SUMMARY OF THE THESIS

The main aim of the project was to develop a methodological approach of in-vitro chondrogenic graft preparation. This graft should utilise mesenchymal stem cells (MSC) seeded in a hyaluronan based scaffold.

A review of known hyaluronan derivatives with a potential for biological applications was prepared in the first phase of the project. (Sedova, Knotkova et al. 2007) As a result of the review, a hydrogel based on tyramine derivative of hyaluronan cross-linked by peroxidase (HA-TA scaffold) was chosen for the following research. A sufficiently rich, reliable and ethically acceptable source of MSCs was required. Washed bone marrow collection sets remaining after bone marrow collection for transplantation purposes was demonstrated to meet these needs. (Dvorakova, Hruba et al. 2008) MSCs reveal specific features, namely low seeding density and morphological changes during culture, which may affect the outcome of cell viability measurements in experiments. In order to solve this complication, three different methods of cell viability determination were compared, and the luminiscent detection of intracellular ATP was recommended for MSC applications. (Vistejnova, Dvorakova et al. 2009) Hyaluronan influence on chondrogenic differentiation of MSCs was evaluated in a pellet micromass system at first. The study has shown, that regardless of native hyaluronan molecular weight (100-1500 kDa), this polysaccharide does not affect the outcome of the differentiation process in this model system. (Dvorakova, Velebny et al. 2008) On the other side, the final study of the project has shown that hyaluronan based HA-TA hydrogel provides a sufficient environment for chondrogenic differentiation of MSCs induced by standard chondrogenic culture medium. This process can be further stimulated by an addition of BMP-5 growth factor; however it may increase the risk of unwanted osteogenic development.

The aim of the study was fulfilled, there was formed a methodological apparatus suitable for development of a chondrogenic graft. This approach was utilised in a project demonstrating that a novel hyaluronan based hydrogel – (HA-TA scaffold) comprises for a convenient material for a MSC seeded chondrogenic tissue substitute.