Chondrocytes and chondrons for tissue engineering of cartilage

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CHAPTER 1

GENERAL INTRODUCTION

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Cartilage defects are a serious public health problem affecting people of all ages. It is a major cause of disability due to symptoms as pain, stiffness and loss of mobility. The most frequently affected sites are the knees, hips and spine[1]. In knees and hips, joint pain often results from degeneration of the articular cartilage due to osteoarthritis or from trauma[2].

The Dutch Institute for Public Health (RIVM) estimated that the prevalence of osteoarthritis was about 4% of the total Dutch population in 2006 according to registration with Dutch general practioners. Based on demographic development alone, it was expected that the prevalence of osteoarthritis will increase by about 50% between 2007 and 2040[3]. Back pain that can develop in association with or as a consequence of degeneration of an intervertebral disc has a lifetime prevalence of 80%[4]. Although cartilage defects are seldom life-threatening, the annual cost in terms of morbidity, lost productivity and medical expenses is significant.

Current treatments attempt to relieve the symptoms rather than repairing the affected tissues. When conservative treatments such as analgesia and physical therapy fail, surgical procedures are performed. Most surgical intervention is directed towards removing the damaged tissue. So far, there is no therapy that restores the tissue and thus the function. As there is a growing consensus that current strategies may not be effective, development of new treatment modalities is critical. Tissue engineering approaches are very promising as they aim at restoring the tissue and its function rather than only pain relieve.

CARTILAGE: COMPOSITION, FUNCTION AND FAILURE

Composition

Cartilage consists of extracellular matrix (ECM) and only a small number of cells, the chondrocytes. The chondrocytes are surrounded by a pericellular matrix, together forming the chondron[5]. The pericellular matrix of a chondron is defined primarily by the presence of relatively high levels of type VI collagen as compared to the interchondron ECM, but it also contains type II, III, IX and XI collagen, hyaluronan, proteoglycans such as aggrecan, biglycan and decorin, and glycoproteins such as fibronectin, link protein and laminin[6-12].

The ECM is composed of collagen, proteoglycans, glycoproteins and glycosaminoglycans[13,14]. Cartilage is classified in three types; elastic cartilage, hyaline cartilage and fibrocartilage, which differ in the amount of the main components of the ECM. Elastic cartilage is found in the external ear, eustachian tube, larynx and epiglottis, it is characterized by the presence of elastin in the ECM. Hyaline cartilage is rich in type II collagen and proteoglycans. Articular cartilage belongs to hyaline cartilage, but it is also

found in the ear, larynx and between the ribs and the sternum. Fibrocartilage is characterized by the presence of type I collagen fibrils. The meniscus and intervertebral discs are examples of fibrocartilaginous tissues.

Function

The cartilage found in joints allows the movements between adjacent bones. Articular cartilage covers the end of bones within synovial joints such as the knee and provides a smooth surface for the movement of articulating bones. In addition the composition of articular cartilage allows it to withstand compressive and shear forces and distributes these forces onto the subchondral bone[1,2,15]. The menisci that are also found in the knee play an important role in load transmission, stability and energy dissipation[16-19].

In the spine, the intervertebral discs that are situated between vertebral bodies function like a joint. They allow flexion, extension, lateral bending and twisting of the spine and they are resistant to compressive loads[4,20]. An intervertebral disc consists of an annulus fibrosus on the outer side and the nucleus pulposus forms its center. The annulus fibrosus contains highly orientated type I collagen fibers. Although nucleus pulposus officially belongs to fibrocartilage, the ECM is rich in type II collagen. It also contains a high amount of proteoglycans, mainly aggrecan. As the proteoglycans are hydrophilic, they retain a lot of water, causing the nucleus pulposus to swell.

Failure

Multiple factors are believed to cause degeneration of cartilage, such as abnormal mechanical loading, injury but also genetic predisposition. Failure of nutrient supply is also thought to contribute to cartilage degeneration. Since cartilage is avascular, chondrocytes have to rely on diffusion for nutrient supply and waste exchange. Conditions that reduce the blood supply to cartilage, such as atherosclerosis or the occurrence of micro-emboli in subchondral vessels, can cause nutrients to drop to levels that no longer support cell activity or viability[19,21-29]. Apart from vascular pathology, nutrients may not reach the chondrocytes if there is sclerosis of the subchondral bone or if the cartilaginous endplate calcifies. Cartilage has a very limited ability to repair itself, no matter what the cause is of the initial degradation.

