CHAPTER 2

Cell-Based Meniscus Tissue Engineering

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Summary

Meniscus tears have an important socio-economic impact on our society. Each year more than 400,000 surgical interventions are performed on meniscal tears in Europe. In the United States, over 1 million interventions are performed. The majority of meniscal tears are situated in the inner avascular zone. In this zone no spontaneous healing is possible. The current therapeutic strategy for this type of meniscus tears is partial or subtotal meniscectomy according to the dimensions of the tear. Because removal of meniscus tissue will finally induce osteoarthrosis of the knee, new therapeutic strategies to substitute or replace the damaged meniscus need to be developed. Current research efforts focus on tissue engineering. Since the long-term biochemical and biomechanical characteristics of a meniscus scaffold are ultimately determined by the cellular phenotype, a combination of a well-characterized cell population with a scaffold, appears to be a logical option. Several candidate cell-types are of interest for meniscus tissue engineering. Of these, progenitor cells have the advantage to be easily expandable without the loss of their differentiation potential. The authors present and discuss their current hypothesis for cell-based meniscus tissue engineering in combination with a scaffold.

Keywords: Meniscus, tissue engineering, fibrochondrocytes, stem cell.

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1. General introduction

The meniscus plays an important role in the complex biomechanics of the knee joint. It has a function in load bearing, load transmission, shock absorption, joint stability, joint lubrication and joint congruity. Loss of this important anatomical structure results in higher peak stresses on the cartilage and eventually leads to cartilage degeneration and osteoarthritis, as observed by Fairbank, over fifty years ago [1].

It is also known that the human meniscus has a poor healing potential, partly due to the absence of vasculature. Blood vessels are present only in the outer 10-30% of the meniscal body [2]. Lesions located in this area can be surgically sutured with a high success ratio [3]. The more frequent lesions, situated in the avascular part, however, show no or only limited tendency to heal and should hence be resected [4]. A partial, instead of total meniscectomy is proposed, knowing that cartilage degeneration is proportional to the amount of meniscus that was removed [5]. Still, partial meniscectomy results in approximately 4% articular cartilage volume loss per year [6].

Each year more than 400,000 surgical interventions are performed concerning meniscal tears in Europe. In the United States, over 1 million interventions are performed. In the majority of these interventions, a partial or total meniscectomy is performed. This therapeutic intervention causes initially good clinical results but will finally lead to osteoarthritis of the knee [1,2].

Multiple different techniques have been investigated to heal central meniscal lesions. These attempts however, remain most frequently unsuccessful [3]. For larger meniscal defects, meniscal transplantation is the golden standard. Deep-frozen, cryopreserved or viable allografts can be used for this procedure [4,5,6,7,8]. Satisfactory clinical results have been obtained on short, middle and long term. These results are independent of the used preservation technique. However, recent histological analyses of biopsies taken after transplantation of human deep-frozen allografts have shown limited and superficial repopulation of the graft by host cells. The core of the graft generally remains acellular. The repopulation by acceptor cells from the synovial lining of a deep frozen human meniscus graft, seems to be more slowly and incompletely than suggested by animal data.

Allogenic meniscus transplantation is confronted with potential problems such as shape incongruency, disease transmission and a limited availability of donor menisci [9]. In 2001 we established a meniscus tissue engineering program at our laboratory. Multiple different therapeutic strategies to heal or replace a meniscal defect are being investigated.

Since ultimately the mechanical and biomechanical characteristics of the graft are determined by the cellular phenotype and the number of (host) cells residing within the graft, several cell types from different origins are currently under investigation, including bone marrow derived mesenchymal stem cells (BMSC), local progenitor cells or differentiated meniscus and cartilage cells. In this paper, we will elaborate the use of progenitor cells for meniscus tissue engineering applications.

