Adipose Stem Cells and Intervertebral Disc Regeneration: Role of the Microenvironment

General Abstract

Current treatments for the degenerated intervertebral disc (IVD) comprise pain management and invasive surgical interventions; both of them are aimed at relieving the pain instead of repairing the tissue and restoring the biological function of the disc. Cell-based tissue engineering may meet the clinical need for the repair or regeneration of a damaged tissue, and it might be ideal to repair the degenerated IVD and restore the joint-like function of the discs. The ultimate goal of this thesis is to develop a one-step procedure to regenerate the IVD by a minimally invasive procedure, which is to inject adipose stem cells (ASCs) into the nucleus pulposus (NP) of the IVD with or without a scaffold.

Stem cells and progenitor cells reside in highly regulated microenvironments called niches, which allow them to maintain a balance of self-renewal and differentiation. Adult stem and precursor cells, as a part of normal regeneration processes, are believed to egress and circulate away from their niches, and then engraft and differentiate within a range of tissue microenvironments. In our strategy for disc regeneration by ASC injection, ASCs would inevitably encounter a NP microenvironment, which consists of cells, extracellular matrix, hypoxia, and other factors. We hypothesized that the fate of the ASCs injected in the NP would be directed by the complex NP microenvironment, thus allowing concepts employing minimally manipulated stem/progenitor cells without exogenous stimulation with growth factors or other stimuli.

NP cells are the sole cell type in the NP. However, the low density of NP cells makes the occurrence of direct cell–cell contacts between NP cells and ASCs a rare incidence after ASCs injection. This poses the question to what extent the ASCs would be affected by the NP cells residing in the NP. Therefore, in **chapter 2** we investigated the interactions between ASCs and NP cells in co-culture studies using transwell systems, thereby preventing direct cell-cell contacts. Moreover, by overcoming the problem of in vitro dedifferentiation of chondrocyte-like cells by culturing the NP cells in a micromass configuration, we were able to show that soluble factor(s) secreted by NP cells are responsible for, and capable of directing the ASCs towards the NP cell-like phenotype when NP cells are in a differentiated state.

Due to the high matrix-to-cell ratio in the NP, most of the ASCs injected will encounter matrix contacts and only indirect contact with NP cells (via soluble factors; see above). In **chapter 3**, we aimed to assess cartilaginous matrix (collagen type II hydrogel)-mediated effects on chondrogenic differentiation in ASCs by comparing them with those mediated by collagen type I gels. We demonstrated that soluble factors released by NP cells can promote chondrogenic differentiation of ASCs in collagen hydrogels, and that combination with a nucleus-mimicking collagen type II microenvironment enhances differentiation towards a more pronounced cartilage/NP lineage relative to collagen type I hydrogels.

Chapters 4 and **5** of this thesis describe our efforts to gain insight in the underlying mechanisms resulting in the differential outcomes when using different scaffolds (e.g. collagen types), and taking into account recent reports on the role of cell shape on lineage differentiation. In **chapter 4**, we demonstrated that β1 integrins play a more important role at the later stages than at the earlier stages of chondrogenesis, as evidenced by our findings that β1 integrin blockage in ASCs surprisingly promoted chondrogenesis at earlier stages but inhibited the chondrogenic gene expression at later stages. We further provided evidence that promoting the onset of chondrogenesis by β1 integrin blockage might be through inhibiting ROCK signaling and shaping round cells. The putative role of the type of collagen of which the 3D hydrogels were made on the β1 integrin-mediated Rho A/ROCK signaling and cell shaping pathway, and the resulting level of chondrogenic gene expression was addressed in **chapter 5.** Our results showed that collagen type II indeed provides the inductive signaling for chondrogenic differentiation in ASCs by shaping round cells through β1 integrin-mediated Rho A/ ROCK signaling pathway. Together, **chapters 4 and 5** suggest that scaffolds can act in concordance with soluble bioactive factors (e.g. transforming growth factor-β1), to direct stem cell differentiation more efficiently towards the chondrogenic lineage by shaping round cells, and to provoke these effects through β1 integrin-mediated Rho A/ROCK signaling.

In **chapter 6**, we summarized the current state of the art of cell-based therapy and the knowledge on microenvironmental conditions possibly influencing treatment outcomes for the intervertebral disc, and concluded that ASC-based cellular treatment might provide a feasible alternative for the currently explored treatment modalities.

In **chapter 7**, the feasibility of injecting freshly isolated ASC preparations (i.e. the stromal vascular fraction; SVF) into the NP to

reverse, halt, or retard degenerative disc disease (DDD) was evaluated in a goat DDD model which was developed recently by our group and is similar in nature to the human pathologic process with respect to biomechanics, geometry, structure, biochemistry and absence of notochordal cells. Surprisingly, in an initial experiment we observed that SVF resulted in rapid and massive cellular infiltration causing severe degenerative changes and osteoclastic activity in surrounding bony structures. In a subsequent animal experiment, primarily designed to provide clues on the possible causes for these adverse phenomena, we demonstrated that the inflammatory response was not observed either in virtually all discs treated with cultured ASCs or density gradient-purified (red blood cell-depleted) SVF. Although this seemed to indicate that red blood cells might be (one of) the causes, this suggestion was contradicted in subsequent studies (see chapter 7 for details). Our current interpretation of the results from our various in vivo studies is that thorough washing of the SVF to get rid of the collagenase used to extract the SVF from the adipose tissue is essential.

Chapter 7 also reports on increased numbers of cells in about 50% of the cell-treated discs. In discs with increased cellularity, a significantly increased expression of the ECM genes collagen type II and aggrecan was observed. Together with the finding that the adipose marker PPAR-γ present in ASCs was consistently elevated in these discs, but not in inflammatory discs generated by high dosages of cABC but without subsequent SVF treatment, it is suggested that the ASCs in the SVF may be actively involved in this anabolic gene upregulation, and that this may already occur within three months after SVF injection.

Conclusion

In summary, this thesis tested the feasibility of the one-step minimally invasive procedure for disc regeneration by investigating ASCs-NP microenvironment interactions, underlying mechanisms by which some scaffolds may provide inductive signals for chondrogenic differentiation, and the feasibility of one-step SVF or ASC injection strategies for disc regeneration in a goat model. We demonstrated that: (i) the native components within the NP microenvironment are able to provide the inductive signals for chondrogenic differentiation in ASCs. (ii) supporting a round cell shape through β1 integrin-mediated Rho A/ROCK signaling is suggested as one of the important criteria when choosing a scaffold for tissue engineering treatment approaches of cartilage-like tissues (e.g. NP).

(iii) The quality and removal of contaminants in SVF mixture are critical when SVF is used for the injection treatment for disc regeneration in a goad DDD model. Although the data provided in this thesis are not yet sound enough to promote the proposed ASCs-based one-step procedure for IVD regeneration into the stage of clinical trial at this moment, and some parameters need to be further evaluated or optimized, we conclude that SVF/ASC injection strategies appear a promising and feasible concept either in one-step or multi-step procedures.