Dynamic stiffness of articular cartilage and potential repair materials

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Contents

Abstract

Cartilage has limited potential for self repair. Therefore, articular cartilage lesions often lead to early osteoarthritis. Early clinical results of cartilage replacement procedures such as autologous matrix-induced chondrogenesis (AMIC) seem promising, but long term results are not available to date. Only longitudinal studies of 20 years and more will show whether the cartilage repair procedures currently in evaluation will prevent the treated patients from developing early osteoarthritis and if the progression of osteoarthritis will be halted. In order to be able to evaluate and compare different methods of cartilage treatment, a thorough understanding of the mechanical properties of intact cartilage and cartilage with early degenerations is needed.

The prime function of cartilage is load bearing. Cartilage absorbs and spreads the applied energy thereby protecting the underlying bone. It also provides diarthrodial joints with an almost frictionless gliding surface. It has been shown in clinic that there is a correlation between the histological quality, the load bearing capacity and the durability of the repair. Thus it seems logical to search for a repair with properties close to that of normal cartilage.

The mechanical behaviour of cartilage is complex, since the tissue structure is a combination of partly porous, viscous and elastic components. This results in deformation rate-dependent stiffness, i.e. how much energy is needed to deform the cartilage (dynamic modulus) and energy dissipation (loss angle) properties. The water movement through or out of the cartilage under a given loading condition makes its response to loading more complex compared to an ordinary viscoelastic solid. To determine these properties, several tests can be performed, i.e. unconfined or confined compression, or indentation tests. In this thesis, dynamic indentation tests were performed because indentation minimizes specimen preparation and has been shown by others to produce meaningful cartilage stiffness data. However, a mathematical model is needed to calculate stiffness data out of those experiments. These models are always a simplification of the real situation, since cartilage is a complex structure with complex mechanical properties. To determine the influence of the mathematical model used on the results the conventional model (Hayes) is compared with a novel method (Kren) in **chapter 2**, which has as a main advantage that it does not assume linear elasticity. Although a difference was found in absolute values calculated with these models, the trends they show were similar when used to evaluate the same set of data. Thus experimental data cannot be compared between these different models, but for comparisons within one model, both models give similar results.

In order to determine cartilage behaviour, preferably healthy human specimens are tested. Unfortunately, these specimens were extremely difficult to obtain. Therefore in **chapter 3**, we investigated whether swine cartilage could serve as a model for human cartilage for mechanical testing. At equivalent anatomic locations, dynamic modulus was similar for human and swine specimens, but a small difference was found in the loss angle. Keeping these differences in mind, swine specimens can be used for ex-vivo testing.

Since mechanical behaviour of cartilage depends on the applied deformation rate and inter- and intra-individual heterogeneity, in **chapter 3** the behaviour of cartilage in swine knee joints was determined as a function of loading mode and anatomic location. We observed a larger heterogenity at fast compared to slow deformation rates. Moreover, no differences were found in the loss angle at slow deformation rate

between locations. These differences highlight the need for using multiple test modes, i.e. loading cartilage at different strain rates.

After expanding the knowledge of dynamic stiffness properties of cartilage, in **chapter 4 and 5** we explored whether double network hydrogels (DN-gels) are suitable as a cartilage repair material. It already has been shown by others that these DN-gels look promising to serve as a cartilage repair material because of its low sliding friction, high wear resistancy, high thoughness and biocompatibility. Current focal repairs have a much lower initial stiffness and strength than the surrounding tissue, which increases early failure potential. In **chapter 4**, we tested the mechanical properties related to surgical use of two kinds of DN-gels. Both DN-gels showed good suture tear-out strength and also pull-off tests with tissue adhesive showed promising results. However, dynamic stiffness of both DN-gels was only about 10% of cartilage stiffness and also its loss angle was much lower.

To increase the potential of these DN-gels as cartilage repair material, its stiffness has to be increased. To achieve this, we adapted the stiffness of one of the two DN-gels tested in chapter 4 by altering the water content in **chapter 5**. The dynamic modulus increased with decreasing water content. No difference in the loss angle was found in slow deformation whereas in fast deformation loss angle was higher in DN-gels with lower water content. The DN-gel with lowest water content had higher stiffness in slow deformation and lower stiffness in fast deformation compared to native cartilage. This difference is caused by the lower loss angle of this DN-gel. Overall it looks promising that DN-gel stiffness can come close to that of native cartilage. However, loss angle differences should be further investigated.

In summary, cartilage is a complex structure and it was shown that not only stiffness, but also energy dissipation is an important mechanical parameter. Both parameters should be investigated at multiple deformation rates to get a complete picture of cartilage mechanics. Also, healthy swine cartilage was shown to be a reasonable substitute for human cartilage in dynamic stiffness evaluations. Finally, DN-gels look promising to serve as a cartilage repair material, since they have good surgical handling properties and their stiffness is close to that of native cartilage.

Publications Arising From This Thesis

Journal Papers

D. Wirz, **S. Ronken**, A.P. Kren, A.U. Daniels, Experimental verification of a non-linear model for computing cartilage modulus from micro-indentation data, submitted to Computational and Mathematical Methods in Medicine

S. Ronken, M.P. Arnold, H. Ardura García, A. Jeger, A.U. Daniels, D. Wirz, A comparison of healthy human and swine joint cartilage dynamic compression behaviour, Biomechanics and Modeling in Mechanobiology, 2011, DOI 10.1007/s10237-011-0338-7

M.P. Arnold, A.U. Daniels, **S. Ronken**, H. Ardura García, N.F. Friederich, T. Kurokawa, J.P. Gong, D. Wirz, Acrylamide polymer double-network hydrogels: candidate cartilage repair materials with cartilage-like dynamic stiffness and attractive surgery-related attachment mechanics, accepted for publication in Cartilage 2011

S. Ronken, D. Wirz, A.U. Daniels, T. Kurokawa, J.P. Gong, M.P. Arnold, Double network acrylamide hydrogel compositions adapted to achieve cartilage-like dynamic stiffness, accepted for publication in Biomechanics and Modeling in Mechanobiology, 2012

Conference Abstracts

Oral Presentations:

S. Ronken, D. Wirz, M. Stolz, A.P. Kren, A.U. Daniels, Experimental verification of a viscoelastic model for computing cartilage modulus from microindentation data, 17th Congress of the European Society of Biomechanics, Edinburgh, United Kingdom, July 5 - 8 2010

S. Ronken, M.P. Arnold, A.U. Daniels, D. Wirz, Swine joint cartilage as an ex-vivo standard of comparison for human articular cartilage, 7. Jahrestagung der Deutschen Gesellschaft für Biomechanik, Murnau, Deutschland, May 19 - 21, 2011, *nomination for the young investigator award*

S. Ronken, M.P. Arnold, A.U. Daniels, D. Wirz, A comparison of healthy human and swine joint cartilage dynamic compression behaviour in modes emulating joint function, International Society of Biomechanics, Brussels, Belgium, July 3 - 7, 2011

Poster presentations:

S. Ronken, M.P. Arnold, A.U. Daniels, D. Wirz, Can swine joint cartilage serve as an ex-vivo substitute for human articular cartilage in mechanical tests?, 5th Basel International Knee Congress and Instructional Course, Basel, Switzerland, May 9 - 10, 2011, *best poster award*

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Introduction

Young patients with "old knees" are one of the most demanding patient groups in the outpatient clinic of an orthopeadic surgeon. Cartilage lesions due to traumatic injuries are common, especially in those practicing sports. Total or partial knee replacement results in satisfactory clinical results for most older patients. However, younger patients have higher expectations and an artificial joint cannot get to their standards [99]. Besides artificial joint surgery, other treatment protocols are applied in clinic [69, 109, 113, 141]. The results of these treatments are very variable and the long-term outcome is unsatisfactory. The different types of cartilage lesions and patient's potential to heal are important factors for the success of cartilage repair [7]. However, several lesions lead to osteoarthritis in a later stadium [38]. This heavily increases social and economic burden on the health care systems around the world. Therefore, prevention, treatment and long-term cartilage repairs are widely investigated by researchers and clinicians [111].

In the past decades a tremendous effort has been made in optimizing tissue engineered articular cartilage constructs. However, in order to compare those tissue-engineered constructs and other possible repair materials with native cartilage one has to determine its behaviour. Preferably, these constructs and other repair materials are additionally compared with conventional methods for cartilage repair. To be able to measure mechanical properties of those clinically applied methods, a biopsy is taken from the defect site. The disadvantage of this method is that the newly formed cartilage is disrupted and can only be measured once. An arthroscopic device which is able to measure the mechanical properties non-destructively in vivo would be able to overcome this. Such a device might also be used to detect osteoarthritis. However, before developing such a device it needs to be known what parameters are crucial and have to be measured.

Quantifying mechanical properties of complex structures like cartilage is not straight forward. Mechanical properties of linear elastic materials can be easily determined, but for linear viscoelastic materials it is already more challenging due to their time dependent and rate dependent behaviour. In this thesis we tried to come a step closer to treatment or diagnosis of osteoarthritis by investigating the mechanical behaviour of articular cartilage.

CARTILAGE

Articular cartilage is a connective tissue at the ends of the subchondral bone in diarthrodial joints. It does not have a blood supply, neither a lymphatic drainage, nor a connection to nerves. The specific microstructure and composition of cartilage is thought to give the tissue its remarkable mechanical properties and durability: it provides an almost frictionless joint motion and it absorbs and distributes the applied load to reduce localized stress concentrations in the underlying bone. In most individuals, cartilage is able to do this for 8 decades or even longer [34, 82, 110].

Composition and structure

Articular cartilage is primarily a tissue of extracellular matrix (ECM) with a small number of chondrocytes - specialized cells which are only found in cartilage. The ECM mainly consists of water (65-80%) and the remaining wet weight of the tissue is accounted for principally by two macromolecular materials: collagen and proteoglycans (Table 1.1). Beside these main components, cartilage also consists of lipids, phospholipids, proteins and glycoproteins.

Table 1.1: Composition of articular cartilage. Reprinted from [82] with author's permission.

Quantitatively Major Components		Quantitatively Minor Components
	% wet weight	(less than 5%) *
Water	65-80%	Proteoglycans
Collagen (type II)	10-20%	Biglycan
		Decorin
Aggrecan	4-7%	Fibromodulin
		Collagens
		Type V
		Type VI
		Type IX
		Type X
		Type XI

* Although these components are present in lower overall amounts, they may be present in similar molar amounts compared to type II collagen and aggrecan (for example, link protein), and may have major roles to play in the functionality of the matrix

Chondrocytes

The ECM is built and maintained by the chondrocytes. Chondrocytes are metabolically active and respond to various environmental stimuli. They generally maintain a stable matrix; however, some stimuli may lead to degradation of the ECM.

Water

Healthy cartilage has water contents ranging from 65% to 80% of its total wet weight. About 30% of the water is found in the intrafibrillar space within the collagen, but the majority is found in the molecular pore space of the ECM. By applying a pressure gradient across the tissue, water may move through the ECM. Due to the high frictional resistance against this flow, the permeability of cartilage is very low. This resistance together with the pressurization of the water within the ECM ensures its ability to support very high joint loads. Nutrients are transported within the tissue due to the water flow.

Collagen

Tissue's tensile and shear properties as well as the immobilization of the proteoglycans within the ECM is determined by collagen. Collagen has a triple-helical structure and the fibres vary in width from 10 to 100 nm, although it may increase with age and disease. The collagen in the cartilage tissue is cross-linked, which is thought to add stability to the fibril network. The collagen fibre network does not offer significant resistance to compression, but it is stiff and strong in tension and provides resistance to swelling and tensile strains [144].

Proteoglycans

The size, structural rigidity and molecular confirmation of the proteoglycans affect the mechanical behaviour of articular cartilage. Proteoglycans consist of a protein core with covalently bound polysaccharide (glycosaminoglycan) chains (Figure 1.1a). Aggrecan is the most common proteoglycan in cartilage (80-90%). Aggrecan consist of up to 100 chondroitin sulphate and 50 keratan sulphate glycosaminoglycan chains covalently bound to a long protein core. The N-terminal of this protein core is able to bind to hyaluronate and a macromolecular complex is formed when many aggrecan molecules are bound to a chain of hyaluronate (Figure 1.1b). This macromolecu-

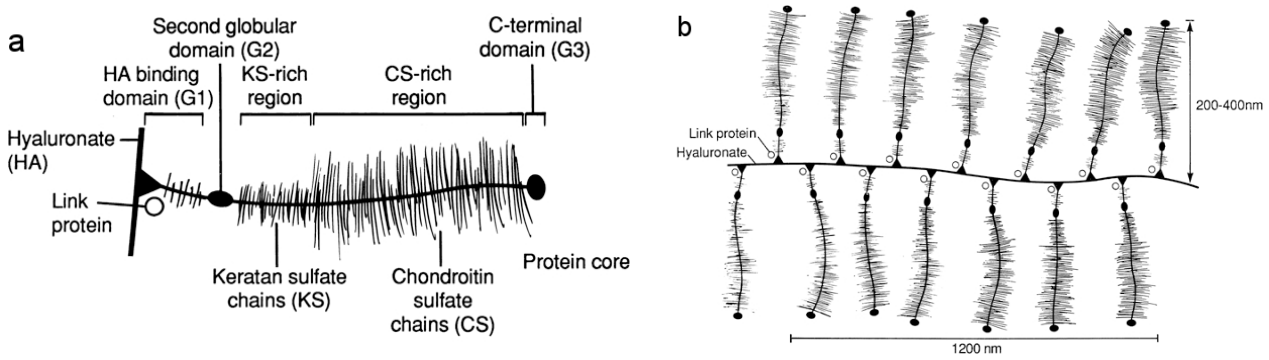

Figure 1.1: (a) Schematic diagram of the aggrecan molecule and its binding to hyaluronate. This binding is stabilized by a link protein. Keratan sulphate and chondroitin sulphate glycosaminoglycan chains are bound to the protein core. **(b)** Diagram of a proteoglycan aggregate; aggrecan molecules bound to a chain of hyaluronate. Reprinted from [82] with author's permission.

lar complex is effectively immobilized within the collagen network.

All the glycosaminoglycan chains are ionized in solution. Positive counter ions are required in the physiologic environment to achieve overall electro neutrality. These free-floating ions within the interstitial water cause osmotic pressure. Because the proteoglycans are packed within one fifth of their free-solution volume in cartilage, fixed-charge groups are only 10 to 15 Å apart. This results in a strong repulsive force.

Location dependency

Depth dependency

Cartilage is not a uniform tissue: its structure and composition vary throughout its depth (Figure 1.2). It can be divided into four zones: the superficial zone, the middle zone, the deep zone and the calcified zone.

The *superficial zone* is the (gliding) surface of the cartilage. It has the highest water content (~80%) and the lowest proteoglycan content. The collagen fibrils are relatively thin and are aligned parallel to the surface. The interconnection between the proteoglycans and the collagen fibrils is very strong. The chondrocytes are extended with their long axis parallel to the surface. In the *middle zone* the collagen fibres have a larger diameter and are randomly distributed. Further, the chondrocytes have a more rounded shape. In the *deep zone* the collagen fibres have a large diameter and are organized perpendicular to the surface. The chondrocytes are arranged in columns and have a spherical shape. In this zone the lowest water content (~65%) and the highest concentration of proteoglycans is found. The *calcified cartilage* separates the hyaline cartilage from the subchondral bone and consists of small cells distributed in a cartilaginous matrix.

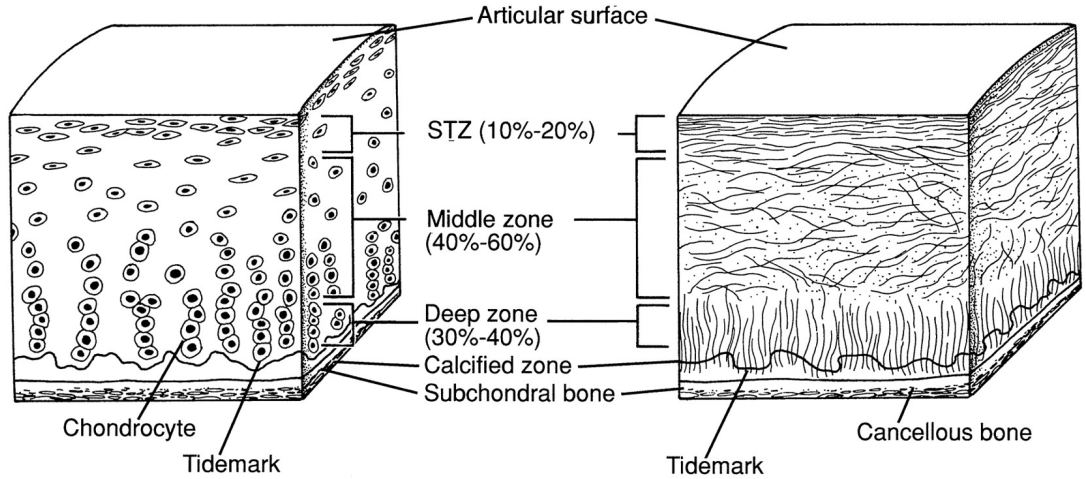

Figure 1.2: Chondrocyte (left) and collagen fiber (right) organization in the different zones: STZ = superficial tangential zone, the middle zone, the deep zone and the calcified zone. Reprinted from [25] with author's permission.

