Chondrocytes and chondrons for tissue engineering of cartilage

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Cartilage degeneration is a major clinical problem nowadays. Current treatment options aim for pain management and removal of damaged tissue instead of repairing the tissue. The ultimate goal for the treatment of cartilage defects would be reconstruction of the tissue by which all the properties of the native tissue are preserved. Tissue engineering aims at restoring the tissue and thereby also the function. This makes cartilage tissue engineering very promising for the treatment of cartilage defects. In this thesis we investigated certain aspects of the repair of cartilage by the use of cell-induced

cartilage formation with chondrocytes. The main focus was on the collagen network as this is often compromised in cartilage reconstruction.

In **chapter 2** we provide quantitative biochemical data from various cartilaginous tissues (e.g. articular cartilage, meniscus, nucleus pulposus and annulus fibrosus) and data on gene expression levels (from genes encoding proteins/enzymes involved in the synthesis and degradation of the ECM) of chondrocytes from these tissues. We found large differences in the biochemical composition of the different tissues and in the gene expression levels by the different chondrocytes. Correct expression of the modifying enzymes by the cells is a requirement for the production of a collagen network with the desired biomechanical properties. The expression of enzymes involved in collagen cross-linking was different between the cell populations under the same culture conditions. This makes collagen-modifying enzymes potent chondrocyte-specific phenotypical markers for cartilage tissue engineering applications.

In **chapter 3** we analyzed the effects of glucose deprivation on extracellular matrix (ECM) production and cell death by various chondrocyte populations and dermal fibroblasts. Chondrocytes responded faster than dermal fibroblasts to glucose deprivation by initiating an endoplasmic reticulum (ER) stress response. A general response by cells to ER stress is a down-regulation of expression of secreted molecules and an up-regulation of chaperones and foldases. We found indeed a down-regulation in the expression of type I and type II collagen, while most of the collagen-modifying enzymes were up-regulated to help with any misfolded or aggregated proteins in the ER. A prolonged situation of ER stress leads to apoptosis. Although chondrocytes are more sensitive to glucose deprivation, less apoptotic chondrocytes were found after chemically induced ER stress than apoptotic fibroblasts, indicating that chondrocytes can cope better with ER stress. Overall, glucose deprivation is unwanted in cartilage tissue engineering approaches as there is no ECM production and the cells undergo apoptosis.

In **chapter 4** we investigated whether the presence of type I or type II collagen affects chondrocyte-mediated matrix contraction and collagen degradation. The chondrocytes contracted the gels composed of type I collagen and this coincided with an increased Mmp-13 and -14 expression as well as matrix degradation.

In **chapter 5** we have shown that maintaining the chondrocyte's native pericellular matrix improves cell-induced cartilage formation. Chondrons secreted higher levels of proteoglycans compared to isolated chondrocytes and the collagen that was deposited by the chondrons was cross-linked. Furthermore, expression levels of Mmp-13 were strongly increased after 18 days of culture in alginate beads by chondrocytes compared to chondrons.

That maintaining the chondrocyte's pericellular matrix prevents collagen-induced Mmp-13 expression and collagen degradation is demonstrated in **chapter 6**. By keeping the pericellular matrix a direct contact between chondrocytes and collagen is prevented. We found that both integrin α1 and discoidin domain receptor 2 modulate Mmp-13 expression after direct contact between chondrocytes and collagen. Furthermore, PKC, FAK, MEK and JNK are involved in collagen-stimulated expression of Mmp-13.

Based on the data presented in this thesis we conclude that chondrons are more effective for cartilage tissue engineering approaches than chondrocytes. The interactions between chondrocytes and their native pericellular matrix seem to provide optimal conditions for cartilage tissue engineering. The pericellular matrix stimulates the production of ECM, it enhances the chondrocytic characteristics of the cells and it prevents matrix degradation.