Porous polymeric elastomers for repair and replacement of the knee joint meniscus
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Chapter 1

INTRODUCTION
Meniscus

Menisci are very important semilunar wedge-shaped fibrocartilaginous structures situated between the upper leg (femur) and the lower leg (tibia) as shown in figure 1. They have long been neglected and often viewed as a poor relation of articular cartilage but meniscal fibrocartilage is a distinct tissue with special mechanical properties such as tensile strength and compression. The number of functions of menisci are multiple. They equalize the incongruence of the articular surfaces of the femur and tibia, and distribute the load over a larger area of the tibia. Additionally, they are able to absorb shocks and they play a role in the stabilization of the knee joint in conjunction with ligamentous deficiency. Furthermore, the menisci help to distribute lubrication fluid between the femur and tibial articulating surfaces.

All these functions are accomplished by the structure of the tissue. Fibrocartilage is a transitional tissue that has structural and functional properties intermediate between those of dense fibrous connective tissue and hyaline articular cartilage. The material is a composite material of an interstitial fluid and a porous matrix containing collagen fibres and a polyelectrolyte called proteoglycan. Fibrochondrocytes are the cells that synthesize and deposit around themselves this matrix of giant molecules. They have a flattened appearance and are similar to those in the superficial zone of articular cartilage. A schematic drawing of the extracellular matrix is shown in figure 2a.

The resilience of fibrocartilage is due to giant proteins called proteoglycans. They are important in determining the viscoelastic properties of (fibro) cartilage. The centre of the molecules consists of repeating disaccharide hyaluronic acid. From this central strand extend

Figure 1. Illustration of the human knee joint. a: front side view, b: upper side view with the menisci (m).
approximately 100 strands of protein. They are attached to the hyaluronic backbone by a link protein. Each strand of protein is itself the place of attachments for numerous polysaccharide chains and are termed ‘the core proteins’ of proteoglycan. In turn each core protein emits about 50 keratan sulfate chains and roughly 100 chondroitin sulfate chains (figure 2b). Since the sugars in the keratan sulfate and chondroitin sulfate carry negative electric charges (figure 3), proteoglycans must be considered as an extremely large but highly ordered array of electonegativity. Water becomes organized in multiple layers or shells around a focus electric charge. This means that proteoglycan structures can contain volumes of water, many times extending their own weight. In addition, water is trapped in holes of the extracellular matrix. This makes water the most abundant component, making up approximately 70% of the total weight\textsuperscript{16}. There is less proteoglycan in fibro- than in hyaline articular cartilage, but more than in pure fibrous tissue, which is \~2.5% of the solid phase\textsuperscript{17}.

The resilience and the viscoelastic properties of fibrocartilage is directly a result of the water structuring property. During compression, water is forced away from the charge domains. Repulsion of the negative groups prevent further compression. In addition, a resistive force is generated by the interaction of the viscous interstitial fluid and the porous-permeable matrix. Proteoglycans do not contribute to the tensile strength of (fibro) cartilage. It is known that the tensile strength of polymers decreases dramatically after absorption of water\textsuperscript{18}. 

Figure 2. A schematic drawing of the extracellular matrix containing collagen fibrils and proteoglycan aggregates (a). Molecular structures of proteoglycan (b and c).
In (fibro) cartilage, collagen fibres are responsible for the tensile strength. They are the dominant features of the extracellular matrix of the meniscus and composing 75% of the dry weight. Both bovine and human meniscal tissue biochemical collagen revealed the vast majority of collagen consist of type I which is typical for tissue resisting tear forces. Type II collagen, which is the major type in articular cartilage, is only present in minor amounts. A differentiation has to be made concerning the inner and outer part of the meniscus. Histologically, the inner one-third of the meniscus resembles hyaline cartilage more closely whereas the outer two-third are more fibrous in appearance. The major collagen type of this inner rim is type II collagen whereas the peripheral part of the meniscus is almost completely composed of type I collagen. In addition to type I and II collagen, small amounts of type III, V and VI are detectable. Little is known about the other proteins in fibrocartilage.
The collagen fibers are organized within layers: superficial, surface and middle (figure 4). In the middle layer collagen fibres, arranged in large bundles of 50-150 µm, are oriented circumferentially, parallel to the periphery of the meniscus. They appear to be continuous with those of the anterior and posterior ligamentous horns that anchor the menisci to the bone. A few radial fibres can be observed in the deep zone. Their function is to resist crack propagation which could lead to longitudinal splitting. The middle layer is surrounded by the superficial layer consisting of fine collagen fibrils woven in a meshlike fashion, which is covered by a thin compactly woven surface layer whose collagen fibres are irregular aligned. As a result of their concave wedge shape, axial loading of the joint causes the menisci to be displaced radially from the knee during weight bearing. Because they are anchored to the bone by the anterior and posterior horn attachments, this displacement generates large circumferential tensile stresses within the meniscal matrix. The arrangement of the collagen fibers is such that they are running parallel to one another in the direction of the principal tensile stress similar to tendons and ligament. The arrangement seems to be ideal for transferring of a vertical compressive load into circumferential stresses.