Chondrocyte proximation

The ECM structure is not only depth-dependent, it also changes depending on the proximity to the chondrocytes. Collagen fibres get thinner closer to the chondrocytes and in close vicinity hardly any collagen fibres are present. The ECM proteoglycan concentration is increased by a factor of two adjacent to a chondrocyte. Since the majority of the ECM is not in close proximity of the chondrocytes, the material properties of the articular cartilage are mainly determined by that part [82, 144].

For cartilage to function normally and provide protection to the bone and joint, each of the components described above must be present in the proper amounts and in the right structure. The chondrocytes must be present for supervising the concentration and condition of the ECM and maintaining the equilibrium between synthesis and degradation. The collagens provide a framework to resist tensile forces and the proteoglycans must sustain the hydration and resist compressible forces. The unique mechanical properties of articular cartilage depend on these components and are sensitive to disruption [21].

CARTILAGE INJURIES

Loading and movement of joints are important to maintain the composition, structure and mechanical properties of human articular cartilage. The intensity and frequency of this loading vary over a broad range. The balance between synthesis and degradation will be disturbed if the intensity and frequency is above or below certain tresholds. This causes changes in composition and structure of articular cartilage. Reduced joint loading, e.g. due to immobilization, results in atrophy or degeneration of the cartilage. Whereas increased joint loading increases the magnitude of the loading or impacts and may damage the cartilage [82]. Thus basically there are two types of articular cartilage damage:
1) Traumatic injuries which are the result of excessive loading, e.g due to a sport-accident or a bad fall. 2) Biological disorders, which causes deterioration of the articular cartilage. This can be initiated by e.g. avascular necrosis and osteochondritis dissecans. Besides this, cartilage damage occurs without knowing the cause.

Traumatic injuries

The amount of stress transmitted to a joint by indirect impact or torsional loading depends on whether the load is expected or unexpected. If the stress is expected, especially during slow movements and impacts, the muscles absorb a lot of energy through contraction while simultaneously stabilizing the joint. If unexpected movements and sudden impacts occur, the muscles are not prepared to stabilize the joint and cannot absorb the energy. Consequently, sudden and unexpected movement or impacts transmit more stress to joint surfaces and are thus more likely to cause articular surface injuries [27]. However, cartilage can be damaged without disrupting the articular surface or surrounding soft tissue [26]. Alterations of the cartilage matrix can occur due to impact loading which is higher than the level during normal activities, but lower than the level necessary to produce cartilage disruption [31].

The risk of an articular cartilage lesion in the knee joint after an anterior cruciate ligament injury is 43% [124]; and in 60% of patients undergoing an arthroscopy, a chondral lesion is found [141]. The most affected areas are the patella and the (medial) femoral condyle. In the majority of the cases the onset of the lesion was traumatic and often occurring during sports participation [8, 53, 124, 141]. However, damage may remain unnoticed, especially in a younger population as long as the individual does not experience sequelae [38].

Degenerative osteoarthritis

Healthy articular cartilage can self-repair and maintain the ECM, but with age this capacity declines. In osteoarthritic (OA) cartilage the equilibrium of degradation and synthesis of the ECM is disturbed. Part of the ECM starts to degrade, which causes an increased synthesis of other matrix components. Many of the mechanisms responsible for the origin and progression of OA are still unknown. In early OA the water content of the superficial zone increases. However, whether this water increase is due to a decrease of proteoglycan content or due to damage in the collagen network is still subject of debate. Due to this water content increase, the cartilage is less able to withstand compressional loading. When OA progresses, disruption and fibrillation

of the cartilage surface commences. As collagen fibers degrade, the proteoglycan molecules are less trapped in the structure and a decrease in cartilage tissue is observed. Eventually, all cartilage will have disappeared and the subchondral bone is exposed. As a side effect, inflammation can occur due to breakdown products of the ECM, which are released in the synovium [24, 68, 82, 137].

CARTILAGE LESION REPAIR

Due to the limited healing capacity of cartilage [58, 81], several techniques were invented to treat articular cartilage lesions - e.g. microfracture, autologous chondrocyte transplantation, mosaicplasty and recently, tissue engineered constructs. Depending on patient specific variables - such as age, demand and other injuries - and defect specific variables - such as size, depth and location of the defect - the most suitable technique is chosen [17, 130]. In general, treatment of fresh defects has a higher chance of healing compared to old defects. The goal of any intervention would be the formation of a durable repair tissue providing symptomatic relief, allowing high physical activity and delaying partial or total joint replacement surgery. To achieve this, the joint needs a stable equilibrium (joint homeostasis), not only of the articular cartilage, but also of the synovium. A cartilage lesion might only be a consequence of a disturbed equilibrium. In this case treating only the cartilage lesion will not recover the equilibrium and the treatment will be less effective [7, 119]. Until now it is not completely understood how to restore joint homeostasis. It is not known whether the cartilage structure needs to be completely normal. It might be that an 80% normal structure is already good enough to restore joint homeostasis. Saris et al. [120, 136] treated patients with symptomatic cartilage defects with chondrocyte implantation or microfracture. Clinical results are comparable for both treatments after 5 years. However, chondrocyte implantation led to better results when the lesion was treated in an early stage. Next, the most commonly used techniques are described, as well as the most recent developments and research areas to treat cartilage defects.

Repair techniques: clinical applications

Chondroplasty

The cartilage in and around a chondral lesion is abnormal. Chondroplasty, also called debridement, is a procedure where all unstable cartilage and the calcified layer are removed from the lesion.

This procedure improves symptoms for five years or more, however, results gradually deteriorate over the five-year period. Whether chondroplasty also improves symptoms in OA is still subject of debate [43, 57, 61, 89, 122, 125].

Microfracture

During an arthroscopy the microfracture technique is performed. This technique has gained popularity because of its low costs, its limited surgical morbidity and the technical simplicity [116, 142]. The lesion is prepared for microfracture by removing the loosely attached cartilage from the surrounding rim and debriding the exposed bone of all remaining cartilage tags. Then multiple holes are made with an arthroscopic awl. Those holes are approximately 3 to 4 mm apart and have a depth of about 4 mm so fat droplets and blood is released from the subchondral bone. A blood clot is formed to provide the optimal environment for a viable population of pluripotent marrow cells to differentiate into a stable tissue within the lesion. The rehabilitation protocol is an important part of the microfracture procedure. Early mobility of the joint with continuous passive motion is advocated in conjunction with reduced weight-bearing for 6-8 weeks.

Most patients suffer less pain and show an increased capacity for activities of daily living after microfracture therapy. Clinical results are satisfactory in lesions up to $2cm^2$. However, microfracture technique does not induce growth of hyaline-like cartilage, but results in fibrous cartilage. Whether this defect filling will become stable over time and support weight bearing is still unclear. Clinical data shows that the results begin to decline 3-5 years after surgery, showing a limited longevity of the repair tissue correlating with the clinical results [120]. The outcome has a higher chance of success in young patients with small cartilage lesions due to trauma who had a high activity level before surgery [18, 59, 69, 88, 96, 127-130].

Autologous chondrocyte transplantation (ACT)

To improve joint function a two-step surgical procedure called autologous cultured chondrocyte transplantation can be performed. In a first arthroscopic operation 300 to 500 mg of cartilage is obtained from the injured knee. Cells are isolated within 6 hours after the operation in a cell-culture laboratory. The isolated cells are cultured for 14 to 21 days in patient's own serum. In a second surgery the lesion is debrided back to the best cartilage available without penetrating the subchondral bone plate. In the classical technique a periosteal flap is harvested, fitted and sutured to the surrounding rim of the lesion, after which the cultured chondrocytes are injected under this periosteal flap [108].

In most cases the integration into the surrounding cartilage is good and the failure rate is only about 16%. In 67% of the patients the defect is filled with hyaline-like cartilage, which is twice as stiff as the fibrous tissue in the other patients. Weight-bearing seems to promote the formation of hyaline repair tissue. The long-term results show that treatment with autologous chondrocyte transplantation results in a durable repair for the majority of patients. However, the time required for the tissue to form and the long rehabilitation period until pain-free weight-bearing is a main disadvantage of this technique [22, 33, 42, 86, 87, 107, 108, 114].

Matrix-Induced Autologous Chondrocyte Implantation (MACI)

A similar procedure to ACT as described above is matrix-induced autologous chondrocyte implantation. Cells are harvested in a first arthroscopic operation and cultured in a laboratory as with ACT. However, the cultured cells are then seeded onto a collagen I/III matrix membrane before implantation. After preparing the lesion in a second surgery, the graft is cut in the correct size and secured in place with fibrin sealant [41].

Short term follow-up shows reliable results for treated cartilage defects. Treated lesions have shown formation of hyaline-like or mixed hyaline and fibrocartilage repair tissue. The advantages of MACI are that it allows a more minimal access approach surgery, less operating time and cell distribution can be ensured. Besides this, the number of revision surgeries is reduced due to less hypertrophy and donor-site morbidity. However, the costs of this procedure is higher and long term follow-up is not yet available [2, 15, 32, 41, 62, 101].

Mosaicplasty

Osteochondral autografting, or mosaicplasty, is usually performed as an open procedure. Although, depending on the location of the defect, it is possible to perform it arthroscopically. Osteochondral plugs are taken with a cylindrical cutting device and used to fill an articular cartilage defect. The defect is first debrided and then measured to determine the number and size of the grafts. Cylindrical osteochondral plugs of about 6 to 11 mm in diameter and 15 to 20 mm long are harvested from non-weight-bearing areas with similar curvature as the defect site. In the defect 1 mm smaller sockets are drilled, in order to press-fit the previously harvested grafts in the right location. At least 80% of the defect needs to be covered by this procedure. Depending on the defect, weight should not be fully applied for 1-8 weeks.

Hyaline cartilage is formed with nice integration into the surrounding cartilage in about 80% of the cases. The advantage of mosaicplasty is that it is a one-stage procedure, it has low costs and morbidity and already living cartilage is implanted. However, it should not be used when (pre)osteoarthritis, inflammatory arthropathies or tumours are present, and in patients over 50. Other concerns of this method are donor site morbidity and the difficulty to produce a smooth, perfectly congruent joint surface [19, 43, 49, 50, 84, 113, 125].

Osteochondral allografts

Besides osteochondral grafts from the patient's own joint, grafts from donors can be used. These osteochondral allografts are preferably fresh; otherwise they need to be fresh stored, to preserve metabolically active chondrocytes [16, 143]. Whole joint specimens are stored in nutritive medium prior to transplantation. Allograft tissue is size-matched to the host with the use of radiographs or magnetic resonance imaging studies. Cylindrical osteochondral allografts of 8-15 mm height are harvested from the donor and press-fitted in the host defect. Weight-bearing is restricted after surgery and a rehabilitation program is needed to restore normal gait.

The functionality of the joint increased after surgery and the cartilage thickness of the

allograft is maintained. Even five and fourteen years after surgery, 75% and 63%, respectively, of the grafts show a good result. However, results are poor for patients age 60 and older. Graft failure can occur due to necrotic bone and articular cartilage fragmentation. The main disadvantages of this technique is the availability of the donor grafts, the possibility of disease transmission between donor and host and the limited cartilage viability over time [16, 40, 45–48, 78, 80, 85, 105, 138, 143].

Repair techniques: research areas

Tissue engineering

The general drawback of e.g. microfracture and autologous cultured chondrocyte injection is that the newly formed tissue lacks the structural organisation of cartilage. This tissue has inferior mechanical properties compared to native tissue and is therefore prone to failure [60]. One ultimate goal of cartilage tissue engineering is to develop a replacement that has a structure and composition resembling native cartilage, yielding similar mechanical behaviour and which fully restores joint functionality [70].

Chondrocytes are the most used cell source, but cells harvested from diseased joints are relatively inactive and less good in forming cartilage [14, 28]. Chondrocytes from older (osteoarthritic) patients are metabolically less active compared to young (animal) chondrocytes [30, 52, 106, 140]. To overcome the limited supply of chondrocytes, multipotent stem cells are also used [20, 126].

There are several factors which influence the growth and tissue formation during cell culture. First, to promote chondrogenic phenotype and to stimulate ECM production a number of growth factors, including transforming growth factor (TGF-β), insulin-like growth factor (IGF-1) and others can be used [1]. Second, to improve the mechanical properties of the tissue-engineered cartilage, the cells are mechanical stimulated during culturing. This is mostly done under direct confined or unconfined compression or hydrostatic pressure [118, 123]. Third, cells can be seeded onto a scaffold, which replaces the function of the native matrix. Scaffold can be synthetic or made from natural materials and can have various architecture, porosity and stiffness. These properties are important, since they influence cell migration, differentiation, tissue growth and diffusion of oxygen, nutrients, waste products and signalling molecules [54, 101, 135].

Big efforts are made to have ECM components in the tissue engineered construct close to native cartilage. It has been shown that proteoglycan content can come close to native cartilage [76]. Unfortunately it is not yet possible to engineer a construct with close to native amount and orientation of collagen [54]. Therefore necessary load bearing mechanical properties of the constructs are still much lower than native cartilage [118]. Also depth depending matrix content, orientation and stiffness still needs to be improved [70].

Double network hydrogels

Double network hydrogels (DN-gels) are developed as possible implant materials for the repair of soft tissues by Gong and her colleagues [11, 44, 56, 94, 95, 133, 145, 146]. A whole family of DN-gels can be made, composed of two kinds of independently interpenetrated polymers (Figure 1.3). Of which the first network is stiff and brittle and the second network is soft and ductile [95]. In this respect they resemble cartilage and other skeletal system soft tissues, which are also high water-content materials or structures with a double-network strategy. Cartilage consists of highly crosslinked collagen-fibres with proteoglycan gel. DN-gels can be created using various synthetic and biological materials, such as acrylamides, collagen and bacterial cellulose [44, 95]. The double network structure results in a high water content material with a much higher stiffness than one of the two components separately. For example the DN-gel consist-

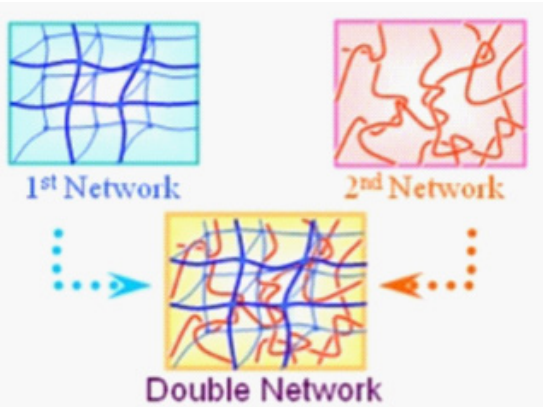

Figure 1.3: Structure of double network hydrogels with a stiff and brittle first network and a second soft and ductile network entangled within the first network.

ing of poly(2-acrylamido-2-methylpropansulfonic acid) (PAMPS) as the first network and poly(acrylamid) (PAAm) as the second network is 43 times stronger than the PAMPS single network gel [44].

To be suitable for clinical use, the implant material has to be biocompatible. Tanabe et al. [133] implanted four different DN-gels: PAMPS/PAAm, PAMPS/poly(N,N-Dimetyl acrylamide) (PDMAAm), Cellulose/PDMAAm and Cellulose/Gelatine, in the muscle and the subcutaneous tissue of rabbits. The Cellulose/Gelatin did not show an inflammation reaction, but was gradually absorbed after 4 and 6 weeks of implantation. Thus the Cellulose/Gelatin gel has the potential to be used as an absorbable implant. The PAMPS/PAAm and Cellulose/PDMAAm gels showed significant inflammation at both time points and are therefore not suitable as an implant material. The PAMPS/PDMAAm gel induced only a mild inflammation after 1 week, and decreased at the same degree as the negative control at 4 and 6 weeks. The stiffness, strength and strain to failure did not change after implantation. This short experiment shows promising results for the PAMPS/PDMAAm gel, however, whether it is suitable for implantation needs to be further investigated [133].

To further investigate the biological response on the PAMPS/PDMAAm gel, Yasuda et al. [145] created osteochondral defects in rabbits and inserted the DN-gel, poly(vinyl alcohol) (PVA) gel or ultrahigh molecular weight polyethylene (UHMWPE) plugs at the bottom of the defect. Hyaline cartilage was regenerated in the defects with the implanted DN-gel, but rarely by the PVA gel or the UHMWPE plugs. However, the depth of the implanted DN-gel plug affected the regeneration effect, which implies that the physical environment may affect hyaline-cartilage regeneration. The cells in the defect with the DN-gel plug highly expressed type-2 collagen, aggrecan and the regenerated matrix was rich in proteoglycan and type-2 collagen at 4 weeks [145]. This is a promising result for cartilage regeneration. Nevertheless, the mechanical properties of the regenerated tissue were not determined and it is unclear whether this mechanism also takes place in older human patients.