In the intervertebral disc, the first changes of degeneration are seen in the nucleus pulposus. Many nucleus pulposus cells undergo apoptosis and there are also biochemical changes; there is a shift from type II collagen to type I collagen synthesis and there is a loss of proteoglycans. The water can no longer be retained and the nucleus pulposus dehydrates causing the intervertebral disc to lose height[30,31]. The disc can no longer function as a shock absorber and the annulus fibrosus and the endplate are loaded by inappropriate stresses[32]. Furthermore, the spine collapses by the reduction in disc height and this can cause pressure on the nerve roots leading to pain[33].

In degeneration of articular cartilage there is also a loss of proteoglycans and type II collagen is degraded by the collagen-degrading enzyme collagenase-3 (MMP-13)[34-40]. An

imbalance between anabolic and catabolic activities can lead to degeneration. The pericellular matrix likely plays a role in this, as changes in the pericellular matrix represent one of the earliest identifiable matrix changes associated with cartilage catabolism. Enlarged chondrons are found in osteoarthritic cartilage with less fibrillar and more type VI collagen in the pericellular matrix. Furthermore an increased number of chondrocytes inside one chondron is found in osteoarthritic cartilage, making the chondron a unit that functions less[41-43]. Once the cartilage is degraded to a certain point, the articular cartilage cannot supply a smooth surface for the movement of articulating bones.

Meniscal tears (ruptures of the meniscus) can occur due to degeneration or trauma. Degenerative meniscal tears are often a typical change of progressive age. These tears are usually horizontal in the meniscus and the segments are remained in their place. Traumatic meniscal tears are usually radial or vertical in the meniscus and are more likely to produce a moveable fragment. Meniscal tears are associated with osteoarthritis in the knee[16-19].

CURRENT TREATMENT OF CARTILAGE DEFECTS

When conservative treatments fail, surgical procedures are performed. In the case of the intervertebral disc, discectomy is a common procedure. This might relieve pain, but it does not restore disc height or its load bearing capacity. Spinal fusion aims at restoring the disc height by making a bony bridge between the vertebrae. Although the height is restored by this procedure, it can result in limited flexibility and possibly in degenerative changes in the adjacent vertebrae. Partial and total disc replacements have been developed in an attempt to preserve mobility[4].

For the meniscus (sub) total meniscectomy was a very common procedure. Although this instantly removes pain, it generates abnormal loads and stresses onto the articular cartilage which lead to the development of osteoarthritic changes. To prevent osteoarthritis, the loose meniscal segment or tear is fixed onto the main body of the meniscus. However, if the tears do not heal or heal too slow, the biomechanical forces might in time lead to failure of the fixation. Another option is the (partial) replacement of the meniscus, but it is very hard to mimic the mechanical properties of the meniscus by artificial materials. And these properties are very important for the functioning of the meniscus and to prevent osteoarthritis in the vulnerable underlying cartilage[16-19].

Cartilage debridement is very often the first treatment after the discovery of articular cartilage lesions. This technique polishes the cartilage and restores the smooth surface, but it does not solve the underlying problems and is most often a temporary solution. Bone marrow stimulation techniques (such as perforations, abrasions, microfractures) penetrate the subchondral bone to stimulate repair from bone marrow-derived cells. However, the defects are often repaired with fibrocartilage and degeneration often occurs in this reparative tissue. With mosaicplasty a piece of cartilage is taken from a non-load bearing region and placed into the defected site. The problem with this technique

is that it is very hard to apply this on large defects and it is difficult to achieve a smooth surface. Moreover, a new defect is created in the cartilage. More directed towards a tissue engineering approach is the transplantation of cells.

The transplantation of autologous chondrocytes (ACI/ACT) is being used in the clinic the last 10 years. This first generation ACI (autologous chondrocyte implantation) does not use a delivery vehicle, but a suspension of cells. However, with this technique the defects are also often repaired with fibrocartilage, which is not the desirable tissue. More studies are now ongoing about the use of different materials to use as a vehicle and about the use of other cell sources such as mesenchymal stem cells in stead of chondrocytes[1,2,15,44-47].

Most of the surgical techniques are directed towards removal of the damaged tissue and the relief of pain. So far, no real regenerative technique is successfully applied. The creation of new cartilage by tissue engineering is very promising as this aims at restoring the tissue and thus also its function.