The orientation of the collagen fibres causes large differences in mechanical properties in circumferential and radial direction. In circumferential and radial direction the Young’s modulus and tensile strength of bovine menisci have been found to be 140-200 MPa, 30 MPa and 10 MPa and 1 MPa respectively.

Although menisci possess excellent mechanical properties, exposing them to abnormal pressure or tension can exceed their elasticity and cause tears. This occurs when the load bearing joint is subjected to a combined flexion-rotation or extension-motion. Since the sporting activities increased in the recent years, there has been a great rise in incidence of meniscal injuries, making (partial) meniscectomy and suturing procedures one of the most frequently performed orthopaedics procedures today.

Figure 5. a: Normal meniscus. Only the perpheral rim of the meniscus (1) is vascularized throughout its attachments to the joint capsule (2) and the meniscal horns. The central two-thirds of the meniscal body is avascular (4). b: All lesions, longitudinal (1), transverse (2) or a combination of these two (3), that have a connection to the meniscal blood supply can heal spontaneously. c: Lesions located in the central part of the meniscus do not heal.
Taking into account the functions of the meniscus, it is not surprising that menisci cannot be removed without consequences. For many years, it was contended that regrowth of the meniscus occurred after total excision. While it is true that a tissue replaces the excised meniscus, an experimental study conducted in dogs showed that this tissue was not fibrocartilage. It consisted of dispersed collagen fibers and the proteoglycan content was lower than in normal menisci. Fairbank was the first who observed degenerative changes of the knee joint after meniscectomy. Since then, it has been demonstrated many times that removal of a meniscus results in degeneration of articular cartilage in a high percentage. Because the degree of degeneration is proportional to the excised part of the meniscus, the method of choice is partial meniscectomy to preserve as much meniscal tissue as possible. Although, the results improve compared to total meniscectomy, the stresses on the underlying cartilage are still higher and osteoarthritis is not prevented.

Repair of the meniscus would be the best option, since in that case all the meniscal tissue can be preserved. However, due to the limited blood supply of the meniscus, healing is a problem. Only lesions in the outer 10-20% vascularized part of the meniscus can be repaired adequately by simply suturing (figure 5). When the lesions are situated in the avascular part of the meniscus there is no tendency for healing and no reliable surgical methods exist. Many researchers have been working on this problem to develop new experimental techniques. Application of a fibrin clot or combination of fibrin glue and endothelial cells, combined with growth factors. The basic principles for the repair of the lesions is to improve the vascularity of the defect by stimulating the ingrowth of vascular tissue. However, reports on repair with meniscus-like tissue, fibrocartilage, are sparse but it has been observed after application of a fibrin clot in dogs. With this technique repair of only small lesions is possible.

Another option is the use of a meniscal prosthesis. Stone claimed that in a fast degrading porous collagen-based prosthesis, a meniscus replica will be formed by the ingrowth of fibrochondrocytes. The consequences of release of the toxic crosslink agent glutaraldehyde were not mentioned. Non-degradable porous prosthesis made of fiber teflon-net showed fibrocartilage ingrowth after 9 months. Implantation of a carbon fibre reinforced polyester did have some cartilage-protective effects but the inflammatory response was severe. A polyurethane coated dacron prosthesis with limited porosity gave similar results compared to meniscectomy and failure was often observed due to insufficient incorporation. Using a cryopreserved allograft as a prosthesis appeared to be not very efficacious. After transplantation the viable cells are restricted to the surface and the areas immediately adjacent to the peripheral attachment.

A new field, tissue engineering, applies the principles of biology and engineering to the development of functional substitutes for damaged tissue. Caplan was the first to show that under the right circumstances embryo chicken limb-bud cells transform into cartilage cells, chondrocytes. The differentiation was controlled by initial plating density. At high density most cells became
chondrocytes, at intermediate density only a few chondrocytes developed and at low densities no development could be observed. The differentiation was also affected by the oxygen level, chemical factors and compressive forces.

