infarcted heart in vivo) was detected by MRi (Fig. 4)

Fig. 4.



CONCLUSION

Method of In-111 and Resovist labelling of rabbit BMMCs were proved working and optimized. This method can be used as base for in vivo studies of tracking of cells delivered into the infarcted rabbit heart.

LITERATURE:

Bindslev L, Haack M, Bisgaard K. Labelling of hunam mesenchamal stem cells witn 111In. Eur J NMI, 2006

Skopalík, Pešl, Starčuk, Štěpán, Scheer (2010) Optimalization and comparrizon of two noninvasive methods for stem cell tracking in infarcted heart (in press)

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