SUMMARY:

Mesenchymal Stem Cells (MSC) are characterized with high pro-regenerative and immunomodulatory potential. The ability to obtain them from adult organism and characterization of their biological properties still are the subject of research for many scientists.

The aim of this study was *in vitro* characterization of biological properties of human MSC, localized in four adult tissue niches: in the bone marrow (BM-MSC), adipose tissue (AT-MSC), skeletal muscle (SM-MSC) and dermis (SK-MSC). The materials used for this thesis were: bone marrow taken from deceased individuals during autopsy, skeletal muscle and adipose tissue obtained as a post-surgical waste and dermis, collected after lower limb amputation.

There were examined similarities and differences in expression of markers characteristic (CD73, CD90, CD105) and negative (CD34, CD45) for MSC, expression of markers involved in regulation of angiogenic process (PDGFRα, CD146) and the markers, that indicated stem and/or progenitor character of studied cells (PW1, CD56). The special attention was paid to expression of *Sox2* and *Oct4* genes, which rates the stemness level, the tumor suppressor *p53* and protooncogenic *c-Myc* genes. The functional features of MSC, that were exhaustively described, were: an ability to secrete various cytokines and trophic factors into supernatant after *in vitro* culture, an ability to differentiate into osteoblasts, adipocytes and chondrocytes and an ability to make spontaneous fusion with cells from other sources.

In the study following methods were used: flow cytometry, immunocytochemical stainings, real time RT-PCR and Multiplex ELISA. Besides the deep characteristic of tissue specific differences of MSC, special attention was paid to characterize the influence of long term *in vitro* culture on their biology. To describe precisely the phenotype changes phenomenon, the analysis of fluorescence intensity dynamic of markers were perform. This analysis refers to density of studied antigens in cells. The observations were made up to passages P9-P10.

It was observed that cells with basic MSC phenotype could be isolated from all of studied tissues. Quickly after passage P1, the cell cultures were characterized with high purity (>90%) of population that expressed CD73, CD90 and CD105 and low (<3%) expression of CD34 and CD45 of hematopoietic markers. However, this scheme did not applied to cells isolated from skeletal muscle, where CD105 expressing population was smaller (78%). These

skeletal muscle isolated MSC were characterized with high heterogeneity, as confirmed by the expression of CD56 which is characteristic for myogenic precursor cells. Typical feature of SM-MSC was also lack of terminal adipogenic ability which suggests their bipotent character, which means that cells isolated from skeletal muscle under applied methodology had more progenitor than multipotent character. The characteristic for BM-MSC was the most stable basic MSC phenotype and the strongest expression of CD146 in passage P10. In contrast, the CD146 expression in MSC from adipose tissue and skeletal muscle decreased between passage P5 and P6. BM- and AT-MSC, but not SM- and SK-MSC were able to secrete high concentrations of various cytokines into post-culture supernatant. The most characteristic were: MCP-1, IL-8, VEGF and IL-6, which the highest observed concentration was after AT-MSC culture.

The new approach to biology of MSC study was analysis of PW1 factor, which so far was identified as characteristic for muscle progenitor cells and mediator of apoptosis. Expression of this marker was observed in isolated MSC from all studied tissues, but not in cells isolated from the bone marrow. In this study it was observed that SM-MSC had strong CD56/PW1 coexpression while fusion. The most recent information's about PW1 suggests, that it can be another marker for stem/progenitor cells. What is the most interesting observation here is lack of PW1 expression in MSC isolated from bone marrow which is considered to be the source of highly undifferentiated MSC.

The source of MSC had its influence on pluripotency genes expression. The highest *Sox2* expression in P1, as compared to iPSC control, was characteristic for MSC isolated from dermis. mRNA expression *of Sox2* in P1 was rather low in BM-MSC, but increased with the number of passages. This gene expression was rather stable in AT- and SM-MSC. Observed expression of *Oct4* in BM- and SM-MSC rise up with the number of passages. The *Oct4* expression was stable for SK-MSC but not for SM-MSC, in which expression of *Oct4* drop down in passage P5. This observation could be related to processes of spontaneous myogenesis. Myogenic process in P5 of SM-MSC could be also related with downregulation of p53, which expression in other tissues was rather stable during long-term culture.

The highest expression of *c-Myc*, as compared to iPSC, was observed in AT-MSC population. Therefore there is a necessity of paying a special attention to MSC from this source if clinical application is considered. The high expression of *c-Myc* could be related to high proliferative capacity of AT-MSC, but potentially higher risk of tumorigenesis can't be ignored.

BM- and AT-MSC both was characterized to be highly potent in cell fusion with SM-MSC. This phenomenon wasn't observed with arrangement of SM-MSC with SK-MSC. This observation suggests, that despite some similarities in basic MSC phenotype of cell from all analyzed tissues there are biological differences (such as lack of fusion ability), that are determined by mechanisms that are yet to be described. The fact, that AT-MSC show equal fusion capacity as BM-MSC suggests another important similarity between the MSC from those two sources. And this feature may suggests another application, in which AT-MSC can be used instead BM-MSC which is characterized with limited accessibility.

The results presented in this study, are the evidence of biological differences between MSC isolated from bone marrow, adipose tissue, skeletal muscle and dermis. Those differences can be important from the point of view of regenerative medicine opening an opportunity of "targeted" therapies, in which MSC from different sources are been chosen depending on their special regenerative potential and genetic stability.