POPULAR SUMMARY

The processes of embryonic stem cell differentiation (ESC) that occur during embryonic development lead to the formation of such different tissues as: epithelial, connective, neural or muscular. The correct process of differentiation guarantees the formation of not only normal cells, but also functional tissues that make up the organs, studying the mechanisms that regulate this process is important for both understanding the normal development and the pathologies that can accompany it.

Obtaining induced pluripotent stem cells (iPSCs), which have embryonic stem cell morphology (ESC), and which can be obtained by reprogramming somatic cells, has opened a new chapter in research aiming on understanding the mechanisms of recovery of pluripotency and additionally opens the possibility of generating patient-specific pluripotent stem cells. iPS cells can be genetically modified and may become available therapy in the future leading to the exchange of the mutated gene for a functioning gene.

However, before the first therapies based on this method arise, the difficulties associated with the way these cells are obtained must be overcome. IPSCs are formed by reprogramming somatic cells by introducing factors responsible for the state of pluripotency, i.e. Oct3/4, Sox2, Lin28A and NANOG (OSLN). This is done by introducing into the host cells using the Sendai virus, a genetic carrier in the form of cDNA encoding the necessary factors for their reprogramming. The advantage of this virus is that, unlike retroviruses, it does not build into the host genome and stays in the cytoplasm without changing the genetic information of the host cells. The use of adenoviruses or adeno-associated viruses also raises many doubts. Although they do not require integration into the host genome and are very effective in delivering genes to cells, in some cases their genetic material may be embedded in the cell. In addition, due to the presence of numerous viral genes, they are still highly immunogenic. This means that there is a need to develop a new, safe method that would allow cells to be reprogrammed without the use of viruses.

In this project, we propose to use the potential of star polymers as carriers in the supply of proteins to cells for reprogramming them into iPS cells. To date, there is no research on the reprogramming of fibroblasts into iPS cells by directly introducing the pluripotency factors: Oct3/4, Sox2, Lin28A and NANOG (OSLN) into cells in a form of proteins. The implementation of this innovative method would increase the efficiency of the reprogramming process, and most importantly, completely exclude the presence of virus in this process. The efficient and effective introduction of these proteins into the cell will be possible thanks to the use of non-toxic, non-viral carriers, such as cationic star polymers. Such carriers are neither immunogenic nor carcinogenic. In addition, they overcome both biological and physicochemical barriers during transport to the cytoplasm and further to the cell nucleus.

The most important application for induced pluripotent cells may be to treat genetic defects. Somatic cells are obtained from sick people and then reprogrammed. The iPS cells obtained in this way are subjected to genetic modification in order to introduce the correct version of the gene. Selected cells with the correct version of the gene are later differentiated into target cells, which allows them to be implanted in the patient's body without the risk of rejection. After transplantation, the cells begin to produce the right gene product and contribute to reducing disease symptoms.

To confirm the possibility of using our iPS cells for genetic modification to repair a non-functional gene, we will use somatic cells from patients suffering from congenital bone fragility (*Osteogenesis imperfecta*; OI). It is a disease caused by a collagen disorder, which makes bones more prone to fragility and brittleness. This disease is a monogenic disorder that results from damage to one gene. In 95% of cases, *Osteogenesis imperfecta* is caused by mutations in the *COL1A1* or *COL1A2* genes that encode the alpha1 and alpha2 chains of type 1 collagen.

Our goal will be to repair the mutation detected in the DNA of the reprogrammed cells, and then to transfect them with polyplexes of normal DNA with the star polymer as a carrier of genetic material. The use of such a polymer will protect DNA from its degradation by cell nucleases, which is a big problem when transfecting eukaryotic cells with other vectors. Clones with the repaired mutation will be differentiated into precursors of osteoblasts and skin fibroblasts that produce procollagen type I, to confirm the effectiveness of the mutation repair method.

The success of this project will make a significant contribution to the use of genetically modified iPS cells, which will make this method more effective and completely safe for patients.