MECHANICAL PROPERTIES OF SOFT MATERIALS

Elastic behaviour

When a force is applied to a material, it will deform the material. This deformation can occur in tension, bending, compression, torsion or shear. The resistance to this elastic deformation is a measure of the stiffness of a material. A measure of this stiffness in compression and tension is the Young's modulus (E; Equation 1.1). The Young's modulus is the stress (σ) divided by the strain (ε). Where stress is the force (F) normalized to the area (A) over which it is applied and the strain is the change in height or length (ΔL) normalized to the original length (L_0).

$$E = \frac{\sigma}{\varepsilon} = \frac{F/A_0}{\Delta L/L_0} \tag{1.1}$$

E is the Young's modulus (modulus of elasticity)
F is the force applied on an object
A_0 is the original cross-sectional area through which the force is applied
ΔL is the amount by which the length of the object changes
L_0 is the original length of the object

The relationship between stress and strain for linear elastic materials is in general referred to as Hooke's Law (Equation 1.2). It also applies in small, elastic deformations of other materials [75].

$$F = -k \cdot x \tag{1.2}$$

F is the force applied
k is a constant called the spring constant
x is the displacement of the spring

When the stress of an elastic material is plotted versus the strain, this results in a straight line. The Young's modulus is the resulting slope of that line. A material with a higher Young's modulus has a steeper slope and is referred to as stiffer material and vice versa (Figure 1.4a).

When a material is compressed in one direction, it usually expands in the other two directions perpendicular to the direction of compression. To what extent this takes place, is dependent on the material. For an incompressible material, the expansion in the perpendicular

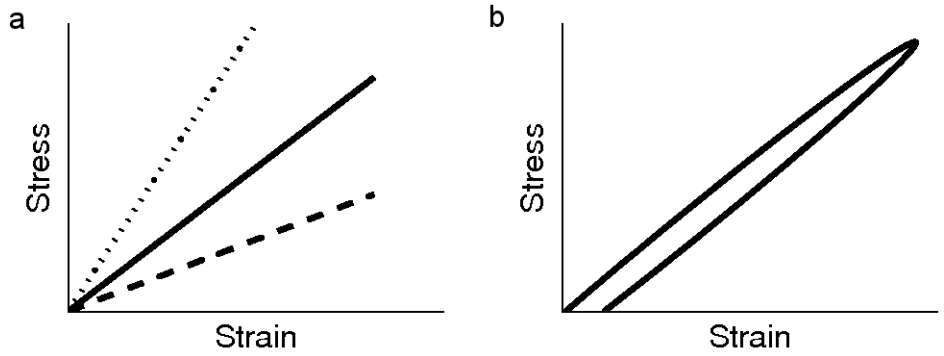

Figure 1.4: Stress-strain diagram of a linearly elastic (a) and linearly viscoelastic (b) material. For an elastic material the slope of the stress-strain curve corresponds to the Young's modulus. For a viscoelastic material the hysteresis-loop shows the energy loss in a loading and unloading cycle.

directions will be larger than in a compressible material. A measure of this effect is the Poisson's ratio (υ). The Poisson's ratio is the ratio of the fraction of expansion divided by the fraction of compression, for small values of these changes (Equation 1.3). A perfectly incompressible material has a Poisson's ratio of exactly 0.5 when it is deformed elastically at small strains.

$$\upsilon = -\frac{d\varepsilon_{trans}}{d\varepsilon_{axial}} \qquad (1.3)$$

υ is the Poisson's ratio
ε_{trans} is transverse strain, perpendicular to the applied stress
ε_{axial} is axial strain, in parallel with applied stress

Viscoelastic behaviour

Viscoelastic materials exhibit both viscous and elastic characteristics when undergoing deformation. Elastic materials deform instantaneously when a force is applied and return to their original state once the force is removed; all the energy is stored (Figure 1.4a). Whereas when a force is applied to a viscous material, it does not deform, it flows like a liquid. When the force is removed it does not return to its original shape, because the force (energy) is dissipated. Viscoelastic materials dissipate part of the energy when a load is applied and then removed. In a stress-strain curve this is observed as hysteresis (Figure 1.4b).

There are several ways to describe these properties. The storage (E') and the loss modulus (E'') represent the energy which is stored and dissipated respectively. The dynamic modulus (E*) is a measure of stiffness for viscoelastic materials. And the loss angle (δ) is a measure of which part of the energy is dissipated. The correlation between these properties is shown in Figure 1.5. This shows that at least two of those parameters are needed in order to describe a viscoelastic material properly.

Since viscoelastic materials have elements of both viscous and elastic properties, they exhibit time dependent strain. Three main characteristics of viscoelastic materials are: *creep, stress relaxation* and *strain-rate dependent* properties.

When a constant stress is applied on a viscoelastic material, the deformation increases in the beginning, but slows down until it becomes nearly constant (Figure 1.6). This phenomenon is called *creep*. *Stress relaxation* is observed when a viscoelastic material is deformed and held under a constant strain, the stress will rise to a peak and decreases continuously with time (Figure 1.7). Viscoelastic materials behave *strain-rate de-*

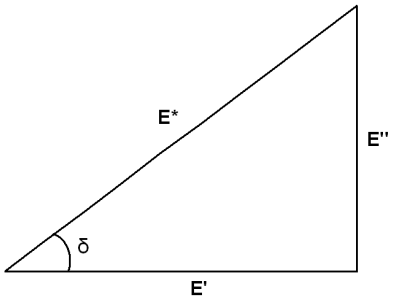

Figure 1.5: Schematic representation of the correlation between the dynamic modulus E*, the storage modulus E' (elastic part), the loss modulus E'' (viscous part) and the loss angle δ in a viscoelastic material.

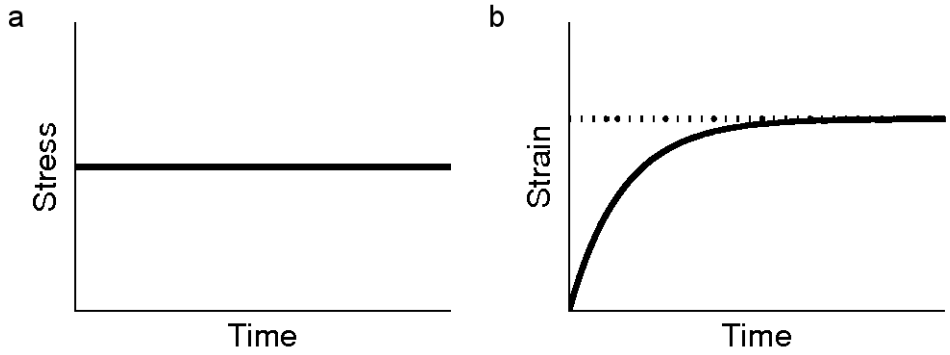

Figure 1.6: Creep: A viscoelastic material where a constant stress is applied (a) shows a continuous deformation (b) until equilibrium is reached (dotted line).

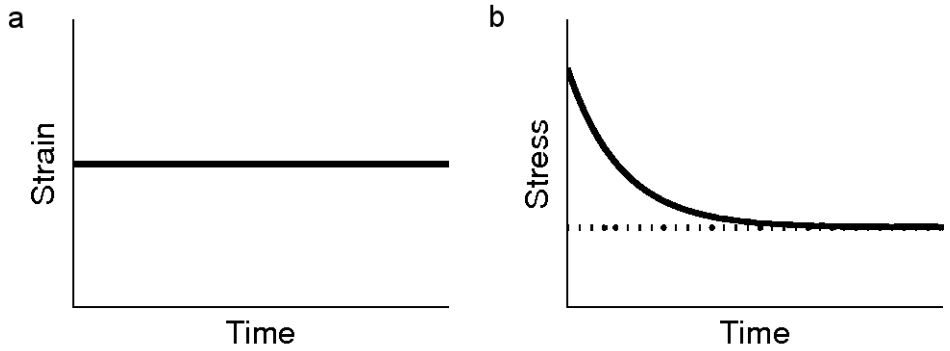

Figure 1.7: Stress relaxation: A viscoelastic material under constant strain (a) shows a decrease in stress with time (b) until equilibrium is reached (dotted line).

pendent. The stiffness increases with increasing deformation rate whereas the part of the energy which is dissipated decreases with increasing deformation rate (Figure 1.8). Viscoelastic materials behave more elastic at high deformation rates and more viscous at low deformation rates [104].

With dynamic mechanical analysis viscoelastic behaviour can be measured. Stress and strain are in phase in elastic materials. The stress-strain response is instantaneous and all energy is stored. Strain lags stress in viscoelastic materials. The loss angle (δ) represents the phase shift between stress and strain (Figure 1.9). A purely elastic material has a loss angle of 0°. The more energy is dissipated, the higher the loss angle will be.

Mechanical properties of articular cartilage

The response of cartilage on mechanical loading determines how load is absorbed and distributed to the underlying bone. If cartilage stiff-

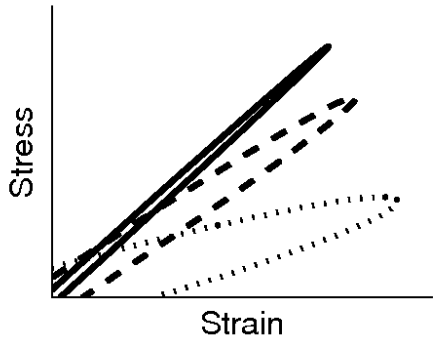

Figure 1.8: Stress versus strain of viscoelastic materials is strain-rate dependent. The stiffness increases and the energy dissipation decreases with increasing strain-rate. Solid line: high strain-rate; dashed line: intermediate strain rate; dotted line: low strain-rate.

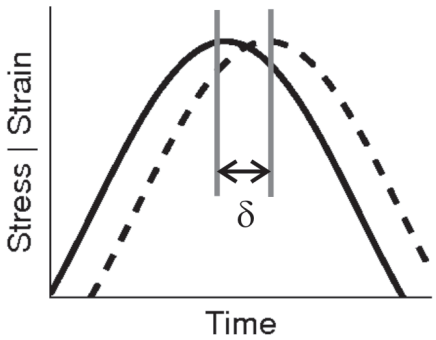

Figure 1.9: Stress (solid line) and strain (dashed line) versus time. The loss angle (δ) is the phase shift between stress and strain.

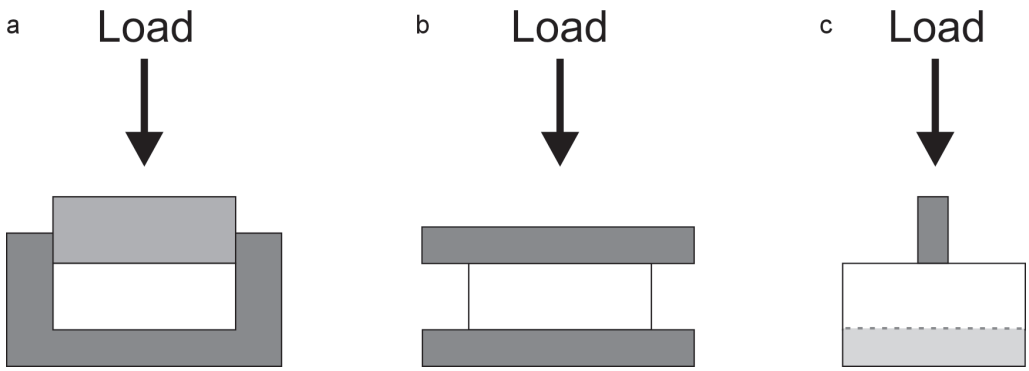

Figure 1.10: Schematic view of measurements performed in (a) confined compression, (b) unconfined compression and (c) indentation to define mechanical properties of cartilage. Within this figure, dark grey indicates impermeable platens or confining chamber, light grey indicates permeable platens and white indicates a cartilage sample. The cartilage sample in indentation (right) is still attached to the subchondral bone (light grey).

ness is too low, the load is transmitted directly to the underlying bone. If cartilage stiffness is too high, a load is likely to become focused in a small region, which can cause tissue damage or pain. Also the energy dissipation of cartilage is important, since it reduces the peak loads in the cartilage itself and the underlying bone.

When cartilage is compressed, the negatively charged aggrecan molecules are pushed closer together, which increases the repulsive force. Non-aggregated proteoglycans would not be as effective in resisting compressive loads, since they are not as easily trapped in the collagen matrix. When the collagen matrix is damaged, the compressive stiffness is also reduced, due to less efficiently trapped proteoglycans. Where the compressive stiffness of cartilage is mainly coming from the proteoglycans, the tensile stiffness of articular cartilage reflects the stiffness of the collagen network in tension [82]. Another response of cartilage on loading is fluid flow through the tissue. When cartilage is deformed or a pressure difference is applied, fluid flows through the cartilage and across the articular surface [77, 83]. So, besides being viscoelastic, cartilage also behaves as a sponge.

Test methods for determining cartilage stiffness

Determination of the mechanical properties of cartilage and other (bio)materials is typically done in confined compression [64, 71, 121], unconfined compression [35, 65, 67, 71, 104] or indentation [3, 65, 71, 79, 90, 102, 103] (Figure 1.10).

In *confined compression* the cartilage layer is placed in a confining chamber and compressed with a permeable piston (Figure 1.10a). Expansion perpendicular to the direction of compression is restrained. It is only possible for fluid to move through the permeable piston, which creates an artificial porous environment. The modulus is determined from the slope of a linear fit of the equilibrium stress versus the strain.

In *unconfined compression* the cartilage layer is placed between two smooth frictionless impermeable plates (Figure 1.10b). The cartilage

Table 1.2: The main advantages and disadvantages of articular cartilage measurements performed in confined compression, unconfined compression or indentation.

	Confined compression	Unconfined compression	Indentation
Data processing/modeling	+	+	--
Sample preparation	--	-	++
Mapping	-	-	++
Original geometry	--	--	++
Original (osteochondral) environment	--	--	+(+)
Measurement flexibility	-	-	++

layer can expand perpendicular to the direction of compression. Here, the modulus can easily be calculated from the stress strain ratio. In both *confined and unconfined compression* the cartilage specimens need to be prepared for the measurements. The subchondral bone has to be removed from the cartilage, which destroys the original osteochondral environment. Further the cartilage needs to fit perfectly in the measurement set-up to get proper results. The contact surfaces have to be completely flat and parallel to each other. Due to the sample preparation the initial geometry is interrupted and likely induces structural changes at the edges of the sample. This interruption of the initial geometry is even worse in unconfined compression due to expansion perpendicular to the direction of compression of the specimens.

During *indentation* measurements a part of the cartilage is compressed with an indenter (Figure 1.10c). This indenter can be plane-ended or spherical and permeable or impermeable. Indentation measurements do not need thorough specimen preparation and can even be performed in vivo [79]. The theoretical analysis of the data is complex and requires a mathematical model. Hayes' method is frequently used to determine cartilage stiffness [51]. However, it is based on the assumption that cartilage is a homogeneous, linear elastic material rather than a complex structure. But even viscoelastic models do not describe cartilage properly, since besides being viscoelastic, cartilage is also porous and water can move within the structure. Indentation behaviour is affected by the inhomogeneity and anisotropy of the cartilage and preferably the model takes all of this into account. But even though these models are a simplification, when the same mathematical model and measurement method is used, data can be used for comparisons. Besides this, it is also useful in increasing the understanding of cartilage behaviour.

Differences found in the mechanical properties determined by the different measurement methods can partly be explained by the different specimen preparation (intact versus not intact cartilage) and mathematical models used. In addition to that, in confined and unconfined compression the whole cartilage tissue is measured, whereas in indentation mainly the superficial zone is measured [63, 68, 71]. The above described advantages and disadvantages of all three test methods are summarized in Table 1.2.

Cartilage response on loading

Two mechanisms are responsible for the mechanical properties of articular cartilage; a flow-dependent and a flow-independent mechanism. The flow-independent viscoelastic behaviour comes from the intermolecular friction in the collagen-proteoglycan matrix. Whereas the flow-dependent behaviour originates from the interstitial fluid flow, which can be seen in creep and stress relaxation experiments [9, 35, 64, 65, 67, 71, 72, 82, 90, 97, 121]. It is shown that cartilage has a long equilibration time and therefore no equilibrium state occurs in daily living because the joints are always moving. This implies that, in normal cartilage, fluid pressurization is an important load-support mechanism.