CARTILAGE TISSUE ENGINEERING

Tissue engineering is the regeneration and remodeling of tissues *in vivo* in order to repair, replace, maintain or enhance organ function, as well as to engineer and grow functional tissue substitutes *in vitro* for implantation *in vivo* as biological substitutes for damaged or diseased tissues and organs. Tissue engineering comprises the use of cells, a supporting structure (scaffold) and molecular signals to trigger a cellular response. For a clinical use of tissue engineering, an autologous cell source is preferred as it will limit the risk of infection, disease transmission and potential immune rejection. The most obvious choice of an autologous cell source is the tissue itself.

The engineered tissue should structurally and morphologically resemble the native tissue to be able to perform similar biological functions. As the functioning of cartilage depends entirely on its ECM, mimicking the native ECM seems essential. The collagen network plays important roles in the biomechanical properties of cartilage[48,49] and failure of the collagen network sets degeneration of cartilage into motion[50,51]. However, the production of this collagen network is the main problem in cartilage tissue engineering; not only are articular cartilage defects often replaced with fibrocartilage, also *in vitro* produced neo-tissue constructs have often a poor quality and quantity of collagen.

Essential for cartilage tissue engineering is the maintenance of the differentiated chondrocyte phenotype. Chondrocytes that are grown in monolayer lose their round morphology as they undergo phenotypic changes; they start producing type I collagen instead of type II and they have a decreased proteoglycan synthesis[52,53]. This protein profile is a characteristic of soft connective tissue rather than cartilage. Maintaining the chondrocytic phenotype can be accomplished by culturing the chondrocytes in a 3-D environment. Culture in alginate is extensively used in *in vitro* applications[54-57].

For *in vivo* cartilage reconstruction collagen-based scaffolds are widely used[58-61], even though it has been reported that collagen can induce the production of MMP-13[62-66]. For tissue engineering purposes, excessive collagen degradation is undesirable, especially the collagen that is deposited in the neo-construct.

AIM OF THIS THESIS

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. This thesis focuses on regeneration of cartilage by the use of cell-induced cartilage formation. The main focus is on the collagen network as this is often compromised in cartilage reconstruction.

To optimally mimic a target tissue, it is important that the properties of the native tissue are known to use as a reference. Although different cartilaginous tissues (e.g. articular cartilage, annulus fibrosus, nucleus pulposus, and meniscus) differ from each other, little quantitative information is available about the biochemical properties of the collagen network in the different tissues and of the phenotypical differences of the chondrocytes within these tissues. The goal of **chapter 2** was therefore to provide quantitative biochemical data from various cartilaginous tissues and to provide data on gene expression levels (from genes encoding proteins/enzymes involved in the synthesis and degradation of the ECM) of chondrocytes from the various tissues to enhance phenotypical characterization of these cells.

Nutrient deprivation is believed to be one of the common factors causing cartilage degeneration. If cartilage degeneration occurred due to a drop in nutrient supply, implanted cells will suffer the same fate if the problem is not solved. The purpose of the study described in **chapter 3** was to examine the effects of glucose deprivation on ECM production and cell death.

Collagen-based scaffolds are widely used for *in vivo* cartilage reconstruction, although there are some known drawbacks. One of them is the cell-mediated contraction of the scaffold which could result in a loss of contact between the implant and the host tissue and thereby decreasing the chances for successful integration. Another drawback is the collagen-mediated induction of MMP-13 which could lead to the destruction of the scaffold and the host tissue. The main goal in **chapter 4** was to investigate whether the presence of type I or type II collagen affects chondrocyte-mediated matrix contraction and collagen degradation.

In **chapter 5** we investigated whether preservation of the chondrocyte's native pericellular matrix has a positive effect on cartilage formation. Isolated chondrocytes are often used for cartilage tissue engineering, but this does not mimic the in vivo situation since the chondrocytes are surrounded by a pericellular matrix in native cartilage and cells are very much influenced by their surroundings. Therefore we hypothesized that retaining the pericellular microenvironment would have a positive effect on the synthetic capacity of

the chondrocytes. Furthermore, retaining the pericellular matrix might prevent collagen-mediated up-regulation of MMP-13 since there is no direct contact between the chondrocytes and fibrillar collagen.

In **chapter 6** we investigated collagen receptors and signalling pathways which are involved in collagen-mediated MMP-13 induction. A better understanding of the mechanisms involved in collagen-induced MMP-13 up-regulation could help in the development of a therapeutic target for the inhibition of excess matrix degradation by this enzyme. Furthermore, it can also have an impact on the use of chondrocyte-seeded collagen-based matrices for cartilage repair as this mostly results in too little collagen in the neo-constructs.

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