Chondrocytes and chondrons for tissue engineering of cartilage

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CHAPTER 7

GENERAL DISCUSSION

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Tissue engineering aims for the replacement, maintenance, and regeneration of tissue or functional enhancement of impaired or defect tissue. This makes cartilage tissue engineering very promising for the treatment of cartilage defects. 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. The specific aim of this thesis was to improve the chondrocyte-dependent deposition of collagen.

Autologous chondrocytes are already being used in the clinic for cartilage tissue engineering approaches (see General Introduction). However, the results are not satisfactory yet as the mechanical properties and the durability of the reparative tissue are less than that of the original tissue. In native cartilage, the chondrocytes are surrounded by a pericellular matrix, together forming the chondron. We hypothesized that the pericellular matrix would positively influence the matrix deposition by chondrocytes.

The pericellular environment of chondrocytes is a highly organized matrix containing high levels of type VI collagen, hyaluronan, proteoglycans and glycoproteins[1,2]. Hyaluronan affects cell metabolism[3] and it plays an important role in the retention of newly synthesized proteoglycans[4,5]. Therefore, it is very likely that the pericellular matrix, or the hyaluronan in it, provides a template that nucleates the organization of newly synthesized proteoglycans. In chapter 5, it is shown that chondrocytes cultured within their native pericellular matrix deposit higher levels of proteoglycans. This is line with observations from other studies where they have also shown that chondrons deposit more type II collagen compared to chondrocytes[6,7]. Proteoglycans and collagens can co-migrate through the pericellular matrix in vacuoles[8].

We concluded from chapter 5 that the type II collagen that is deposited by chondrons does more resemble the type II collagen found in the native tissues as the collagen is cross-linked. These cross-links were absent in the type II collagen produced by the chondrocytes. Thus the pericellular matrix modulates the expression or the activity of enzymes involved in collagen cross-linking. These cross-links are essential in providing tensile strength and mechanical stability of a tissue[9-11]. The modification of collagen, also the modifications which can later be converted to cross-links, is performed intracellular[12]. This means that correct expression of the modifying enzymes by the cells is a requirement for the production of collagen with the desired properties. In chapter 2, we have shown that the expression levels of the collagen modifying enzymes are different between chondrocytes isolated from various cartilaginous tissues. It is very likely that the expression levels of the collagen modifying enzymes are better maintained by preservation of the pericellular matrix. The typical chondrocytic characteristics in terms of type II collagen and aggrecan expression are also better maintained by chondrons than by chondrocytes in

culture (chapter 5)[6,7]. It has to be noted that the pericellular matrix by itself is not sufficient to maintain the phenotype. Chondrocytes can extend lamellipodia through their pericellular matrix and if they get the stimuli for it, they can escape or degrade the pericellular matrix[13]. It is known that this happens when chondrons are grown in monolayer[13]. We did not observe, however, degradation of the pericellular matrix or empty chondron shells when chondrons were cultured in alginate beads.

Changes in the cell-ECM interactions by removal of the pericellular matrix do not only affect matrix production, but also matrix degradation. Direct contact between chondrocytes and fibronectin fragments[14-16] and/or fibrillar collagen[17-21] stimulates the production of MMP-13. As direct contact between chondrocytes and collagen induces MMP-13 expression, it is questionable whether the use of chondrocyte-seeded collagen matrices is the best choice for reconstruction of cartilage defects. In chapter 4 we have shown that contraction of collagen gels can even further enhance MMP expression and matrix degradation by chondrocytes. The problem is not limited to collagen scaffolds only. In chapter 5 we showed that the contact of the chondrocytes with type II collagen that is produced by them also induced Mmp-13 expression. Thus prevention of direct contact between chondrocytes and fibrillar collagens seems useful for tissue engineering applications. In chapter 6 we showed that this can be accomplished by maintaining the native pericellular matrix of chondrocytes.

As the interactions of chondrocytes with their pericellular matrix seem very important in the prevention of excessive collagen degradation, one might even speculate that changes of the pericellular matrix can lead to the onset of osteoarthritis. Changes in the pericellular matrix represent one of the earliest identifiable matrix changes associated with cartilage metabolism[22-24]. In osteoarthritic cartilage, the expression and the distribution of type VI collagen are disturbed[22-26] and proteases that can degrade type VI collagen are highly expressed[27-29]. Furthermore, Col6a1-knockout mice show an accelerated development of osteoarthritic changes[30]. So the pericellular matrix, and especially the type VI collagen in it, seems important for healthy cartilage.

For cartilage tissue engineering purposes, the interactions between chondrocytes and their native pericellular matrix seem to be superior; it stimulates the ECM production, the chondrocytic phenotype is maintained and it prevents collagen degradation. However, the number of cells that can be obtained from cartilage remains low and expansion of both chondrocytes and chondrons leads to dedifferentiation. Although dedifferentiated chondrocytes can redifferentiate again, they do not automatically form a new native-like pericellular matrix. Certain factors, such as scaffold properties, age of the chondrocytes, and culture conditions, are known to have an influence on the formation of the pericellular matrix[31-34], but the mechanisms behind this are still unknown. It would be of considerable interest to study how the formation of a native-like pericellular matrix can be stimulated.

An alternative approach is the use of a scaffold that resembles the chondrocyte's native pericellular matrix. In this way, the interactions between chondrocytes and the scaffold are similar to those found in native cartilage. Although type VI collagen is the

most abundant component of the pericellular matrix, the pericellular matrix is a highly organized and it is likely that the whole network determines the optimal surrounding for chondrocytes. Therefore, it can be argued whether the incorporation of type VI collagen into a scaffold is sufficient to acquire all the benefits from the native pericellular matrix.

CONCLUSION

This thesis proposes that the interactions between chondrocytes and their native pericellular matrix provide the optimal circumstances for cartilage tissue engineering. The pericellular matrix stimulates the production of ECM (both quantitatively and qualitatively), it enhances the chondrocytic characteristics of the cells and it prevents matrix degradation. For tissue engineering applications the interactions between the chondrocytes and the pericellular matrix can possibly be acquired by the formation of a new native-like pericellular matrix or by the use of a scaffold that resembles the native pericellular matrix.

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