The mechanical properties of articular cartilage are deformation rate-dependent. Cartilage stiffness increases and its energy dissipation decreases with increasing deformation rate [82, 104]. Park et al. [104] showed that the dynamic modulus of cartilage increases by a factor 2 due to its viscoelasticity when the deformation rate is increased from 0.1 to 40 Hz. Their theoretical studies suggest that flow-dependent viscoelasticity is less significant than flow-independent viscoelasticity at higher frequencies [55]. At 40Hz the loss angle reduced to zero, which implies that above this frequency cartilage behaves principally as an elastic solid. No further increase in dynamic modulus is to be expected in this case [104]. Fulcher et al. [37] showed that the storage modulus increases with increasing frequency. However, this increase levels out into a plateau before a frequency of 92 Hz is reached. However, the loss modulus stayed constant over the whole frequency range tested (1 to 92 Hz). Thus, the dynamic modulus increased with increasing frequency, but levelled out into a plateau. The loss angle decreased with increasing frequency until the plateau is reached, but did not decrease to zero. At all frequencies a much higher storage modulus than viscous modulus was observed, which means that more energy is stored by the tissue than dissipated and that this effect is greater at higher frequencies [37]. A possible reason for increased stiffness, with increasing deformation rate, is that the time for fluid flow in

the proteoglycan gel is short compared with the periodic time associated with high-frequency sinusoidal loading. However, these viscous effects cannot only be explained by fluid flow.

Not only are the mechanical properties of cartilage deformation rate dependent, they are also depth dependent. The equilibrium confined compression modulus is the lowest at the surface and increases with depth. Thus the actual mechanical behaviour of cartilage is different from that predicted under the assumption of tissue homogeneity [121]. However, the tensile modulus is the highest at the surface and decreases with depth, because of the high concentration and high degree of orientation of collagen fibrils in the superficial zone [82].

Cartilage is an adaptive tissue, which can be seen when looking at the mechanical properties in different joints, and on different locations within one joint. Several studies found location dependent properties [3, 10, 39, 79, 117, 132]. In several studies the highest stiffness was found in the load bearing areas of the condyles, while the tibial and patellar joint surfaces had softest cartilage [10, 79, 132]. Cartilage of the tibial plateau was thinner and the instantaneous stiffness was higher on locations covered by the meniscus [134]. Appleyard et al. found the highest dynamic shear modulus in the lateral outer region and the lowest in the medial inner region of ovine tibial plateaus. A high variation in cartilage stiffness was found between individuals and between locations, whereas the energy dissipation was found to be relatively constant on ovine tibial plateaus [3].

A possible explanation for these differences is that there are variations in the cartilage composition. It is shown that the compressive stiffness of cartilage in creep experiments increases as a function of the glycosaminoglycan content. However, no correlation was found between compressive stiffness and collagen content [66, 117]. Besides the ECM also the water content influences the mechanical properties of cartilage. As the water content increases, cartilage becomes less stiff and more permeable [6].

Changes in mechanical properties in osteoarthritis

Early detection of osteoarthritis (OA) is necessary to prevent or reduce long-term disability. Both morphological and mechanical properties are important, since they determine the functional behaviour of cartilage. Magnetic Resonance Imaging (MRI) is shown to be useful in obtaining morphology data of healthy and progressed OA cartilage. Unfortunately, early OA does not lead to detectable morphological changes.

It is widely accepted that the mechanical properties of cartilage depend on its composition and structural characteristics. Thus a lot of effort is made to determine whether changes in these characteristics due to OA could be detected in mechanical tests. Several studies were performed to investigate the changes in mechanical properties due to cartilage degradation. There are three types of studies performed; 1) OA-like changes were investigated, where some components of the cartilage was modified by using a degradation medium; 2) OA-like changes in animal models, with induced or spontaneous OA; 3) spontaneous occurring OA in vivo in humans [68].

In the *first* group the proteoglycan content decreased between 60% and 90%, mainly in the superficial zone. A correlation was found between cartilage stiffness and the proteoglycan content. In some cases an increase in collagen type II was found. And structural changes were observed when a collagen degenerating medium was used [74, 97, 98, 112, 139]. In the *second* group an increase in water content was observed and a proteoglycan content decrease, whereas no collagen changes were found. In most cases a decrease in cartilage stiffness was found, although in some cases this decrease was only temporarily and stiffness increased to near normal again [3, 103]. In the *third* group the results were comparable with those found in the animal models concerning the decrease in mechanical properties and proteoglycan content and the increase of water content. More structural changes were observed as well as degraded collagen [13, 100, 115].

These studies showed that a decrease in stiffness was found in static and dynamic measurement methods. However, a mechanical testing device should have a high accuracy and reproducibility to detect small changes in stiffness in early OA. Besides this, the high inter-subject and location variation in stiffness of articular cartilage will complicate the detection of early OA as is shown by Brown et al. [23] The stiffness of visually normal, artificially degraded and naturally osteoarthritic articular cartilage of bovine

patellae using a micro-indentation device was investigated. They found a 25% decrease in stiffness after proteoglycan depletion, however, when compared to the stiffness of visually normal cartilage, only 17% of the samples lie outside the normal range. Because of the high variability in the stiffness of normal samples, indentation data cannot accurately distinguish between normal and abnormal articular cartilage samples [23].

Stolz et al. [131] examined cartilage biopsies from seven patients undergoing total hip or knee replacement using atomic force microscopy. It was not possible to distinguish between healthy and osteoarthritic cartilage by determining the micro-stiffness, whereas the nano-stiffness decreased from 83 kPa (healthy) to 5.6 kPa (osteoarthritic). These changes were clearly depicted before any morphological changes could be observed using current diagnostic methods. Although this is a clear difference, it might be challenging to be able to detect early changes of osteoarthritis due to an increase in cartilage nano-stiffness with age. It might be difficult to distinguish healthy cartilage from early osteoarthritic cartilage from older patients, since age increases the stiffness, whereas osteoarthritis decreases the stiffness [131].

Modelling cartilage behaviour

In order to improve understanding of cartilage behaviour several groups made an effort in modelling articular cartilage [4, 5, 12, 29, 36, 90-93]. Mow et al. [90-93] modelled cartilage as a mixture of fluid and solid components. In this modelling, all of the solid-like components, e.g. proteoglycans, collagen and cells, are taken together to constitute the solid phase. The fluid phase, consisting of the interstitial fluid, is free to move through the matrix. Typically, the solid phase is modelled as an incompressible elastic material, and the fluid phase as incompressible and without viscosity [92]. The biggest drawback of this and other biphasic/poroelastic models [5, 36, 92] is that cartilage is seen as a homogeneous material. Therefore efforts were made to incorporate the inhomogeneous nature of cartilage into these models [29]. Here it was clearly seen that the stiffness increased from the superficial to the deep zone and that the value of the homogeneous model lies in between these values.

Bae et al. [12] modelled the effects of indenter geometry and indentation depth on intratissue strain, in order to predict damage which can occur performing these indentation tests. As expected, indenting deeper into the cartilage increases the strain magnitude, whereas indenter geometry only slightly influences the peak strain. Above described models give more insight in articular cartilage behaviour. However, a major drawback is that a model always will be a simplification of the real situation. Therefore the results will be just an approximation of what really happens.

AIM OF THIS THESIS

Because of the particular micro-architecture of biologic materials, techniques to measure their mechanical properties are complex. Indentation measurements need a mathematical model to calculate the stiffness out of the force and displacement data. The most commonly used model (Hayes) assumes that cartilage is a linear elastic material and it requires calculation of a factor κ, a complex function depending on indentation depth, cartilage thickness and Poisson's ratio [51]. Since cartilage is not linear elastic, the results are compared with another mathematical model (Kren) in which linear elasticity is not assumed. However, this other model does not take cartilage thickness into account and treats the cartilage as a material rather than a structure [73].

Many studies have been performed to study the mechanical properties of healthy and osteoarthritic cartilage. Most of these studies focussed on how cartilage stiffness changes due to variation in ECM components, structure, grade of degradation or location within a joint. But since cartilage is a viscoelastic material, not only stiffness is an important material property, also energy handling is a key property (Figure 1.5). Detecting early osteoarthritis might be improved by not only looking at the stiffness, but also at the energy dissipation. The variation in energy dissipation is smaller [3] and early changes in human osteoarthritic cartilage are increased water content and decreased proteoglycan content [82]. Besides this, cartilage has strain rate dependent behaviour (as other viscoelastic materials) and it undergoes different loading patterns in daily living. Thus, both cartilage stiffness and energy dissipation at different deformation rates were investigated.

However, this does not solve the problem of the lack of sufficient treatment possibilities of cartilage defects. Therefore, other possible cartilage repair materials need to be further investigated. Since DN-gels showed promising results to function as a cartilage repair material [11, 133, 145], dynamic stiffness and surgery-related attachment mechanics were determined. Further, it would be advantageous when the stiffness parameters of those DN-gels would be tuneable to mimick the mechanical properties of cartilage.

The aim of this thesis is to increase the understanding of the mechanical behaviour in articular cartilage specimens, including energy dissipation and to further investigate the feasibility of DN-gels to become a cartilage replacement material.

OUTLINE OF THIS THESIS

Hayes' method [51] is widely used to determine articular cartilage mechanical properties. The main disadvantages are that it assumes linear elastic behaviour and requires calculation of a factor κ, which is a complex function of indentation depth, cartilage thickness and Poisson's ratio. In **chapter 2** Hayes' method is compared with a new, simplified mathematical model to determine stiffness by Kren [73]. This model does neither assume linear elasticity nor determination of κ. Both models were applied on indentation data on swine cartilage specimens. Differences were found between the determined dynamic modulus. The modulus determined by the models correlated well together, which confirms that one model should be used in order to be able to compare different specimens. To reduce the number of assumptions and to be able to use the same model for both articular cartilage and nonlinear elastic materials, Kren's model is used in the rest of this thesis to calculate the mechanical properties.

In **chapter 3** the mechanical properties of swine cartilage were compared with those of human cartilage. Since fresh healthy human articular cartilage is not readily available, we tested whether swine cartilage could serve as a suitable substitute for mechanical comparisons. Cartilage stiffness was tested under different loading conditions related to function: fast impact and slow sinusoidal mode. For equivalent anatomic locations, there was no difference in dynamic modulus. However, the loss angle of the human cartilage was ~35% lower in fast impact and ~12% higher in slow sinusoidal mode. These differences seem attributable to age (young swine cartilage and older human cartilage) but also to species anatomy and biology. Test mode-related differences in human-swine loss angle support use of multiple function-related test modes. And keeping loss angle differences in mind, swine specimens could serve as a standard of comparison for mechanical evaluation of e.g. engineered cartilage or synthetic repair materials.

Nowadays in focal repair of joint cartilage and meniscus, initial stiffness and strength of repairs are generally much less than the surrounding tissue. This increases early failure potential. Secure primary fixation of the repair material is also a problem. In **chapter 4** it was evaluated whether acrylamide polymer double-network hydrogels (DN-gels) could serve as a repair material. Mechanical properties related to surgical use were tested in two types of DN-gels and the results were compared to that of swine meniscus and cartilage. Remarkably, these >90%-water DN-gels exhibited dynamic modulus values approaching swine meniscus (up to 50%). However, the energy-absorbing capability of these DN-gels was much lower than that of swine meniscus. Also, fine 4/0 suture tear-out strength approached cartilage. Initial strength of attachment of DN-gels to cartilage with acrylic tissue adhesive was also high. DN-gel strength and toughness properties stem from optimized entanglement of the two network components. DN-gels thus have obvious structural parallels with cartilaginous tissues, and their surgical handling properties make them ideal candidates for clinical use. However, the initial stiffness of these DN-gels is still lower than cartilage stiffness.

DN-gels have shown to be an attractive repair material for skeletal system soft tissues. They exist in a very wide range, with different compositions, with corresponding differences in stiffness, biocompatibility, etc. In chapter 4 it has been shown that their surgical handling properties as well as the ability to attach them to the surrounding tissue make them very good can-

didates, but in the stiffness and energy handling properties there was still room for improvement. In **chapter 5** it was investigated whether it was possible to create a DN-gel, which is as stiff as cartilage. Stiffness properties of three different water content DN-gels were determined and compared. The dynamic modulus increased with decreasing water content in both testing modes and resembles that of cartilage. The loss angle increased with decreasing water content in fast-impact, but not in slow-sinusoidal deformation, and is still much lower compared to cartilage. This results in a different rate dependency. It is possible to adapt the chemical composition of DN-gels in such a way that most of their biomechanical properties are close to those of healthy cartilage.

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2

Experimental verification of a non-linear model for computing cartilage modulus from micro-indentation data

Neither linear-, visco- nor poro-elasticity is a correct model for cartilage stiffness behaviour. Therefore, a new, simplified mathematical model is proposed for determining from indentation data the stiffness of hyaline cartilage and related materials with non-linear elastic behaviour. In the literature a traditional mathematical model proposed by Hayes et al. [4]--which assumes linear elasticity--is in general use for calculating cartilage stiffness, although cartilage is not linearly elastic. The Hayes approach also requires calculation of a factor κ which is a complex function of indentation depth, cartilage thickness and Poisson's ratio. The newer Kren [7] model does not require either the linear elasticity assumption or determination of κ. Sinusoidal indentation data were obtained at 0.1 Hz from osteochondral plugs of fresh swine cartilage from 8 knees. Using the same assumed Poisson's ratio (0.45) in both cases, the calculated moduli at indentation depths of 0.05 and 0.1 mm were respectively 4.2 ± 1.1 MPa and 4.9 ± 1.3 MPa (Hayes) and 7.0 ± 2.1 and 8.7 ± 3.0 MPa (Kren). Linear regression analysis between the two models showed a correlation of 0.97 (99% confidence interval). For the Kren model in contrast to other models, it is not necessary to assume linear-, visco- or porovisco-elasticity to calculate a modulus from indentation data. This is a strength since cartilage is not a material but instead an inhomogeneous, non-isotropic structure. The Kren model is perhaps best used for indentations <10% of cartilage thickness to avoid effects on modulus of the stiffness of underlying bone.

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INTRODUCTION

In recent years, a lot of effort is put in finding an articular cartilage repair material, e.g. tissue engineered constructs. To be successful, such engineered tissues must approach not only the morphology but also the mechanical properties of healthy natural cartilage, in order to bear the heavy loads occurring in the human body. The primary mechanical measurements have been non-destructive assessments of stiffness since this determines how cartilage bears and distributes loads which are functional (i.e. non-traumatic) when applied to healthy tissue. Methods for measuring the stiffness of cartilage in confined or unconfined compression have been widely used but are disadvantageous for several reasons (i) it is very demanding to prepare geometrically correct specimens (e.g. cylinders), a condition that has to be met in order to get proper measurement results, (ii) the cartilage layer is usually only 1-3 mm thick which makes it difficult to measure the stiffness for small strains, (iii) initial specimen geometry is lost during unconfined compression, due to lateral bulging of the specimens, (iv) confined compression requires use of artificial porous confinements and (v) preparation of geometric samples destroys the specimen's original osteochondral environment.

Another approach to determine the stiffness of cartilage is to measure with indenters. No preparation of the cartilage is needed and stiffness mapping of a cartilage surface is possible on a smaller scale than with compression specimens. Also, a comparison by Korhonen et al. [6] suggests that indentation provides more functionally meaningful results than either confined or unconfined compression. Further, to allow in vivo measurements e.g. during a knee arthroscopy, indentation is the only possibility.

Hayes et al. [4] proposed a mathematical model allowing stiffness calculations (e.g. E-modulus) from indentation measurements using flat or spherical indenters. Equation 2.1 shows the Hayes' solution for spherical indenters:

$$E = (1-\nu^2)\frac{P}{2\kappa\alpha\sqrt{\alpha(2r-\alpha)}}\int_0^1 \alpha(\tau)d\tau \quad (2.1)$$

With E = E-Modulus, ν = Poisson's ratio, r = indenter radius, α = indentation depth. κ is a nondimensional parameter depending on cartilage thickness, α and r. τ is a normalized radial coordinate.

One of the major drawbacks of the Hayes model is the fact that linear elasticity is assumed. At the beginning of the discussion, Hayes warns that: "Articular cartilage is a viscoelastic material and any dynamic analysis must treat it as such."[4].

THEORETICAL ANALYSIS AND MODELING

We therefore propose a simplified, alternative approach for calculating stiffness parameters of linear elastic and viscoelastic materials from indentation data gathered with a spherical indenter. One first assumes that the force acting against the (spherical) indenter may be separated into an elastic component P_e and a viscous component P_v (Equation 2.2) [7]:

$$P = P_e + P_v \quad (2.2)$$

It is then necessary to assume that the elastic component of contact force obeys the Hertz law [5] (Equation 2.3):

$$P_e = \frac{4E^*}{3(1-\nu^2)}\sqrt{r\alpha^3} \quad (2.3)$$

In addition, the viscous component (Pv) depends directly on viscosity η, and the velocity of intrusion V (Equation 2.4):

$$P_v = g(\alpha)V \quad (2.4)$$

where g(α) is an arbitrary positive function and V the current velocity of the indenter.

