

## **Abstract:**

Type I diabetes mellitus is primarily an autoimmune disease resulting from selective destruction of insulin producing pancreatic beta cells. Transplantation of purified pancreatic islets is an alternative method for standard insulin therapy of these patients. Though the islet transplantation represents a promising approach for a selected group of patients, there is still a number of problems to be solved and one of them, perhaps the most important, is the lack of a reliable method of transplanted islets monitoring.

Our group developed a direct method that allowed for the long-term visualization of the intrahepatically transplanted islets with the use of magnetic resonance imaging (MRI), based on their previous labeling with superparamagnetic iron oxide nanoparticles (SPIO).

The main focus of this thesis was to elaborate a protocol that would be might used for efficient and safe islet labeling in clinical practice. Therefore we investigated the effect of iron labeling on islet function in vitro as well as in vivo and the time behavior of the labeling process and iron accumulation in different islet cell types in order to optimize the labeling process.

For islet study the clinically approved contrast agent ferucarbotran (Resovist®) with particle size 62 nm and carboxydextran coating was used. Islets were labeled in tissue culture media supplemented with ferucarbotran for 1, 4, 8, 12 and 24 hours. Iron uptake by islet cells was visible as distinct hypointense spots on MR images. The sufficient superparamagnetic effect was already achieved at the 1 hour labeling period and did not change considerably after extending culture time. Using light microscopy, we demonstrated the iron incorporation in both islet beta cells as well as islet macrophages. With the use of electron microscopy we have described in detail the process of iron endocytosis into different cell types. After 1 hour of labeling, the iron nanoparticles were inside islet macrophages and only after 4 hours labeling we found the iron nanoparticles in endosomes of the all endocrine cells. Massive iron accumulation in islet macrophages and minor iron accumulation also in islet alpha, beta and delta cells was seen with the prolonged culture time. Incorporation of iron nanoparticles into the cells did not influence the quality of islets in vitro and also did not impair the ability of the transplanted islets to treat experimental diabetes. We did not observe any significant ultrastructural changes of labeled cells in comparison to control cells.

The results of this experimental study lead to the improvement of the labeling protocol in experimental transplantation and to its translation into clinical research. While different methods such as PET and SPECT will be soon available for short-time in vivo follow-up of transplanted pancreatic islet, magnetic resonance imaging represents so far the only available technique able to provide a direct, long-term and reliable visualization of the transplanted islets with sufficient spatial resolution. At present, is this method ready for clinical application.