It has been shown that culturing of chondrocytes is only possible when they grow in a three dimensional matrix otherwise they differentiate into cells with a fibroblast-like appearance. Langer et al. showed that cartilaginous tissue can be regenerated by culturing chondrocytes on fibrous polyglycolic acid (PGA) scaffold. The resulting implants can potentially be used to repair articular cartilage, nasoseptal- and tracheal replacement. After subcutaneous implantation the chondrocyte seeded scaffold in rats, mechanical properties of the neocartilage approached that of normal cartilage over time. Implantation of scaffolds without seeded cells did not show cartilage formation whether these implants are useful as meniscal prosthesis or as meniscal repair material has not yet been determined.

Our approach for repairing the meniscus is quite different but in essence it is also tissue engineering. We also use porous biodegradable polymer implants for the regeneration of tissue. After healing of the lesions and ingrowth of newly formed fibrocartilage in the implant, the polymer is allowed to degrade leaving a healed meniscus. These implants, however, are not seeded with cells before implantation.

After it had been shown that an access channel, connecting a lesion in the avascular part of the meniscus to the periphery can result in regeneration of meniscal tissue, Veth, Pennings et al. laid the basis for the present studies. Repair of large meniscal lesions can be achieved by implanting a porous polymer with an open-pore structure in the connecting defect (figure 6). Not only ingrowth of connective tissue and blood vessels was observed but also areas of fibrocartilage were found. This was rather surprizingly because these newly formed fibrocartilage areas had never been seen before. When the access was left open, both the lesion and channel were filled with fibrous tissue not

Figure 6. a: A longitudinal lesion in the avascular part of the meniscus does not heal. b: After connection this lesion to the vascular periphery by using a second access defect according to Arnoczky et al. and Veth et al., vascular tissue can reach the longitudinal lesion and healing can be observed. When the access defect is left open, both lesions are filled with fibrous tissue not resembling meniscal fibrocartilage. c: After implantation of a porous polymer in the wedge-shaped defect, healing with fibrocartilage is observed.
resembling meniscal fibrocartilage. Evidently the porous implant plays an essential role in the formation of fibrocartilage. It is surmised that under the right circumstances, the ingrown fibrous tissue transforms into fibrocartilaginous tissue\(^2,4^7\). It is likely that properties of implant influence the transformation into fibrocartilaginous tissue.

The first meniscal reconstruction materials were made of porous polyesterurethane/poly(L-lactide) mixtures reinforced with fibers of carbon\(^77,78\). By reinforcing a polymer with fibres, the structure of fibrocartilage was imitated. Materials were prepared by a repeated dipcoating procedure or a modified saltcasting process. In the grafts, larger pores of 200-250 \(\mu\)m were dispersed in a more dense matrix with pores sizes up to about 60 \(\mu\)m. Carbon fibres, however, lead to the development of synovitus because of the release of small carbon particles in the knee joint. PLLA fibers appeared to be a promising alternative\(^79,80\).

Porous materials

Additionally to dipcoating and salt casting as described above, many other techniques are available to prepare porous polymers\(^81,82\), depending to a great extent on the material used and the desired morphology.

One common method is to introduce gas bubbles into the liquid polymerizing monomer or polymer melt\(^81,82\). Solidification of the polymer traps the bubbles, resulting in a foamed material. The gas can be introduced in a variety of ways including mechanical stirring, or mixing a blowing agent with the polymer. Physical blowing agents can either be inert gases, such as carbon dioxide or nitrogen which under high pressure act as solvent for the polymer melt and expand by reducing pressure, or low melting liquids such as chlorofluoro-carbons which evaporate on heating to form bubbles. Chemical blowing agents are additives which decompose to gases upon heating. The final structure of the foams can either be open- or closed-cell. In order to stabilize the structure and to control the pore sizes, additives (surface tension depressants) have to be added.

Joining together spheres, granules or fibers is another method of polymeric foam production\(^82\). Emulsions can be used to produce foams by incorporation polymerizing monomers in the oil phase of a water-oil emulsion. Instead of a gas or solvent, water serves as the pore-former around which the polymeric cell develops\(^83\).