Obviously P_v is equal to zero when P is equal to zero and/or V is equal to zero. For indentation measurements P is equal to zero at the moment of indentation--i.e. where α = 0 and V is equal to zero at the end of the active stage of indentation when $\alpha = \alpha_{max}$. Consequently the total force at α_{max} is as follows (Equation 2.5).

$$P_{a_{max}} = \frac{4E^*}{3(1-\nu^2)}\sqrt{r\alpha_{max}^3} \quad (2.5)$$

For a given spherical indenter of radius r, if $P_{\alpha max}$ and α_{max} are measured and ν is known or assumed, then by rearrangement of (Equation 2.5) the dynamic modulus can be determined:

$$E^* = (1-\nu^2)\frac{3P_{a_{max}}}{4\sqrt{r\alpha_{max}^3}} \quad (2.6)$$

MATERIALS AND METHODS

Eight pig knees were obtained at a local butcher shop. All pigs were of similar size and age and were freshly slaughtered on the day of testing. After preparation of the knee joints, cylindrical osteochondral plugs from the lateral condyles were obtained with a diamond core drill with a diameter of 7.45 mm (Synthes, Oberdorf, Switzerland). Cartilage thickness was measured to the nearest 0.1 mm using a calliper. The indentation tests were performed with a mechanical test machine (Synergie 100, MTS Systems, Eden Prairie, MN) equipped with a 2.5 N loadcell (Burster 8432, Burster Praezisionsmesstechnik GmbH & Co, Gernsbach, Germany) and a spherical indenter with a radius of 1.585 mm. Force and displacement data were measured under sinusoidal displacement control at 0.1 Hz until a maximum indentation depth of either 0.05 or 0.1 mm. Indentation depth was kept below 10% of cartilage thickness. Cartilage modulus was calculated using the method described above as well as the method of Hayes et al. [4]. A Poisson's ratio of 0.45 was assumed for both methods. It needs to be pointed out that values different Poisson's ratio would not change the relative values of the moduli obtained by the two methods. Statistical analysis (Wilcoxon rank sum test and linear regression analysis) was made with R (R Foundation for Statistical Computing, Austria).

RESULTS

The moduli from swine hyaline cartilage at two different indentation depths (0.05 and 0.1 mm) calculated using Hayes' and Kren's models are shown in Table 2.1. The paired Wilcoxon rank sum test showed that there is a significant difference ($p<0.01$) between the values of the modulus measured at the two different indentation depths using either the Hayes or Kren model. However, the moduli at a given depth determined by the two models differ significantly. The modulus determined by the Hayes model is ~40% smaller at both depths, but it is a proportional difference. That is, linear regression analysis shows a correlation coefficient of 0.97 between Hayes' and Kren's modulus values that is statistically significant (99% confidence interval).

Table 2.1: Moduli determined using Hayes' and Kren's model

Indentation depth [mm]	E Hayes [MPa]	E* Kren [MPa]
0.05	4.2 ± 1.1	7.0 ± 2.1
0.1	4.9 ± 1.3	8.7 ± 3.0

DISCUSSION

In 1972 Hayes et al. [4] proposed a mathematical model to measure stiffness of cartilage in indentation mode. In this model cartilage is assumed to be linearly elastic, although Hayes acknowledges that this is not actually the case. The Hayes approach also requires calculation of a factor κ which is a complex function of indentation depth, cartilage thickness and poisson's ratio. The model has been widely and is still used to calculate stiffness from indentation data at various strain rates and for stress relaxation measurements [3]. This was done even though Hayes et al. warned that this model is only valid for predicting the instantaneous response to step loads and predicting asymptotic deformation.
Much later, different models where proposed in order to elaborate a constitutive model of cartilage, including a poroelastic model reinforced with a network of elastic fibres [2]. But it should be noted that the actual fibres in cartilage, i.e. the collagen fibres, are also not linearly elastic.

Because neither linear-, visco- nor poro-elasticity is a correct model for cartilage we propose a new method taking non-linear elastic behaviour of cartilage into account, based on the work of Kren [7]. With this approach only Poisson's ratio has to be assumed. When moduli are calculated from the same data using the Kren approach and the traditional Hayes approach, there is a significant difference between the modulus values. However, both models showed similar behaviour.

First, both models showed a higher modulus at an indentation depth of 0.1 mm compared to 0.05 mm. The deformation rate is also higher at an indentation depth of 0.1 mm, since the frequency used is the same. This higher modulus is expected since viscoelastic materials and cartilage both become stiffer with increasing deformation rate [8]. In addition, it has been

shown elswhere that articular cartilage stiffness increases with depth. Cartilage closer to the surface is less stiff compared to that close to the subchondral bone [9].

Second, the results of the two models correlate perfectly. When a high modulus is calculated using Hayes' model, Kren's model also gives a high modulus and vice versa. This suggests that changes in moduli due to differences in e.g. specimens or deformation rate calculated with one model will have similar effects on moduli calculated using the other model. Clearly however, absolute values of moduli determined using one model should not directly compared with values obtained using the other model to determine effects of variables.

There is a possible explanation for why the Kren model yields higher modulus values at both depths than the Hayes model. As noted above, cartilage stiffness increases with depth; cartilage closer to the surface is less stiff compared to that close to the subchondral bone [9]. Kren's model only uses the force and deformation values obtained at maximum indentation depth.

A limitation of Kren's model is the fact that the thickness of the cartilage is not taken into account. This is in contrast to Hayes' model, which includes a factore κ which depends on cartilage thickness, indentation depth and radius of the indenter. As a result, when using the new model, it seems appropriate that the indentation depth should not exceed 10% of the cartilage thickness. According to Bueckle [1], this ensures that the indentation force/displacement response is not influenced by the presence of a much stiffer underlying support (e.g. subchondral bone).

CONCLUSION

The results suggest that a much simplified approach based on the work of Kren can be used for calculating dynamic stiffness of cartilage from indentation data--providing that indentation depth does not exceed 10% of specimen thickness. Another strength of the new model is that in contrast to other models, it is not necessary to assume linear-, visco- or porovisco-elasticity to calculate a modulus from indentation data.

Finally, as show through the results reported here, older data determined using the Hayes model can still be considered highly accurate and useful in evaluating the response to variables such as indentation depth. However, when evaluating the effects of controlled variables the absolute values of moduli based on different models should not be compared.

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3

A comparison of healthy human and swine articular cartilage dynamic indentation mechanics

Articular cartilage is a multicomponent, poroviscoelastic tissue with nonlinear mechanical properties vital to its function. A consequent goal of repair or replacement of injured cartilage is to achieve mechanical properties in the repair tissue similar to healthy native cartilage. Since fresh healthy human articular cartilage (HC) is not readily available, we tested whether swine cartilage (SC) could serve as a suitable substitute for mechanical comparisons. To a first approximation, cartilage tissue and surgical substitutes can be evaluated mechanically as viscoelastic materials. Stiffness measurements (dynamic modulus, loss angle) are vital to function and are also a non-destructive means of evaluation. Since viscoelastic material stiffness is strongly strain rate dependent, stiffness was tested under different loading conditions related to function. Stiffness of healthy HC and SC specimens was determined and compared using two nondestructive, mm-scale indentation test modes: fast impact and slow sinusoidal deformation. Deformation resistance (dynamic modulus) and energy handling (loss angle) were determined. For equivalent anatomic locations, there was no difference in dynamic modulus. However, the HC loss angle was ~35% lower in fast impact and ~12% higher in slow sinusoidal mode. Differences seem attributable to age (young SC, older HC) but also to species anatomy and biology. Test mode-related differences in human-swine loss angle support use of multiple function-related test modes. Keeping loss angle differences in mind, swine specimens could serve as a standard of comparison for mechanical evaluation of e.g. engineered cartilage or synthetic repair materials.

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INTRODUCTION

Joint surface hyaline cartilage is comprised mostly of extracellular material that is produced by a small number of chondrocytes. These cells have no direct blood supply and receive nutrition from synovial fluid and the subchondral bone plate [28]. Cartilage must withstand millions of dynamic loads each year that are often multiples of body weight, and it protects the sensitive, nerve-filled underlying bone from receiving loads which are concentrated enough to result in pain and/or damage. In the majority of individuals, joint cartilage is able to do this for decades after skeletal maturity without undergoing appreciable damage or wear itself.

One consequence of the above is that no synthetic implantable materials or structures are yet available which come close matching the mechanical properties and durability of joint cartilage. As a result, surgical repair of the joint surfaces is reliant on either (a) joint replacement with metal, ceramic and polymer components, (b) autograft, allograft or xenograft transplantation, (c) medical and surgical treatments designed to rejuvenate cartilage or (d) surgical resurfacing of portions of the joint surfaces with engineered tissue [6, 9, 12, 17, 30]. Assuming that repair products for damaged cartilage should mimic the biomechanical properties of healthy human cartilage, the best relative measure of their success is to determine to what extent the resultant structures have mechanical properties which resemble those of healthy human joint cartilage. But this poses another problem--such human tissue is not readily available for ex-vivo use as a standard of comparison--i.e. to be subjected to the same mechanical tests as candidate synthetic structures, grafts, rejuvenated cartilage or engineered cartilage. In contrast, healthy animal joint cartilage is readily available for such purposes. In order to draw conclusions from animal models, one then has to ascertain the extent to which such animal cartilage has the same mechanical properties as human cartilage.

The response to non-damaging dynamic compressive loads has two aspects. The first is stiffness, or the amount of deformation in response to load. If cartilage stiffness is too low it can become so thin under load that the load is effectively transmitted directly to the underlying bone. If cartilage stiffness is too high, a load is likely to become focused in a small region and can cause pain or tissue damage. The second response is energy handling, or the extent to which energy imparted by deformation is either stored or dissipated. If cartilage stores energy it rapidly springs back to shape when a load is removed. If it instead dissipates some of the deformation energy (as heat) it returns to shape more slowly, and the peak loads in the cartilage itself and in underlying bone are reduced. This is a means of damage protection.

Cartilage is a complex "material" because it behaves in some ways like a sponge (porous), like a spring (elastic) and like a liquid (viscous). Thus cartilage can be described as a "poroviscoelastic solid". The stiffness and energy handling of such complex materials depends on the rate at which they are deformed. As a result there are special definitions for stiffness--E^* = dynamic modulus, and energy handling--δ = loss angle, methods for measuring them, and reasons for doing so under different conditions which mimic cartilage dynamic mechanical function. The correlation between the storage modulus (E'), the loss modulus (E"), the dynamic modulus (E^*) and the loss angle (δ) for a viscoelastic material is shown in Figure 1.6. This shows that at least two of those parameters are needed in order to describe a viscoelastic material properly. In previous studies, the focus was mainly on determining the stiffness [10, 15, 22, 24, 32, 33], and only a few studies also determined which part of the energy was dissipated [1, 27, 29]. Determining the mechanical properties of cartilage is typically done in confined compression [13, 16, 32], unconfined compression [8, 13, 15, 16, 29] or indentation [1, 14, 16, 22, 23, 26, 27]. Most of these studies determine the stiffness of the cartilage in slow compression, e.g. stress relaxation [8, 13, 16, 25, 32] or creep [4, 14, 15, 23]. However, besides undergoing slow quasi-cyclic deformations (e.g. while someone stands in place), cartilage also functions in sudden transient deformations which occur during gait. This is important, because as mentioned above dynamic stiffness parameters of poroviscoelastic materials like cartilage are extremely dependent on deformation rate.

The aims of this study were to (a) further develop the authors' methods of measuring E^* and δ of cartilage in both slow quasi-cyclic deformation and fast impact indentation, and

(b) to determine and compare results for healthy swine specimens and healthy human cartilage. The overall goal was to establish whether swine specimens can serve as a standard of comparison for evaluating the extent to which joint cartilage replacement and repair strategies achieve cartilage-like mechanical behaviour.

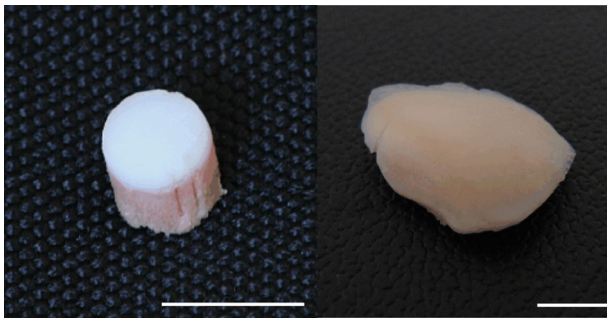

Figure 3.1: Example of a swine (left) and a human (right) piece of cartilage. White bar is 1 cm.

MATERIALS AND METHODS

Materials

Swine cartilage
Swine cartilage (SC) was obtained from the knee of 10 nine months old swine. Cylindrical osteochondral plugs of 7.6 mm in diameter (Figure 3.1; left) were harvested using a standard diamond core-drill designed for mosaicplasty (Synthes, Oberdorf, Switzerland). Plugs were harvested from the lateral patella (LP), the medial and lateral patellar groove (MPG & LPG) and from the medial and lateral condyles (MC & LC). The samples were stored in phosphate buffered saline (PBS) and frozen at -24°C. Prior to testing, the samples were thawed until room temperature and kept wet with PBS during testing.

Human cartilage
Human articular knee cartilage (HC) tissues were collected from full-thickness biopsies of the lateral femoral condyle of 8 fresh human cadavers (2 male and 6 female, median age: 57 years, range: 42-69) at the Department of pathology of the local University Hospital following informed consent by relatives and in accordance with the requirements of the Local Ethical Committee. Tissue was only harvested from knees without macroscopic signs of degenerative arthritis (Figure 3.1; right). The samples were stored in PBS and frozen at -24°C for later use. Prior to testing, the samples were thawed in PBS at room temperature and kept wet during testing.

Methods

Two micro-indentation methods were used as previously described [2, 3, 35] to determine the dynamic stiffness parameters (dynamic modulus E* and loss angle δ) of cartilage, meniscus and possible implant materials. The dynamic stiffness parameters of poroviscoelastic materials, i.e. cartilage, are extremely strain rate dependent. Besides that, cartilage functions in two different loading regimes -- the sudden transient deformations which occur during gait, and the slow quasi-cyclic deformations which cause fluid to move in and out of cartilage and thus provide a means for nutrition. Thus both a Fast Impact Mode and a Slow Sinusoidal Mode test method were developed. The dynamic modulus was calculated as described by Wirz [35] and Kren [18]. The loss angle was calculated directly from the phase shift between the displacement and load curves.

Fast Impact (FI) mode
To simulate the impact velocity in gait a fast impact micro-indentation instrument was used. This is a modified version of an instrument developed at the Minsk Institute of Physics [18]. A pendulum-mounted spherical indenter (diameter: 1.0 mm; 1.7 g) falls down on the specimen under gravitational force. The motion of the indenter is captured electromagnetically during indentation and rebound. On each specimen 10 replicate measurements were performed on the same spot at ~20 second time intervals. Resultant E* and δ were calculated for each impact and then each set was averaged to get one set of specimen values.

Slow Sinusoidal (SS) mode
To simulate nutrition in cartilage specimens a Synergie 100 MTS® mechanical testing instrument was used to perform slow sinusoidal micro-indentations. A spherical indenter (diameter ~3.2 mm) was moved sinusoidally under computer software control. The frequency was 0.1 Hz and the indentation depth was ~0.05 mm. The maximum speed was ~0.015 m/s. The same specimens were measured as in FI-mode. On each specimen

5 replicate measurements were performed at intervals of ~2 minutes on the same spot. Resultant E* and δ were averaged to get one set of specimen values.

Statistical analysis

A one-sided Wilcoxon Rank Sum test was performed on both the E* and loss angle δ data sets ($p < 0.05$). To quantify the spread in the data, the median absolute deviation was calculated and divided by the median to normalize and thus allow comparisons of the spreads within the data. Statistical analysis was accomplished using R (R Foundation for Statistical Computing, Austria).

RESULTS

In FI-mode, for both HC and SC specimens, both force and displacement vs. time traced a portion of an essentially sinusoidal curve but with peak force and peak displacement not occurring at the same time (Figure 3.2). This phase difference was attributable to the essentially viscoelastic behaviour of cartilage at high strain rates.

In SS-mode the applied displacement was sinusoidal as specified by the software driving displacement. There was again a difference in the time at which force and displacement reached a maximum, again attributable to viscoelasticity. In addition, however, for both HC and SC specimens the resultant force-time curves were not sinusoidal.

The E* and δ calculated from the raw SC data are shown in Figure 3.3. At any anatomic location E* was significantly higher and δ was significantly lower in FI-mode compared to SS-mode. In cartilage and also most synthetic viscoelastic materials faster deformation rates result in higher E* and lower δ. Also, some significant differences in these parameters were found among the various locations on the swine knee for both testing modes as follows.