2. Organisation of normal meniscal tissue

Normal human menisci have been found to be composed of extracellular matrix (ECM), water (72%) and cells. The ECM consists of collagen and glycosaminoglycans. 90 % of the collagen is collagen type I. The other collagen types are type II, type III, type V and type VI. Due to the large amount of collagen type I, the meniscus has properties of fibrous and cartilaginous tissue. Type II collagen is expressed in significant amounts in the inner region of the meniscus but is absent in the outer vascular region. Aggrecan is a major proteoglycan. Minor glycosaminoglycans are decorin, biglycan and fibromodulin [10,11]. These collagen fibers and proteoglycans are organized in a complex architecture which provides the tissue-specific biomechanical characteristics. This structure is distinctly different from articular cartilage.

3. Histology of the normal human meniscus

The meniscus is defined as a fibrocartilage, because of the round or oval shape of most of the cells and the partially fibrous appearance of the ECM. Ghadially *et al*. classified cells in the meniscus as chondrocytes, fibroblasts or cells of intermediate morphology, based on their shape and the presence or absence of a territorial matrix [12]. Type I collagen is the major fibrillar collagen in the meniscus in contrast to articular cartilage, where the major collagen is type II. This difference in cell associated matrix protein expression is used as a molecular criterion in the distinction of fibrocartilage and hyaline cartilage and thus of meniscus cells and chondrocytes. In 1985, the term fibrochondrocytes has been introduced to show the typical characteristics of these cells [13].

Fibrochondrocytes from the inner part of the meniscus are round to oval shaped and show a clear cell associated matrix (CAM) with an intense pericellular proteoglycan staining. They synthesize large amount of fibrous type I collagen and lower but significant amounts of type II collagen and aggrecan. In comparison to this, hyaline chondrocytes produce mainly

collagen type II and aggrecan. For this reason they introduced the term "fibrochondrocytes" (Figure 1) [14].

Fibroblast-like cells do not have a cell associated matrix (CAM) and are located in the peripheral region of the meniscus. These cells have long cell extensions which are in contact with other cells and different parts of the matrix [15].

A third population of cells in the meniscus is situated in the superficial zone which contains cells, known as *superficial zone meniscus cells*. These cells do not have cell extensions. This zone could possibly contain specific progenitor cells with healing potential.

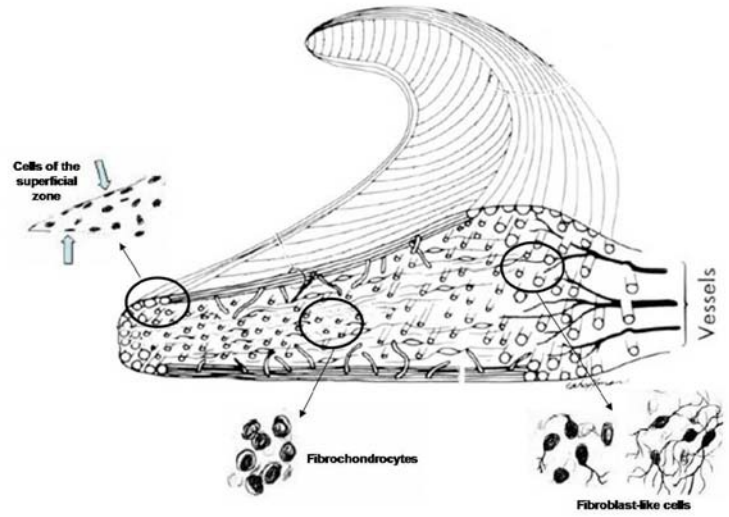

Fig.1. Schematic representation of the human meniscus showing the distinct cell type populations and their regional distribution. Fibrochondrocytes are round cells with no cellular projections, located in the avascular portion of the meniscus, while fibroblast-like cells are located in the vascular portion and reveal thin cytoplasmatic projections. Cells from the superficial area are fusiform.

Recently, we characterized a *CD34+ meniscus cell* residing in this superficial zone, as well as in the vascular zone and in the subsynovial lining. Preliminary data on normal and pathological menisci makes us hypothesize that this cell type could be implicated in tissue homeostasis and repair/regeneration.