Thermally-induced phase separation (TIPS) is a technique, applicable to many polymers, which is used to produce open-cell foams. The process utilizes freeze-drying in the latest stage and the main requirement is polymer solubility\(^84,86\). The TIPS process begins with a single-phase, polymer solution at high temperature. Upon cooling phase separation will occur. Phase separation can be divided into liquid-liquid phase separation, which may occur prior to freezing of the solvent, and liquid-solid phase separation, which occurs when the solvent freezes. Then, freeze-drying is pursued to remove the solvent from the polymer solution. Sublimation preserves the morphology developed as a result of induced phase separation, leaving behind an open-cell polymeric foam.
For many biomedical applications, there is a need for porous polymeric biomaterials. They are valuable for artificial blood vessels, wound covering, artificial skin, time-modulated drug delivery systems, as matrix for in-vitro tissue regeneration and meniscal reconstruction.

Aim and survey of this thesis

The aim of this study is optimization of porous materials that can be used for meniscal reconstruction, in order to achieve healing of the lesions and accomplish ingrowth of fibrocartilaginous tissue in the implant. The influence of the pore structure, porosity, mechanical properties of the porous materials on ingrowth and healing were determined. An additional aim is to investigate whether these materials can be used as meniscal prosthesis. It is very important to gain insight in the optimal chemical structure of the polymer used for these applications, since the chemical structure determines the mechanical properties, degradation rate and degradation products of the polymer.

Chapter 2 describes a freeze-drying/salt-leaching technique to produce porous polyurethanes with highly interconnected pores. With this technique homogenous, porous materials with a controllable and reproducible morphology were prepared. Three different polyurethanes were used: a methylenediphenyl diisocyanate based polyurethane, a lysine diisocyanate based poly(urethane) and a poly(ε-caprolactone) based poly(urethane). The compression behavior of the porous materials could be changed by varying the polymer and casting material concentration.

In Chapter 3 PLLA-fibre reinforced aromatic PU/PLLA composites, PU/PLLA and PU foams were implanted in severe meniscus lesions in dogs. The results of these implants with different pore structure and porosity were evaluated.

In Chapter 4 porous linear PU, synthesized by curing a poly(ε-caprolactone) and 1,4-trans-cyclohexane diisocyanate based prepolymer with cyclohexanedimethanol was used for meniscal reconstruction in dogs. Compared to the aromatic PU used in chapter 2 and 3 the degradation products of this polymer are less toxic. A porous meniscal prosthesis was developed to replace a total meniscus. The prosthesis was made of an aliphatic PU network to prevent stress hysteresis. Due to the low tear resistance of the polymer network a complex suturing technique was used.

Chapter 5 describes the use of porous high molecular weight 50/50 copoly(L-lactide/ε-caprolactone) for meniscal reconstruction. In contrast to the aromatic PU and aliphatic PU used in respectively chapter 3 and 4, the degradation products of this polymer are non-toxic. Due to the high molecular weight the maximal compression modulus of the materials was 100 kPa. Two series porous materials with compression moduli of respectively 40 and 100 kPa were implanted in the knees of dogs. The influence of the compression modulus/density of porous material on the fibrocartilage formation was determined. Additionally, the in-vivo and in-vitro degradation behavior of the copolymer was determined.
In Chapter 6 a technique is developed to prepare stiff porous materials with a high molecular weight 50/50 copoly(ε-caprolactone/L-lactide). In chapter 5 it was shown that a compression modulus of 100 kPa was too low to accomplish 100 % fibrocartilage formation. Materials were prepared by agglutinate copolymer microspheres. Three series of copolymer prostheses with compression moduli of 150, 400 and 650 kPa were implanted into knee joints of goats 102.

Chapter 7 is concerned with the synthesis of polyurethanes with high tear strength. In chapter 4 it was concluded that the low tear strength of the polymer network was responsible for dislocation of meniscal prosthesis. Polyurethane ureas were synthesized by terminating a 2000 g/mol molecular weight poly(ε-caprolactone) prepolymer with three different diisocyanate: L-lysine-ethylester-diisocyanate (LDI), 1,4-butanediisocyanate (BDI) and 1,6-hexanediisocyanate (HDI). Mechanical and thermal properties of the polymers were determined. These polymers are expected to release non-toxic degradation products. Porous materials with a compression modulus of 750 kPa could be made of BDI based PUI 103.

In the Appendix porous 50/50 copoly(L-lactide/ε-caprolactone) is used as a bottom and middle layer in a triple-layer artificial skin system for the treatment of full thickness wounds. Two series materials, with different pore structure, were implanted in pigs. After 1,2 and 3 weeks the middle layer was removed and replaced by a split thickness skin graft. Attachment of the skin graft to the bottom layer and contraction over a period of two years were verified 104.

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