The E* in FI-mode on the LC and the MC was lower compared to the LP, the LPG and the MPG. In SS-mode fewer differences were found. The E* on the LC was lower than on the

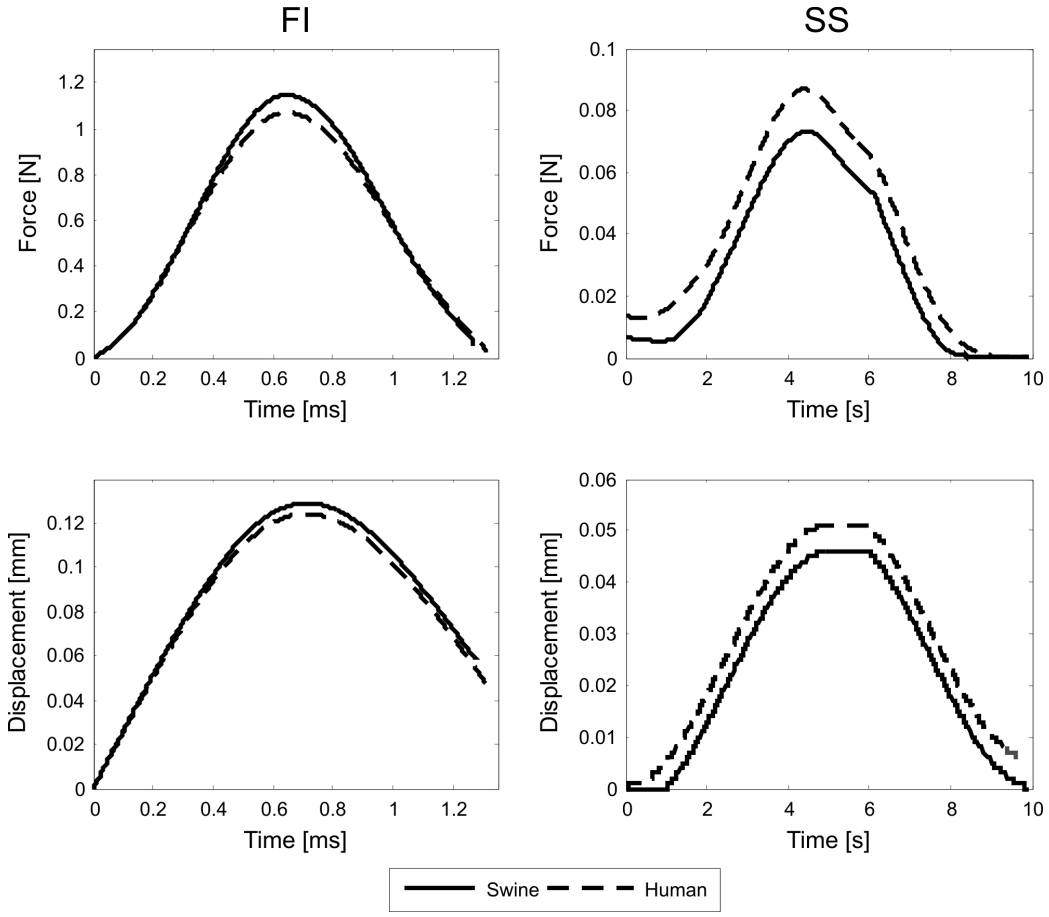

Figure 3.2: Typical example of the force and displacement curves of a swine (line) and human (dotted line) sample in fast impact (left) and slow sinusoidal (right) mode.

LPG and the MPG and also lower on the MC compared to the MPG.

The δ of the LC was lower compared to the LP, LPG and MPG and lower on the MC than on the LP in FI-mode. In SS-mode there were no differences in δ among the various locations.

For both test modes there was no statistically significant difference in E* between human and swine LC (Figure 3.4). However, the δ was significantly higher in FI-mode and lower in SS-mode for the HC compared to the SC.

The median absolute deviation was calculated and divided by the median to normalize and thus allow comparisons of the spreads within the data (Table 3.1). The normalized spread in E* of the HC specimens was 26% for both FI- and SS-mode. For the SC the normalized spread was between 10% and 52% depending on location and test mode. The overall normalized variability within the SC samples was 36% for FI-mode and 34% for SS-mode. The normalized spread was lower for the δ compared to the E*. For the HC specimens the normalized spread was 21% in FI-mode and 8% in SS-mode. In the SC specimens the normalized spread was between 5% and 19%. The overall normalized variation within the SC samples was 16% in FI-mode and 7% in SS-mode. The normalized spread in δ was lower in SS-mode compared to FI-mode.

DISCUSSION

Effect of specimen dimensions

To ensure that tissue specimen thickness would not influence results, indentation in both modes was limited to less than ~10% of tissue thickness [5]. Further, Niederauer et al. [24] showed that cartilage stiffness is not correlated with cartilage thickness. We also used a small indenter diameter compared to the diameter of the osteochondral plugs, in order to minimize specimen dimension effects in directions parallel to the specimen articular surface.

Effects of test mode on force-time and displacement-time data

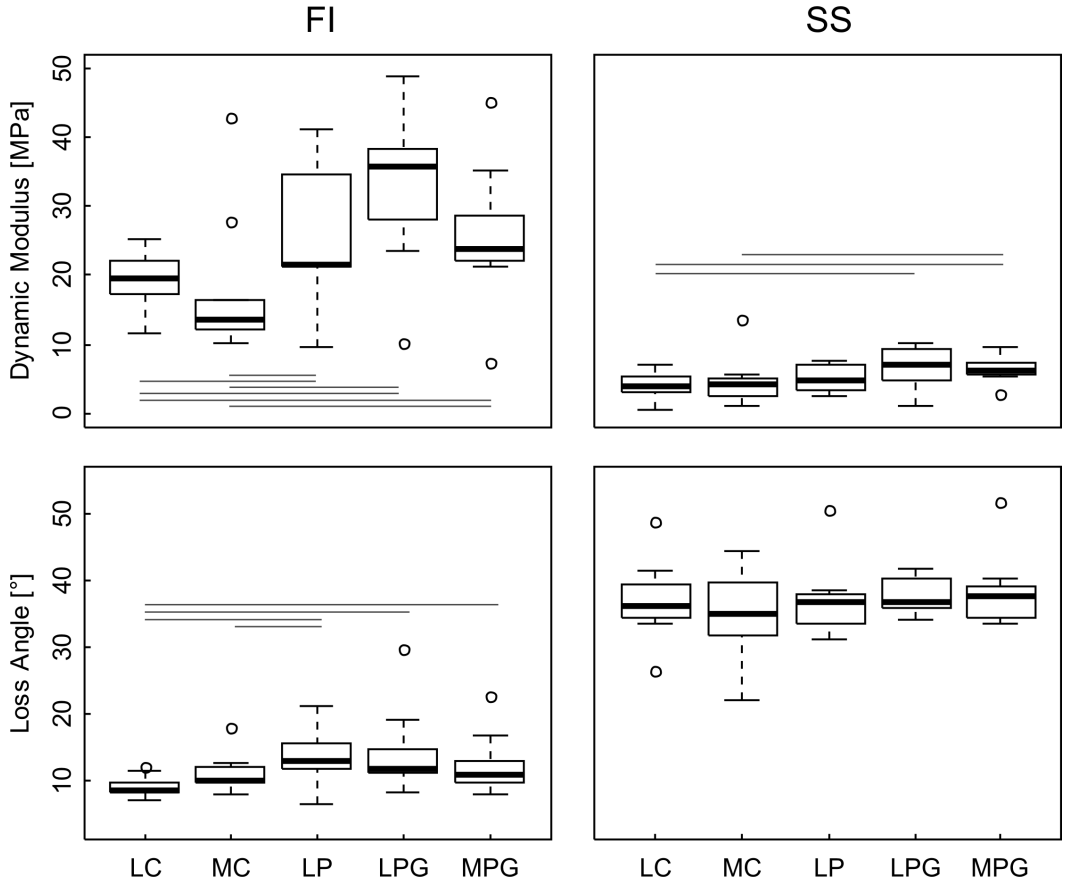

Figure 3.3: Box-and-Whisker plot of the dynamic modulus (up) and the loss angle (bottom) of the swine specimens at fast impact (left) and slow sinusoidal (right) mode. LC= lateral condyle; MC= medial condyle; LP= lateral patella; LPG= lateral patellar groove; MPG= medial patellar groove. Horizontal lines indicate a significant difference with $p < 0.05$.

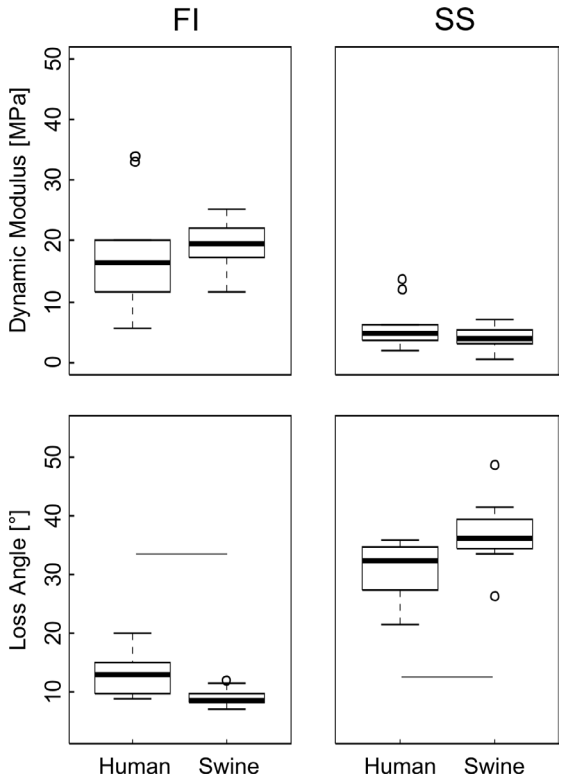

Figure 3.4: Box-and-Whisker plot of the dynamic modulus (up) and the loss angle (bottom) of the human and swine lateral condyle (LC) specimens at fast impact (FI) and slow sinusoidal (SS) mode. Horizontal lines indicate a significant difference with $p < 0.05$

As expected, since cartilage is partly a viscoelastic material, the maxima in force-time and displacement-time data (Figure 3.2) occurred at different times in both FI- and SS-modes. This phase shift is due to energy losses resulting from internal friction. In FI-mode the high strain rate coupled with the low permeability of water through cartilage resulted in essentially viscoelastic rather than more complex poroviscoelastic behaviour, and thus smooth changes in both force and displacement with time were observed. In contrast in SS-mode, the strain rate is low, and at 0.1 Hz there is time for water to move through the structure in response to load in spite of the low permeability. The response of cartilage is then poroviscoelastic. In SS-mode --and thus under poroviscoelastic response conditions and at smaller displacements than in FI-mode-- discontinuities were observed in both the displacement-time and force-time curves. The discontinuities in the displacement-time curves were due to measurement artefacts. These were uniform throughout the measurements. The measurement system was unable to resolve the small changes in displacement with time occurring near the peak of the applied sinusoidal wave form. In contrast the non-sinusoidal variations in the force-time curves was seen only with cartilage specimens and not seen with essentially elastic synthetic materials (data not shown), indicating that this was not an artefact. For cartilage, near the peak of displacement, the change in the indentation depth with time was extremely small. Therefore, the non-sinusoidal decline in force during this time can likely be attributed to energy loss of the cartilage.

Effects of cartilage test mode and anatomic location on E* and δ

As expected E* was higher and δ was lower at all swine anatomic locations in FI-mode compared to SS-mode (Figure 3.3). This is the normal response of materials exhibiting some viscoelastic behaviour when tested at a high strain rate (FI) vs. a low strain rate (SS).

There were also marked differences in E* as a function of anatomic location in both FI- and SS-modes. However, there were more significant differences in E* as a function of anatomic location for FI-mode than for SS-mode,

Table 3.1: Median absolute deviation divided by the median from the human and swine specimens on the various locations. LC= lateral condyle; MC= medial condyle; LP= lateral patella; LPG= lateral patellar groove; MPG= medial patellar groove

Parameter	Mode	Human	Swine					
		LC	LC	MC	LP	LPG	MPG	All
E*	FI	26%	14%	21%	52%	16%	10%	36%
	SS	26%	24%	31%	43%	33%	14%	34%
δ	FI	21%	11%	19%	18%	8%	19%	16%
	SS	8%	6%	12%	6%	5%	6%	7%

and only FI-mode detected any significant differences in δ as a function of anatomic location. Thus FI-mode seems to provide a more sensitive measure of cartilage stiffness parameters. This is somewhat surprising since as described above, the high strain rate and short duration of the FI-mode limits the motion of water through cartilage. As a result, one might expect that the FI-method would be less sensitive to changes in the proteoglycan portion of the cartilage structure and thus less sensitive overall. The fact that this was not the case points up the importance of evaluating cartilage stiffness parameters at substantially different strain rates that are related to the spectrum of strain rates that occur in vivo. Also, as discussed later, there are other phenomena which are only revealed by using more than one strain rate.

It is already known that the stiffness of cartilage varies at different locations in human [22] and sheep [1] knees. Lyyra et al. [22] found the highest stiffness in the load bearing areas of the condyles, where we found the highest E* in the lateral patellar groove and the lowest E* on the lateral and the medial condyles. This difference might be due to a different loading pattern in the swine knee compared to the human knee.

To quantify the normalized spread within the different specimens, the median absolute deviation was divided by the median. The results showed a large spread between the various samples (Table 3.1). The normalized spread in E* between the HC samples was 26%. Lyyra et al [22] determined the coefficient of variation which was 29% between individuals. The E* of the HC specimens did not correlate with age in this study. The spread might be due to the cartilage being exposed to another load pattern, e.g. sports, body weight. Another reason could be differences in cartilage thickness, although thickness and stiffness were not shown to be correlated in the study of Niederauer et al [24].

The normalized spread between subjects and between the different locations was smaller in δ than in E* (Table 3.1). The spread was the smallest in the δ in the SS-mode. In the early stage of osteoarthritis more proteoglycans are produced by the chondrocytes [7], which changes the capability of the cartilage to hold the water. Besides the increase in proteoglycans during osteoarthritis, cartilage collagen declines [20, 31] and damage occurs in the existing collagen in the superficial and upper mid zone [11]. Due to the smaller variation in δ and the changes occurring in osteoarthritic cartilage, osteoarthritis might be easier to detect by looking at the δ instead of the E*.

Differences in E* and δ between human and swine LC specimens

The E* of the HC and SC were not significantly different in either FI- or SS-mode. This was encouraging as a purpose of this study was to evaluate whether healthy SC can serve as a stand-in for healthy HC in stiffness parameter tests. However the trends were that SC was stiffer than HC in FI-mode but less stiff in SS-mode. In contrast to the E* results, the δ of SC was significantly (~35%) lower in FI-mode and (~12%) higher in SS-mode compared to HC. Overall, the swine vs. human differences were not enormous, and that is encouraging. However, the results do raise two questions. First, why were the results were somewhat different for HC and SC, and second why were the swine vs. human relationships opposite for FI mode compared to SS-mode?

The relatively small swine vs. human differences in a given test (FI or SS) seem likely to be due to a difference in composition of the cartilage. Composition differences could be expected for two reasons. First, the swine specimens were from young, healthy animals (age ~10 months) while the human specimens--although visually judged to be healthy--were from humans far past skeletal maturity (age 42-69 years). In addition the swine data for various anatomic locations show considerable variation, no doubt reflecting load-related needs. In this light, it may be that differences in the stance and gait of swine vs. humans result in different load-related needs on the LC and thus differences in structure and mechanical properties between swine and human LC cartilage.

The second question remains: why were the swine vs. human relationships for LC cartilage in both E* (trend) and δ (significant) opposite for FI-mode compared to SS-mode? These opposite results suggest the following.

In FI-mode there is little or no movement of water and thus the E* values may mostly reflect mechanics of cross-linked collagen. Cross-linking of collagen or other polymers (e.g. latex)

generally causes them to behave more like rubber--less viscously and more elastically (higher E* lower δ). Thus in FI-mode, the lower E* and higher δ for HC compared to SC might have been attributable to a relatively lower amount of cross linking of collagen in the (older) HC than in the (younger) SC. However, it is reported that collagen cross linking is likely to increase with age [21]. Also it has been shown that the collagen fibre arrangement is similar in HC and SC [19]. Thus the difference found is perhaps attributable to a species difference in the amount of collagen cross linking.

Conversely, in SS-mode HC exhibited a higher E* (trend) and lower δ (significant) compared to SC. As described above, a great part of the cartilage mechanical response during slow deformation (0.1 Hz in this case) is due to the nature of the motion of water in the proteoglycan portion of the cartilage structure. A relatively lower amount of water in the proteoglycans could be expected to result in less viscous behaviour--i.e. higher E* and lower δ--as seen here for HC (older) specimens vs. SC (younger) cartilage specimens. Indeed, a decline in proteoglycan water content of cartilage with age has been reported [34].

