One of the most difficult challenges of modern medicine is to obtain therapeutically active stem cells, optimally matched to the specific disease both in terms of their ability to differentiate into desired cell types and rebuild the tissue, as well as to maintain a limited proliferative potential for extended period of time. As numerous studies show, it can be obtained or even strengthened by the proper selection of cell type and various modifications of their *in vitro* culture conditions (so-called prelicensing). The development of cell therapy mainly with mesenchymal stem/stromal cells (MSC) application confirmed their strong secretory and immunomodulatory properties, however their regenerative (tissue restorative) effect has been confirmed only for a few medical applications in orthopedics, dermatology or ophthalmology. The neural stem cells, e.g. fetal neural stem cells (fNSC), represent the population of cells with significant potential for advanced stages of differentiation toward the specific tissue. Unfortunately, their survival and proliferation after transplantation are significantly limited due to the lack of proper stimulation with growth factors and the strong local inflammatory response caused by allogeneic transplantation. In this context, it is important to use the unique properties of Wharton jelly mesenchymal stem/stromal cells (WJ-MSC), whose immunomodulatory effects have been confirmed by using them as the second line of treatment in graft-versus-host disease (GvHD). Another important aspect enabling the use of both of these cell types in future clinical therapy is their similar, post-embryonic developmental stage, which means that they have already passed the developmental barrier of genetic instability and the associated developmental tumorigenicity.

The aim of the project is to examine the bilateral interactions of both above-mentioned types of therapeutic cells in terms of their involvement in the reconstruction of injured neural tissue. We anticipate the synergistic benefits of direct and indirect interaction of WJ-MSC/fNSC on expansion and differentiation both in *in vitro* cell culture and in experimental model of injured rat brain *in vivo*. In our project the dominant mechanisms of these cell-cell interactions will be analyzed, especially in the aspect of determining the most physiological environment (niche) favoring the differentiation and incorporation of fNSC into the injured brain.

During the project, we plan to conduct tests in accordance with FDA guidelines, starting from *in vitro* experiments. In co-cultures of WJ-MSC / fNSC the indirect interactions will be investigated using trans-well technique enabling spatially separated culture of two types of cells. This model of experiment will allow better analysis of soluble (paracrine) factors affecting differentiation and migration of NSCs as well as changes in gene expression occurring during differentiation.

In order to learn about direct interactions (cell-cell), mixed neurospheres consisting of WJ-MSC and fNSC will be established. They will represent both spatial and component model of the cell niche.

The aim of the next stage of the project will be to assess the ability of fNSC transplanted into the injured brain tissue to migrate and integrate in the presence of co-cultivated WJ-MSC. In these experiments, organotypic rat hippocampal slice culture (OHC) will be used. This model is devoid of a humoral response, often a barrier to xenogenic transplants, while maintaining cytoarchitecture and vascular scarring of normal tissue and natural projection fibers that are the pathway of migration for newly emerged neural progenitors. In order to restore the *in vitro* injury that occurs as a result of cerebral ischemia, the slices will be temporarily deprived of oxygen and glucose (OGD). The fNSC transplanted on the slices surface will be stimulated with growth factors and anti-inflammatory cytokines secreted by co-cultivated under the membrane WJ-MSC. In this step the following problems will be assessed: fNSC migration pathways depending on the transplant site (DG vs. CA region), migration dynamics depending on transplantation (tx) time after injury (early injury vs. late) and the integration of fNSC into the hippocampus cytoarchitecture.

Observations in such a model are, however, limited due to the relatively short time of slice culture survival (maximum 2-3 weeks). The only model that allows long-term observation of transplanted cells remains the animal model. To this end, research will be carried out in an experimental model of cytotoxic brain injury in rats (Domańska-Janik et al. 2008, Kozłowska et al., 2007). The fNSC cells will be administered intraparenchymal to the lesion site (brain cortex and striatum), while WJ-MSC will be administered to the cerebrospinal fluid.

Due to the WJ-MSC secretory properties, we aim to observe increased regenerative properties of fNSC, their extended survival, as well as targeted migration to places of injured brain, their colonization and partial tissue regeneration.