4. In vitro behavior of human meniscus cells

A recent study assessed survival and proliferation of human meniscus cells in different culture conditions and characterized the ECM, produced by these cells [33]. The composition of this ECM offers a variable to define the distinct meniscus cell phenotypes.

Human meniscus cells were isolated enzymatically from visually intact lateral and medial menisci. Cells were cultured in monolayer conditions or in alginate gel. The composition of the cell associated matrix (CAM) accumulated by the isolated cells during culture was investigated and compared to the CAM of articular chondrocytes cultured in alginate. For this we used flow cytometry with FITC-conjugated monoclonal antibodies against type I collagen, type II collagen and aggrecan. Additional cell membrane marker analysis was performed to further identification of different meniscus cell populations in the alginate culture conditions and meniscus tissue sections. Proliferation was analyzed using the Hoechst 33258 dye method. In some experiments, the effect of TGFβ-1 on some of these variables was investigated.

The CAM of monolayer cultured meniscus cells is composed of high amounts of type I and II collagen and low amounts of aggrecan. A major population of alginate cultured meniscus cells on the other hand synthesized a CAM containing high amounts of type I collagen, low amounts of type II collagen and high amounts of aggrecan. This population is CD44+, CD105+, CD34- and CD31-. A minor cell population in the alginate culture did not accumulate ECM and was mainly CD34+.

The proliferation of meniscus cells increased in monolayer culture conditions. The cell numbers decreased slightly in the alginate culture, but could increase after the addition of TGFβ-1. Articular chondrocytes cultured in alginate synthesized a CAM, composed of low amounts of type I collagen and high amounts of type II collagen and aggrecan. The amount of type II collagen and aggrecan increased after addition of TGFβ-1 to the culture medium.

These results demonstrate that the human meniscus is populated by different cell types which can be identified by a distinct CAM composition and membrane marker expression. Unlike the monolayer culture conditions, the alginate culture conditions appear to favour a more fibrochondrocyte-like cell accumulating with a cell associated matrix resembling the native tissue composition. This CAM composition is distinctly different from the CAM composition of phenotypically stable articular cartilage chondrocytes, cultured in the same alginate matrix.

5. Rationale for meniscal replacement

Substantial research has already been performed to substitute the resected meniscus in case of a total or partial meniscectomy, in order to (1) prevent or delay cartilage degeneration, (2) to improve biomechanics and (3) to diminish pain. The use of different autologous or allogenic tissues has been investigated in different surgical approaches. These tissues can be tendon, pediculated Hoffa fat pad, periosteal tissue, perichondral tissue, small intestine submucosa or meniscal allografts. Meniscus substitution can also be performed by a meniscal scaffold based on native polymers (collagen and hyaluronic acid) or purely synthetic scaffolds such as poly-lactic acid, poly-glucuronic acid or poly-urethane [3,5,16,17,18,19]. Beside meniscal allografts and a collagen type I based meniscus scaffold (CMI®, Regen Biologics, Franklin Lakes, NJ, USA), none of these materials have been advanced to human clinical use. These surgical approaches are based on the concept of a colonization of the acellular scaffold or the allograft tissue by host cells in time. These cells are probably derived from the synovium and joint capsule (Figure 2).