The above-described contrasting, structurally-explainable results for E* and δ for the FI vs. the SS test modes supports the idea that cartilage mechanics should be evaluated over a range of representative load deformation conditions. Otherwise the picture is incomplete and misleading conclusions could be drawn. Results here are for two extremes. They suggest that even more information related to age and disease induced variations in cartilage structure might be revealed by a more thorough exploration of the effects of rate and amplitude of deformation. The results also point up that besides the modulus, the loss angle is an important dynamic stiffness parameter of cartilage and should therefore be a part of every test set that is meant to define the mechanical quality of cartilage or suitability of a cartilage repair material.

CONCLUSIONS

Swine cartilage dynamic modulus and loss angle varied significantly with anatomic location within the swine knee. More significant differences were seen in FI (fast impact) mode than in SS (slow sinusoidal) mode. In fact, in SS-mode no significant differences were seen in loss angle as a function of anatomic location. This was surprising since one might expect that structure-related differences in cartilage permeability might be an expected difference as function of anatomic location. It seems that such a difference would be more evident during SS deformation since movement of water is possible in this mode. No ready explanation was found for this apparently anomalous result.

Differences in human-swine loss angle trends for FI (fast impact) vs. SS (slow sinusoidal) test modes support the need for using multiple function-related test modes to more completely understand cartilage mechanical behaviour.

Finally, keeping loss angle differences in mind, swine specimens could serve as a standard of comparison for mechanical evaluation of e.g. surgically repaired cartilage, engineered cartilage or synthetic repair materials.

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4

Acrylamide polymer double-network hydrogels:

Candidate cartilage repair materials with cartilage-like dynamic stiffness and attractive surgery-related attachment mechanics

In focal repair of joint cartilage and meniscus, initial stiffness and strength of repairs are generally much less than surrounding tissue. This increases early failure potential. Secure primary fixation of the repair material is also a problem. Acrylamide polymer double-network (DN) hydrogels are candidate-improved repair materials. DN-gels have exceptional strength and toughness compared to ordinary gels. This stems from the double-network structure in which there is a high molar ratio of the second network to the first network, with the first network highly crosslinked and the second loosely crosslinked. Previous studies of acrylic PAMPS/PDMAAm and PAMPS/PAAm DN-gels demonstrated physicochemical stability and tissue compatibility as well as the ability to foster cartilage formation. Mechanical properties related to surgical use were tested in 2 types of DN-gels. Results: Remarkably, these >90%-water DN-gels exhibited dynamic impact stiffness (E*) values (~1.1 and ~1.5 MPa) approaching swine meniscus (~2.9 MPa). Dynamic impact energy-absorbing capability was much lower (median loss angles of ~2°) than swine meniscus (>10°), but it is intriguing that >90%-water materials can efficiently store energy. Also, fine 4/0 suture tear-out strength approached cartilage (~2.1 and ~7.1 N v. ~13.5 N). Initial strength of attachment of DN-gels to cartilage with acrylic tissue adhesive was also high (~0.20 and ~0.15 N/mm^2). DN-gel strength and toughness properties stem from optimized entanglement of the 2 network components. DN-gels thus have obvious structural parallels with cartilaginous tissues, and their surgical handling properties make them ideal candidates for clinical use.

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INTRODUCTION

Repair of Cartilage Lesions

Cartilage and meniscal lesions have limited potential for spontaneous repair [9, 17]. Specifically, joint surface lesions with surface areas larger than 4 cm^2 are now believed to inevitably lead to degenerative arthritis [11]. This is especially the case in the knee, with serious consequences (debilitating pain and markedly restricted mobility), often leading to the need for major surgery, that is, total joint arthroplasty. As a consequence, in recent years, much effort has been devoted to developing methods for repairing such lesions.

The pioneering work of Peterson et al. [14-16] showed that a suspension of the patient's own previously harvested and expanded chondrocytes, injected behind a periosteal flap, is able to build up hyaline-like cartilage tissue. However, this method has its disadvantages, mostly the time required for the tissue to form and a consequent long rehabilitation period, up to 1 year, until pain-free full weightbearing is possible and joint homeostasis is re-established. As a result, alternative methods have been proposed and pursued. The main approach has been to place harvested and expanded chondrocytes in a scaffold material and stimulate them in vitro to begin cartilage formation in the scaffold prior to implantation. The resultant "construct" is then implanted [22]. Using this approach, the rehabilitation period can be shortened considerably.

However, such tissue-engineered constructs still do not have mechanical properties (e.g., stiffness, strength) at the time of implantation that are even remotely similar to natural articular cartilage. As a result, the rehabilitation period must still be on the order of several months in order to establish repaired tissue capable of bearing cyclic impact loads in the knee of the magnitude and frequency associated with normal daily activity [9, 10, 11, 18].

In order to further shorten the rehabilitation period needed after a cell-based cartilage repair, a tissue-engineered scaffold with cartilage-like initial mechanical properties (and of course the ability to foster cartilage formation) would be an attractive solution. Alternatively, for small repairs, one could also consider using plugs of a completely artificial solid material with cartilage-like mechanical properties rather than a scaffold. In this case, the plugs must also be extremely durable (lasting years) in order to be of clinical use. In either case (scaffold or plug), it is also necessary to have a means for securing the implant to the osteochondral bone and the surrounding intact cartilage, which can be particularly challenging in defects that are not well contained. After initial surgical wound healing, scaffolds or plugs with cartilagelike mechanical properties would be able to immediately distribute gait-related biomechanical impacts nondisruptively to the surrounding natural cartilage and also protect the sensitive subchondral bone.

Double-Network Hydrogels

Double-network hydrogels (DN-gels) are a new family of candidate materials for potential use in the repair of skeletal system soft tissues. They have been developed for these and other purposes by Gong et al. at Hokkaido University and reported in the literature starting in 2003 [5]. Ordinary single-network hydrogels containing 85% to 95% water do not have cartilage-like compressive strength. For example, articular cartilage is reported to have a compressive fracture strength of approximately 36 MPa [7]. In contrast, an example of a single-network 92%-water gel, based on an acrylamide polymer, poly(2-acrylamido-2-methylpropanesulfonic acid), that is, PAMPS, which is highly crosslinked (~4 mol %), has a compressive fracture stress of only 0.4 MPa. However, when a large molar ratio of a second acrylamide polymer, poly(acrylamide), that is, PAAm, is added to PAMPS and controlled to be lightly crosslinked (e.g., 0.1 mol %), the result is a 90%-water PAMPS/PAAm DN-gel with a markedly higher compressive fracture stress, 17.2 MPa, which is 43 times higher than the PAMPS gel [5]. In addition, a PAMPS/PAAm hydrogel with a >90%-water content does not fail until compressive strain is over 90%. For comparison, a commercially available PVA hydrogel is reported to have a compressive fracture stress in the range of only 1.4 to 2.0 MPa and fails at 47% to 62% compressive strain [19]. The tough 90%-water PAMPS/PAAm DN-gel studied here thus approaches the compressive strength of articular cartilage. It should be noted that cartilage and other skeletal system tissues are also high water-content materials and employ crosslinking and a double-network strategy (e.g., highly crosslinked

collagen plus proteoglycan gel) to achieve their mechanical properties.

Gong et al. have performed and reported a variety of preliminary, promising biomechanical and biological studies of DN-gels over the past few years [2, 5, 12, 13, 20, 26]. Recently, a study of the repair of induced osteochondral defects in rabbit knees with a DN-gel composed of a PAMPS first network and a PDMAAm, that is, poly(N,N'-dimethylacrylamide), second network was performed [25]. The PAMPS/PDMAAm DN-gel used had been shown previously in a rabbit model to exhibit no decline in stiffness, strength, or strain at failure at 6 weeks [2] and to elicit little inflammatory response [20]. For the repair study [25], the defects created in the patellofemoral groove of the femoral condyle were 4.3 mm in diameter and 15 mm deep, thus extending approximately 12 mm or more into osteochondral bone. The bony part of the defect was partially filled with a cylindrical plug of the same diameter made from the PAMPS/PDMAAm DN-gel, leaving the last 1.5 to 2.5 mm of depth (relative to the original cartilage surface) empty. After 4 weeks, the empty space (above the DN-gel plugs) had become completely filled with white, opaque tissue. It appears that hyaline-like cartilage was formed on top of the PAMPS/PDMAAm cylinders in the osteochondral defects.

Tissue adhesives are increasingly being evaluated and used as an alternative to sutures for small-scale repairs. They offer the potential advantage of distributing the load over a much larger interfacial area than is possible with sutures and thus markedly reducing the focal stresses created by sutures. They also offer speed and simplicity compared to sutures, and the repairs have been found to be sufficiently durable to allow subsequent healing in many applications. The inflammatory response to clinically approved adhesives is acceptably low. A recent orthopedic surgery-related in vitro study compared a clinically approved tissue adhesive, Histoacryl (primary active ingredient of N-butyl-2-cyanoacrylate; B. Braun Melsungen AG, Melsungen, Germany), with sutures in the repair of knee meniscal tears [1]. They found Histoacryl (B. Braun Melsungen AG) significantly increased the force required to produce a 2 mm gap in the repairs. Because of the technically easier handling for the surgeon, it might be a good idea to develop and use cartilage-like repair materials that can be safely glued into the defect, thus avoiding the need for sutures.

Because the DN-gels studied here are based on acrylamide polymers, it seemed likely that an acrylic tissue adhesive might work well to bond them to other surfaces, which is why we chose to investigate whether this was the case. As a result of reviewing the intriguing work of Gong and her group, we were fortunate to establish a collaboration with her to study further the properties of DN-gels that might be of interest before their clinical use.

The aims of this study of two types of acrylic polymer DN-gels were the following:

- to measure dynamic stiffness and the ability to dissipate energy using function-related mechanical techniques previously established by the authors [4, 24];
- to devise and use methods to measure the surgical attachment strength that can be achieved with 1) sutures and 2) a surgical tissue adhesive; and
- to compare the results for the 2 DN-gels and compare the properties with those of natural cartilage where applicable.

MATERIALS

For this study, two different acrylamide polymer DN-gels, known as PAMPS/PDMAAm and PAMPS/PAAm, were provided. The preparing ratios and water content of the two DN-gels are given in Table 4.1. While the second-network component was different for the two gels, (DMAAm v. AAm), the only difference in preparing ratios for the two gels was for the ultraviolet initiator for the second-network component (0.03 v. 0.01 mol%). As shown, both DN-gels contained more than 90% water. However, the difference in water content (94.0% v. 90.9%) means that the PAMPS/PDMAAm gel contained only approximately 66% as much polymer as the PAMPS/PAAm. The methods for producing the DN-gel structures from the polymeric components are described elsewhere [5, 23]. The DN-gels were then placed in normal saline and shipped to Basel by ordinary post, where they were kept in saline at 4 °C to 6 °C before testing at room temperature. Specimen dimensions for each test mode are described later.

Table 4.1: Preparing ratios and water content of the two double-network (DN) polymer hydrogels

DN-gel	Components: first network			Components: second network			
	Monomer	Cross-linker	UVI	Monomer	Cross-linker	UVI	Water[a]
PAMPS/ PDMAAm	AMPS 1 mol/l	MBAA 4 mol%	0.1 mol%	DMAAm 2 mol/l	MBAA 0.01 mol%	0.03 mol%	94.0%
PAMPS/ PAAm	AMPS 1 mol/l	MBAA 4 mol%	0.1 mol%	AAm 2 mol/l	MBAA 0.01 mol%	0.01 mol%	90.0%

Note: Prepared from the following: AMPS = 2-acrylamido-2-methylpropanesulfonic acid; DMAAm = N,N'-dimethyl acrylamide; AAm = acrylamide; MBAA = N,N'-methylenebisacrylamide; UVI = ultraviolet light initiator.
[a]DN gel equilibrium water content in normal saline (0.91% w/v NaCl).

METHODS

Dynamic stiffness by millimeter-scale microindentation

The Basel authors have previously developed and used microindentation methods for determining the dynamic stiffness parameters (dynamic modulus [E*] and loss angle [δ]) of cartilage and meniscus [4, 24]. The methods were employed here on the DN-gel specimens. Briefly, the methods are designed to recognize two things: 1) the values of E* and δ for poroviscoelastic materials are extremely dependent on deformation rate; and 2) articular cartilage must function in two extremely different loading regimes: the sudden transient deformations that occur during gait, and the slow quasicyclic deformations that cause fluid to move in and out of cartilage and thus provide a means for nutrition. Thus, both a "gait mode" and a "nutrition mode" testing procedure have been developed. In both tests, described briefly below, it is possible to measure the loss angle directly (Figure 1.10).

In our gait mode, evaluation of dynamic stiffness is accomplished by fast impact (FI) microindentation (MI), using a modified version (Figure 4.1) of an instrument developed at the Minsk Institute of Physics [8]. FIMI does not precisely duplicate the complex impact loading/unloading patterns seen in gait. However, the indenter velocity at impact is in the gait range: approximately 0.3 m/s. Briefly, the dynamic motion (distance v. time) of a falling microindenter (steel, 1.0 mm diameter spherical tip; 1.7 g mass of indenter) is captured electromagnetically. The velocity at impact is among the parameters captured by the electromagnetic coil through which the indenter moves.In these tests, the mass- and gravity-produced acceleration of the indenter results in nondestructive indentations having depths of 0.1 to 0.2 mm. From the dynamic motion data and indenter mass and geometry, it is possible to calculate the same parameters as in cyclic loading tests: E* and δ. Ten DN-gel specimens were tested; they were 3 mm thick and about 10 × 20 mm in lateral dimensions. They

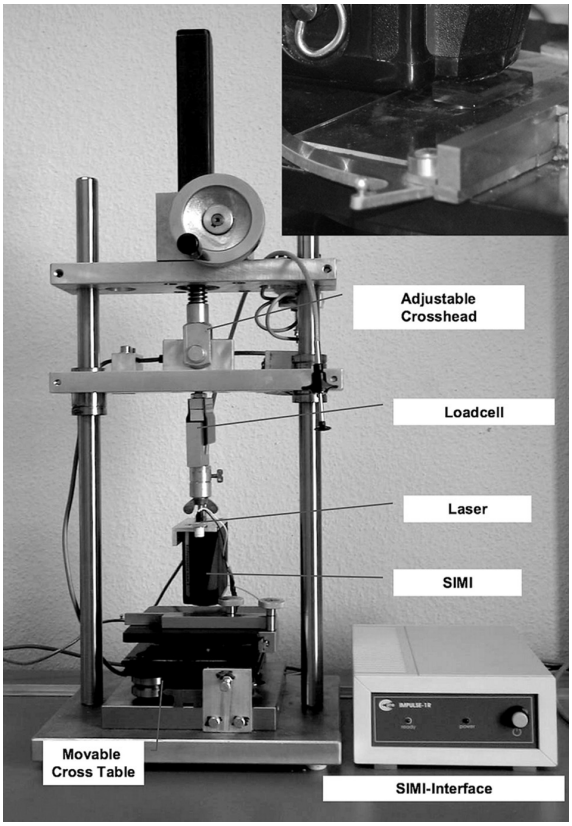

Figure 4.1: Fast impact (FI) mode modulus and loss angle measurement device, mounted on a stable loading frame, equipped with a load cell and laser positioning system.

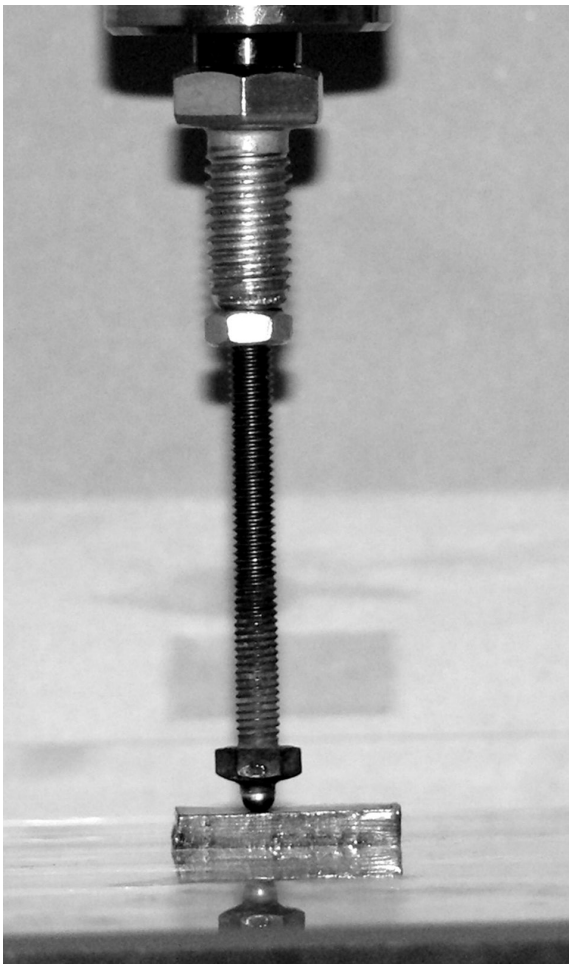

Figure 4.2: Spherical steel indenter, 3.2 mm in diameter, mounted on a material testing system (MTS Synergie 100, MTS Systems Corporation, Eden Prairie, MN), indenting a 3-mm-thick PAMPS/PDMAAm hydrogel specimen. This configuration was used for the slow sinusoidal (SS) microindentation tests.

were kept moist with saline during testing at room temperature. For a given specimen, 10 replicate impact tests were performed at the same spot at intervals of approximately 20 seconds, and the resultant E* and δ were averaged to produce E* and δ values for a given specimen.

