Ischemic stroke is the second leading cause of death after cardiovascular disease, overtaking even cancer. However, nowadays, the only commonly used method of its treatment is thrombolysis with use of recombinant plasminogen activator (rtPA). This method, however, requires administration of the drug within 4.5 hours after stroke, which significantly limits its effectiveness and causes that many patients who survive stroke suffer from a number of dysfunctions. Therefore, there is an urgent need to find therapies which overcome the negative effects of the disease in stroke survivors.

Mesenchymal stromal cells derived from tissues such as bone marrow, Wharton's jelly and dental pulp, are a group of cells intensively studied for their use in the regeneration of damaged nerve tissue. Of these, the population with the greatest potential to support the reconstruction of the nervous tissue are dental pulp stromal cells (DPSCs). This is due to the fact that the pulp in the course of embryonic development arises from the progenitor cells of the neural crest, and thus from the ectoderm – the germ layer from which neurons and glial cells develop. While regenerative strategies based on the differentiation of DPSCs cells into neurons require a lot of further research, it is confirmed that DPSCs can stimulate endogenous regenerative mechanisms of damaged tissues by exerting paracrine effects. Among these effects, not only DPSCs stimulate target cells to proliferate and differentiate, but they are able to modulate immunological response. As is known in the course of stroke and other neurodegenerative diseases, chronic inflammation causes serious damage. Because one of the postulated mechanisms of paracrine DPSCs action is their influence on target tissues through the activity of both their own chemokines signaling, as well as, modulation of chemokine secretion by target tissues, the project will investigate this paracrine DPSCs interaction. Antagonists of selected chemokine receptors will be used to inhibit effects exerted by particular chemokines. **The research hypothesis assumes that the regenerative properties of DPSCs cells depends on the activity of chemokines – CXCL12, CCL2 and CX3CL1, while their modulation through the use of antagonists for their receptors will affect the neuroprotective properties of DPSCs on organotypic hippocampal cultures (OHC) subjected to the commonly accepted experimental ischemic stroke model. C**XCL12 is a chemokine secreted by stromal cells, which plays an important role during neurogenesis by influencing the migration and proliferation of neuronal progenitor cells. Therefore, we postulate that its modulation may negatively affect the paracrine effect exerted by DPSCs. CCL2 is a chemokine promoting inflammation, hence inhibition of its receptor should positively affect the effects of DPSCs. While the increased expression of CX3CL1 is associated with the learning process, hence it is postulated that its role is promoting the improvement of cognitive functions after stroke.

The model proposed in the project will be based on the use of DPSCs culture derived conditioned medium for OHC subjected to the oxygen-glucose deprivation (OGD) ischemic stroke model. To characterize DPSCs flow cytometry and qRT-PCR will be applied. Determination of conditioned medium secretome will be carried out by means of mass spectrometry. Organotypic hippocampal cultures are a research model reflecting the complexity of real tissue because they contain various types of brain cells (including neurons, microglia and astrocytes). The OGD model will be obtained by placing inserts with OHC in a medium that does not contain glucose in the atmosphere with an oxygen concentration of 0% for 40 minutes. These conditions form a model of ischemia where the brain is deprived of oxygen and essential glucose. In the next stage, by means of confocal fluorescence microscopy, the morphology of OHC after OGD and after modulation of CXCL12, CCL2 and CX3CL1 receptors through antagonists will be investigated. Also, measurement of the binding forces of chemokines to their receptors under OGD conditions and after modulation of their receptors, using atomic force microscopy working in force spectroscopy mode will be performed. This will allow to determine how the activity of receptors will be change after ischemia, modulation using antagonists and DPSCs paracrine action.

The main project contribution to the development of science will be hypothesis verification whether CXCL12, CCL2 and CX3CL1 chemokines play an important role in the paracrine effects of DPSCs on nerve tissue after ischemia. The OHC model will provide a comprehensive examination of the interactions, not only like often studied in this context for neurons, but the entire *ex vivo* nervous tissue. Understanding the role of selected chemokines and their modulation - and particularly inhibiting the pro-inflammatory activity of CCL2 through the effects of Irbesartan may contribute to the development of more effective cell therapy.