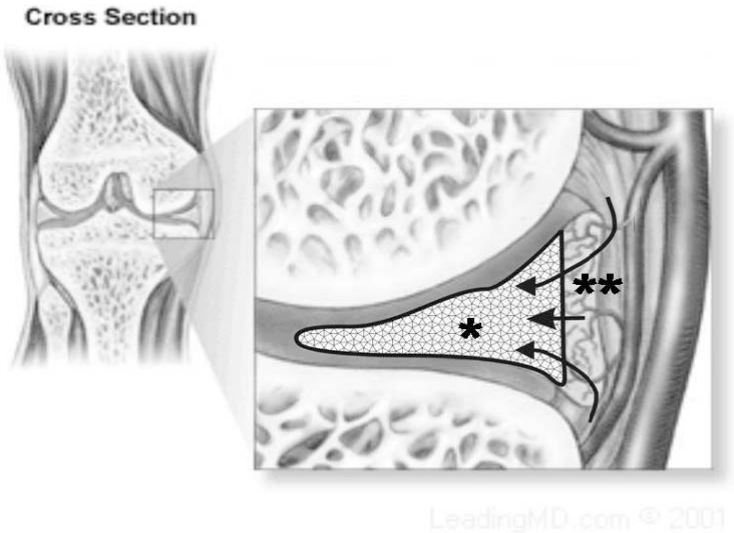

Fig. 2. Acellular meniscus grafts or scaffolds (*) are colonized by host cells (arrow) which are probably derived from the synovium and the joint capsule (**).

The phenotype of these host-derived scaffold-colonizing cells ultimately determines the biochemical composition and biomechanical behavior of these repopulated scaffolds or

tissues. Another critical variable in this approach is the time needed for colonization of the scaffold or tissue. Since these scaffolds are biodegradable, the colonization and healing by host cells should be faster than the degeneration process (Figure 3).

Previous animal studies have provided evidence that fresh meniscus allografts are quickly invaded by host cells within one month after transplantation [20]. In the human model, however, only limited data is available. A previous study has provided evidence that this process of colonization is considerably different in the human model. Histological sections on specimens taken from transplanted deep-frozen allografts showed a decreased cellularity after transplantation, indicating insufficient repopulation of the graft or scaffold [21].

An increase of the initial cell number in the meniscal substitute can be accomplished by (1) transplantation of an *in vitro* cultured 'viable' meniscal allograft or by (2) seeding autologous cells, with a proven meniscus repair potential, on or in a biodegradable scaffold prior to implantation.

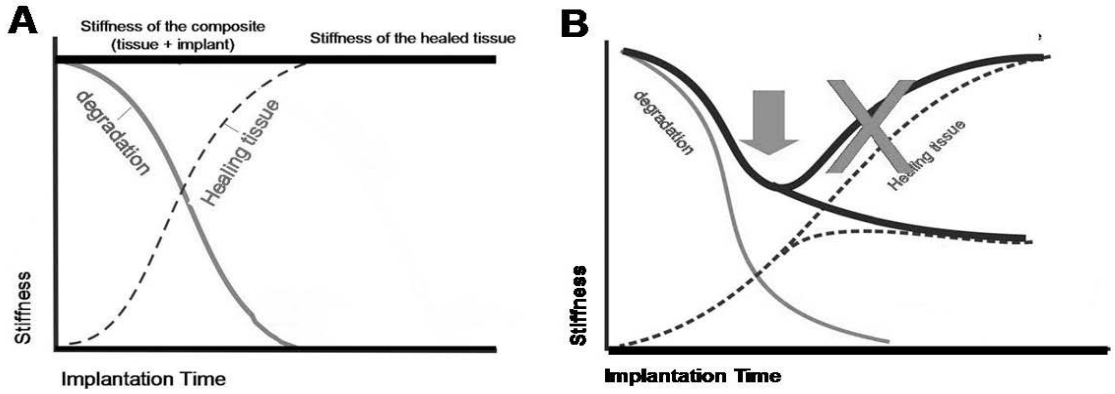

Fig. 3. (A) Ideal degradation kinetic of a resorbable scaffold (grey line) related to tissue healing (dashed grey line). The sum of these processes (black line) guarantees the stiffness of the construct. (B) In the human model, tissue healing is considered much slower than the resorption of many grafts and scaffolds, resulting in reduced stiffness (arrow) and early failure of the construct.

