P7. **Assessment of perilipin expression by microscopy, flow cytometry and molecular biology techniques**

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Introduction: Perilipin is involved in the energetic metabolism as it functions as a lipid gatekeeper that controls access to of the lipases to the intracellular lipids in a phosphorylation-dependent manner. The expression of perilipin is correlated to the adipose phenotype and it is traditionally assessed as an adipogenic marker for mature adipocytes. In this context, the aim of our study was the *in vitro* genic and protein evaluation of perilipin in induced human adipose derived stem cells (hASCs) for developing knowledge regarding the adipogenesis kinetics. Additionally, the original adipogenic medium was designed to modulate the differentiation of hASCs in the view of tissue engineering applications.

Materials and methods: Third passage hASCs were induced towards adipogenesis using an original differentiation medium. The protein expression of perilipin was qualitatively and quantitatively evaluated by immuno-cytofluorescence techniques, namely fluorescence microscopy and flow cytometry respectively. In this view, cells were fixed with paraformaldehyde, permeabilised and incubated over night at 4°C with the primary antibody, washed with PBS buffer, incubated with the secondary antibody labelled with rodamine, washed again and stained with DAPI. In order to evaluate the genic expression of perilipin the following procedures were performed: i) RNA extraction, ii) determination of RNA concentration, purity and integrity, iii) revers transcription and iv) qRT-PCR. Quantitative data were analysed using Prism 3.03 Software.

Results: Perilipin wasn't detected at any level at the initiation of the adipogenic process in hASCs. The first detection in genic expression of perilipin was registered after one week of adipogenesis, while the protein expression was detected for the first time at 10 days post induction. In addition, the expression of perilipin increased in time, during the adipogenesis, probably due to the increasing number of cells undergoing the process and to the increasing volume of the intracellular lipid deposits inside the mature cells.

Conclusions: The adipogenic medium formulated sustained the adipogenic process and could be used for further tissue engineering applications.

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