6. Possible useful cells for meniscus tissue engineering

Two different cell options could be used to be seeded on a meniscus implant. We use differentiated cells which can synthesize their original ECM and regenerate the tissue [22]. Or, we can use progenitor cells which can differentiate *in vivo* to the desired cell phenotype.

i. Fibrochondrocytes

Fibrochondrocytes are able to proliferate in a three-dimensional matrix *in vitro*. In these circumstances they express there phenotype with the synthesis of their original ECM. Immunohistological staining of this tissue shows fibrocartilagineous tissue [22]. In the clinical setting autologous fibrochondrocytes could be harvested by biopsy and isolated. These can be taken from the contralateral healthy meniscus or from the torn ipsilateral meniscus which will be torn more by this action. Cells could also be isolated from the resected part from the torn meniscus but these cells seem to be of minor quality. By consequence the amount of useful cells is little. These fibrochondrocytes have already been characterised by flow cytometry. Fibrochondrocytes cultivated in alginate produce mainly collagen type I and aggrecan and type II collagen in a smaller amount [33]. Fibrochondrocytes, cultivated in monolayer do synthesize collagen type I and II but no aggrecan [33]. These results need to be further investigated and can be useful for the further research in meniscus tissue engineering. Due to the difficulty to harvest a sufficient number of cells, we can conclude that fibrochondrocytes may not be the most ideal cells for meniscus tissue engineering.

ii. Articular chondrocytes

Porcine chondrocytes have been investigated for their use in meniscus tissue engineering. In this study, performed by Peretti, autologous chondrocytes have been seeded on allogenic meniscus fragments. These meniscus fragments were implanted in the avascular part of the meniscus. The investigator described that these chondrocytes were able to synthesize meniscal tissue in an *in vivo* situation [23]. For this reason it is useful to further investigate the use of these cells in meniscus tissue engineering strategies.

iii. Fibroblasts

After implantation of an acellular scaffold or a deep frozen allograft, invasion of these scaffolds or grafts by fibroblasts from the synovium, has been observed [24]. In an animal

model these cells initially produce a fibrovascular tissue which is transformed into fibrocartilagineous tissue [3,25]. Immunofluorescent staining in healthy menisci shows CD34+ cells with cell extensions in the peripheral zone of the meniscus. These cells need to be further investigated.

iv. Mesenchymal stem cells

Stem cells have the potential to differentiate into different tissues and have the ability of self renewal [22]. Mesenchymal stem cells (MSCs) are able to differentiate into different mesenchymal tissues such as bone, cartilage, tendon, fat, neuronal tissue, muscle and bone marrow [26,27,28,29]. Bone marrow is one of the most wide spread sources of MSCs used in tissue engineering. Besides this, they can also be isolated from other mesenchymal tissues such as muscle, fat and synovial membrane [30]. BMSCs can be isolated easily due to their potential to adhere in a monolayer culture system. In this situation these cells seem to maintain there multipotency [29].

MSCs can differentiate into different tissues in an *in vitro* setting, depending on the culture medium and culture conditions. This culture system is based on very dense cell cultures, called micropellets. The cells will give rise to cell-cell interactions by forming aggregates. If MSCs are cultured as a micropellet in medium supplemented with dexamethasone and TGF-β, a (fibro)chondrogenic differentiation will be induced [31,32].

In an experimental setting, autologous MSCs mixed with hyaluronan have been injected in a meniscectomised goat knee. This technique resulted in some cases in the neoformation of meniscus. Currently, it is hypothesized that the neoformation could be the consequence of the intrinsic regeneration, induced by the extrinsic trophic effect of these cells [30].

v. CD34+ meniscus cells

Recently, we discovered a possible progenitor cell in the normal human meniscus and the subsynovial tissue. These cells, which are CD34+, seem to disappear, from the superficial zone, in menisci with a tear or in menisci from osteoarthritic knees [33]. In case of meniscal tears, the cells from the superficial zone seem to differentiate into a myofibroblast with alpha smooth muscle actin formation [34]. Alpha smooth muscle actin positive cells have been seen in torn rotator cuffs, in dermal and tendon scar tissues, and in menisci from the arthritic knee [35]. In an animal model, alpha smooth muscle actin positive cells have the capability to

migrate into a deep frozen meniscus plug [36]. Our hypothesis is that these CD34+ cells are essential for the homeostasis and intrinsic repair of human menisci. Therefore, these cells may be of interest for tissue engineering applications.

6. Growth factors

The differentiation capacity and the production of ECM is determined by an optimal environment. Sufficient nutrients, growth factors, cytokines, mechanical stimulation and the optimal oxygen concentration are necessary [25]. Growth factors can address certain cells to a certain phenotype. Subsequently, we will give a short overview of growth factors with a known fibrochondrogenic potential on different cells.

Transforming Growth Factor - β1 (TGF-β1) is involved in the development of bone and cartilage. In an *in vitro* cell culture of MSCs, TGF-β1 induces the matrix production (e.g. collagen type I, II and aggrecan) and induces chondrogenesis [37].

Platelet Derived Growth Factor bb (PDGF bb) influences the chondrogenic potential of BMSC [25]. This was investigated by submitting these cells in a medium with TGF-β1 in combination with PDGF. These growth factors seem to stimulate the proliferation rate of these BMSC's [38]. PDGF also stimulates the proliferation rate of chondrocytes in a cell culture based system of meniscal tissue [39,40].

Insuline Growth Factor-I (IGF-I) is the most important anabolic factor for the growth of hyaline cartilage. As the inner part of the meniscus resembles articular cartilage rather closely, the effect of IGF-I on meniscus tissue has to be investigated in the future [3]. IGF-I induces an increased migration if meniscus cells and an increased synthesis of proteoglycans.

Fibroblast Growth Factor 2 (FGF2) seems to be a strong modulator for stem cell proliferation. FGF2 keeps the stromal bone marrow fraction immature. This means that osteochondrogenic progenitor cells retain their multipotent character in a cell culture based system [41].

Bone Morphogenetic Protein – 6 (BMP-6) seems to stimulate the chondrogenesis in a subpopulation of stromal bone marrow cells [42].

7. Cells and scaffolds

For meniscus tissue engineering, cultivated cells need to be seeded on a scaffold. Different experiments have been performed. Mueller *et al*. cultivated meniscal cells, seeded on a CMI,

in an *in vitro* experiment [35]. After three weeks there was a shrinkage of 50% of the collagen scaffold. Histological analysis with immunohistology showed the presence of α-smooth muscle actin positive cells [35]. In another *in vitro* experiment the combination of human bone marrow derived stem cells with a type I collagen scaffold was investigated. Different cell-seeding protocols were performed. This study demonstrated that human bone marrow derived MSCs attached and were able to undergo fibrochondrogenic differentiation when seeded onto the type I collagen scaffold or injected into it. Injection of the cells resulted in a significantly higher number of cells present within the scaffold compared to superficial seeding. The clinical application of a cell-scaffold construct injection would result in a higher number of cells at the site of injury and thus a decrease in the time needed to completely repopulate the scaffold [43].

8. Conclusion

A functional meniscus is of paramount importance for the homeostasis of the knee joint. In case of resection, meniscus substitution by an allograft is a proven concept with satisfactory clinical long-term results. Due to specific drawbacks related to the use of allogenic material, current research efforts focus on tissue engineering using different cell-sources, scaffolds, growth-factors, or a combination thereof. Currently, limited data is available on the histological outcome after scaffold implantation. These data show that scaffolds or allografts are repopulated by synovium-derived cells and cells derived from the remnant meniscus. The number of repopulating cells appears to be limited and their phenotype unknown. Since the long-term biochemical and biomechanical characteristics of the graft/scaffold are ultimately determined by the cellular phenotype, a combination of a well-characterized cell population with a scaffold appears to be a logical option. As it stands now, the applicability of growth-factors remains very limited in clinical practice. Several candidate cell-types are of interest for meniscus tissue engineering. Of these, progenitor cells have the advantage to be easily expandable without the loss of their differentiation potential.

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All subjects enrolled in this research have responded to an informed consent which has been approved by the Ethics Committee on Human Research and this protocol has been found acceptable by them.

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