In our nutrition mode, evaluation of dynamic stiffness is accomplished by slow sinusoidal microindentation (SSMI). The SSMI tests are performed with a MTS Synergie 100 mechanical testing instrument (MTS Systems Corporation, Eden Prairie, MN), programmed to perform a series of single sinusoidal cycles at 0.1 Hz. The microindenter (steel, ~3.2 mm diameter spherical tip) moves under sinusoidal displacement control with a maximum speed of 0.015 m/s to a depth of approximately 0.1 mm (Figure 4.2). The same 10 DN-gel specimens were tested as in gait mode. They were again kept moist with saline during testing at room temperature. For a given specimen, 10 replicate slow sinusoidal tests were performed at the same spot at intervals of about 20 seconds, shown to be sufficient to allow dimensional recovery. The resultant E* and δ were averaged to produce E* and δ values for a given specimen.

Suture tear out

There are clinical situations in which cartilage defects are not perfectly contained. In such cases, inserting a simple unsecured plug of repair material is not an adequate repair technique. It is thus an advantage if a repair material can be secured with sutures. DN-gels are known to be highly resistant to propagation of a preinduced slit in a standardized test of tear resistance [21]. Therefore, high suture tear-out forces could be expected. To test this hypothesis, again, the MTS Synergie 100 test instrument (MTS Systems Corporation) was used. DN-gel specimen dimensions were 3 × 10 × 20 mm; 3 specimens of both types of DN-gel were tested. One end of a DN-gel specimen was fixed using acrylic adhesive in an aluminum fixture matching the thickness and exceeding the width of the DN-gel specimens. A small-diameter (4/0 = 0.15 mm) surgical suture was passed through the other end of the DN gel specimen, laterally centered and approximately 3 mm from the end of the specimen, using the needle integrated with the suture by the manufacturer. The suture was Vicryl 4/0 (Ethicon, Johnson & Johnson Medical GmbH, Neuss, Germany), a type used in fine-scale soft tissue approximation. It is composed of a braided bioabsorbable copolymer (Polyglactin 910 = glycolide-L-lactide random copolymer) coated with another bioabsorbable copolymer (Polyglactin 370 = 65/35 mole ratio lactide-glycolide copolymer). The suture is further coated with calcium stearate to promote easy passage through tissues, precise knotting, and so on. After passage through the gel, the suture was then tied to itself to form a loop. The loop was slipped through a hook fixture attached to the test machine platen. The aluminum fixture was attached to a 100 N load cell attached to the crosshead of the test machine. Specimens were kept moist with saline during testing at room temperature. The crosshead was moved upward at a speed of 1 mm/s, while recording load cell force versus crosshead

motion, until the suture completely tore through the DN-gel specimen.

The MTS Synergie 100 test instrument (MTS Systems Corporation) was also used for the tissue adhesive tests. The adhesive used was Histoacryl (B. Braun Melsungen AG) (see above). Three specimens of both types of DN-gel were tested; the dimensions were 3 × 10 × 20 mm. One end of the specimen was secured with acrylic glue in a slot opening in a small aluminum fixture with slot dimensions matching the thickness and exceeding the width of the DN-gel specimens. The fixture was attached to the load cell/crosshead of the test machine. A drop of Histoacryl (B. Braun Melsungen AG) was applied to the other end of the DN-gel specimen, which was then lowered to contact a test surface secured to the test machine's fixed platen. Test surfaces were either ordinary plate glass (precleaned with ethanol) or articular cartilage in the form of osteochondral plugs 7.6 mm in diameter, taken from the knees of 9 month old swine, obtained from a retail meat vendor. These specimens were fresh frozen in 0.9% saline solution and thawed before testing. The cartilage surface was used without any cleaning except removal of surface moisture with a soft paper tissue. After allowing a minimum 60 seconds for adhesion to become secure, the crosshead of the test machine was raised at a speed of 1 mm/s while recording force and crosshead displacement. The results were normalized to the apparent contact area between DN-gels and material surface.

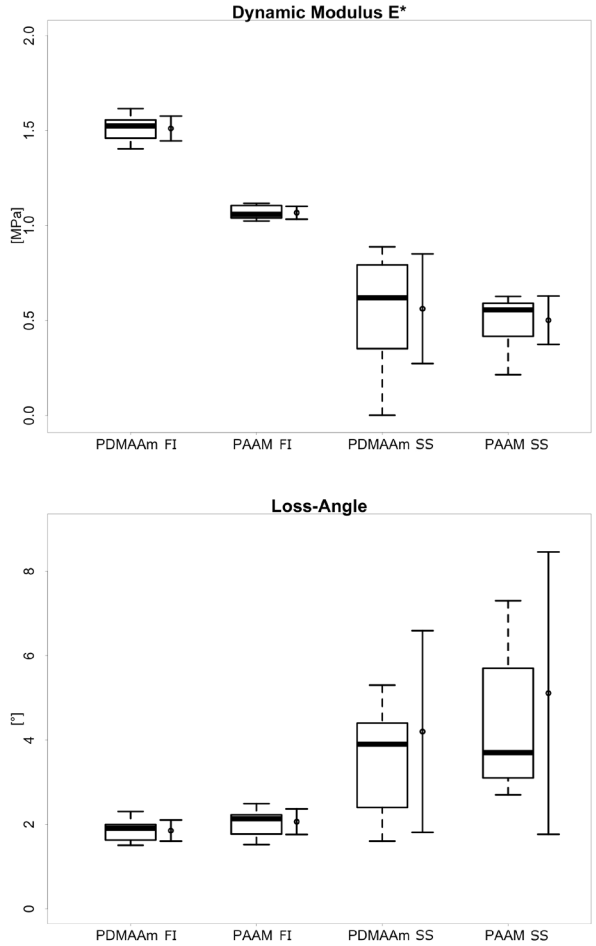

Figure 4.3: Comparison of stiffness properties of PAMPS doublenetwork hydrogels (DN-gels) with different second-network components (PDMAAm and PAAm). Dynamic modulus E* and loss angle δ measured in both fast impact (FI) and slow sinusoidal (SS) microindentation. See text for complete descriptions of test modes. For each DN-gel and mode, the left bar shows box and whisker plot showing median and quartiles, and the right bar shows mean and standard deviation.

Table 4.2: Dynamic modulus E* of double-network hydrogels in fast impact (FI) and slow sinusoidal (SS) mode tests

Dynamic modulus E* [MPa]	Mean	Standard deviation	Median
PAMPS/ PDMAAm FI	1.52	0.07	1.53
PAMPS/ PAAm FI	1.07	0.04	1.05
PAMPS/ PDMAAm SS	0.85	0.09	0.87
PAMPS/ PAAm SS	0.5	0.14	0.5

Note: Number of replicate specimens (n = 10)

Table 4.3: Loss angle δ of double-network hydrogels in fast impact (FI) and slow sinusoidal (SS) mode tests

Loss Angle [δ]	Mean	Standard deviation	Median
PAMPS/ PDMAAm FI	1.75	0.36	1.91
PAMPS/ PAAm FI	2.03	0.34	2.14
PAMPS/ PDMAAm SS	1.43	1.2	1.42
PAMPS/ PAAm SS	5.84	7.3	3.04

Note: Number of replicate specimens (n = 10)

Statistical Analysis

Wilcoxon rank–sum and signed–rank tests were performed on stiffness data ($p < 0.05$). Statistical analysis was performed and created using R (http://www.R-project.org).

RESULTS

Stiffness

For dynamic modulus E* and loss angle δ, the two DN-gels were measured in two modes: FIMI and SSMI (Tables 4.2 and 4.3; Figure 4.3). In general, it is expected for viscoelastic materials to have a higher E* and a lower δ at more rapid deformation rates. The E* for both DN-gels was significantly higher at the more rapid (FI) deformation rate. However, δ did not change significantly between the two deformation rates employed.

A paired Wilcoxon rank–sum test revealed a significant difference for each DN-gel between FI- and SS-mode values for E* but not for δ. The differences between the two gels were significant for E* but not for δ.

Suture Tear Out

Recordings of resultant force versus test instrument crosshead position are shown in Figure 4.4. In these exploratory experiments, only three replicate tests for each DN-gel were possible, so statistical comparison of results for the two gels was not feasible. The median maximum tear-out force for 94%-water PAMPS/PDMAAm was approximately 2.1 N, and the median maximum for 90.9%-water PAMPS/PAAm was approximately 7.1 N.

Attachment to Surfaces Using a Tissue Adhesive

Figure 4.5 shows the force versus crosshead position for the adhesion of DN-gels to a glass plate using Histoacryl (B. Braun Melsungen AG) tissue adhesive. Also in these exploratory experiments, only three replicate tests for each DN-gel were possible, so statistical comparison of results for the two gels was not feasible. The median maximum pull-off force of PAMPS/PD-

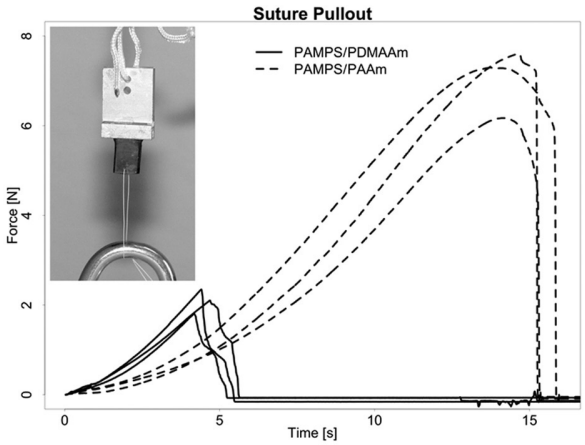

Figure 4.4: Tear-out forces for 4/0 (0.15 mm diameter) braided suture versus crosshead displacement. Three replicate tests for each 3 mm thick gel specimen. Median maximum force of PAMPS/ PDMAAm was 2.1 N, and median maximum force of PAMPS/PAAm was 7.4 N.

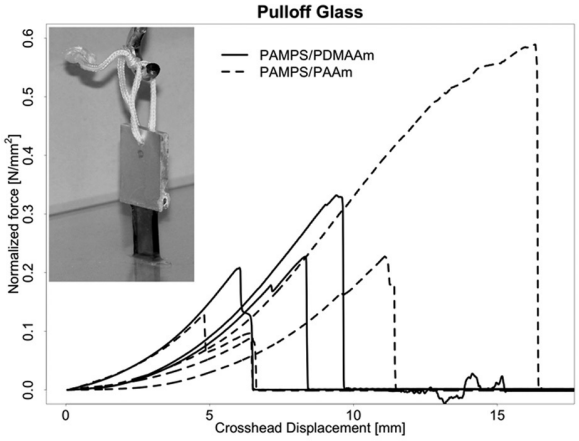

Figure 4.5: Pull-off force normalized by contact area versus crosshead displacement for double-network (DN) specimens attached to a glass plate using an acrylic tissue adhesive. Nominal area of contact = 3 × 10 mm. Three replicate tests for each DN-gel. Median maximum pull-off force of PAMPS/PDMAAm was 0.23 N/mm^2, and median maximum pull-off force of PAMPS/PAAm was 0.18 N/mm^2.

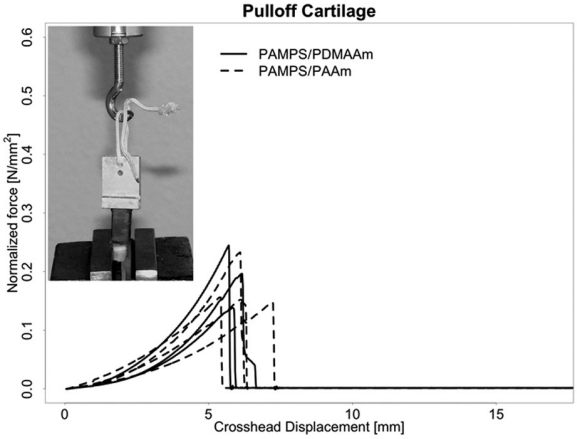

Figure 4.6: Pull-off force normalized by contact area versus crosshead displacement for double-network hydrogel (DN-gel) specimens attached to porcine articular cartilage using an acrylic tissue adhesive. Three replicate tests for each DN-gel. Median maximum pull-off force of PAMPS/PDMAAm was 0.20 N/mm^2, and median maximum pull-off force of PAMPS/PAAm was 0.15 N/mm^2.

MAAm was 0.23 N/mm^2 (range, 0.21-0.59 N/mm^2), and median maximum pull-off force of PAMPS/PAAm was 0.18 N/mm^2 (range, 0.09-0.23 N/mm^2). To the naked eye, pull off always occurred at the interface between the gel and the glass surface rather than in the gel substance.

Similarly, Figure 4.6 shows force versus crosshead position for the adhesion of DN-gels to porcine articular cartilage using Histoacryl (B. Braun Melsungen AG) tissue adhesive. Again, only three replicate tests for each DN-gel were possible. The median maximum pull-off force of PAMPS/PDMAAm was 0.20 N/mm^2 (range, 0.15-0.23 N/mm^2), and median maximum pull-off force of PAMPS/PAAm was 0.15 N/mm^2 (range, 0.14-0.24 N/mm^2). To the naked eye, pull off always occurred at the interface between cartilage surface and the DN-gels rather than in either the cartilage or gel substance. It is interesting to note that the forces required to produce adhesion failures approached the forces required to produce single-suture tear out.

DISCUSSION

The DN-gels showed very promising results, being almost as stiff as normal cartilage and allowing for a safe fixation to the surrounding tissue, either by suturing or gluing. The results of a given test of fixation (e.g., suture tear out or attachment of specimens to surfaces with tissue adhesive) showed a wide spread. This was due to the fact that it was intended to simulate the variable situation in the operation room. For example, for the attachment with cyanoacrylateglue to cartilage, we dried the surface with a paper towel, added a drop of glue, and placed the gels by hand on the cartilage surface.

Stiffness Parameters

The PAMPS/PDMAAm gel was significantly (~41%) stiffer (higher E*) than the PAMPS/PAAm gel under fast (FI-mode) deformation. PAMPS/PDMAAm was also stiffer (~12%) in slow deformation (SS-mode), but the difference was not significant. In a separate study [20, 25], we have evaluated the stiffness of swine knee articular cartilage and meniscus in the same two test modes. The DN-gel E* values were only about 10% of cartilage values in either FI- or SS-mode. However, the PAMPS/PDMAAm gel was approximately 50% as stiff as swine meniscus in both FI-mode and SS-mode. It seems likely that PAMPS/PDMAAm DN-gels with lower water content, closer to cartilage (e.g., 65%-80% rather than >90%), may have E* values more closely approaching that of the articular cartilage surface.

The two DN-gels exhibit some characteristics of a viscoelastic material because their moduli decline when the deformation rate is decreased (i.e., from FI-mode to SS-mode). However, one measure of viscoelasticity, that is, the loss angle, of the two gels did not differ significantly, and both DN-gels were relatively "rubbery," that is, more energy storing, in that the loss angle δ was low even at 0.1 Hz in the slow, cyclic N-mode tests. In both modes, median values were below 4° for both gels. In general terms, the low loss angles of these DN-gels in slow (0.1 Hz) cyclic deformation can be attributed to the fact that their structures are chemically crosslinked. Therefore, deformation does not entail much sliding between polymer chains, and there is thus little of the attendant frictional dissipation of energy that is known to result from such sliding. This less viscoelastic, more energy-storing character of these acrylamide DN-gels is particularly evident in comparison to swine articular cartilage and meniscus [25]. The FI-mode loss angle for both these tissues is approximately 12°, and the N-mode loss angle is approximately 37° for cartilage and approximately 26° for meniscus [25]. Thus, the tissues, in spite of being stiffer, can be viewed as better in absorbing and dissipating energy than the two DN-gels. What difference this might make in implant use and whether it would be possible to produce a DN-gel structure comparable to either of these tissues in both dynamic stiffness and viscoelasticity have not been explored yet.

It is interesting to note that the PAMPS/PDMAAm gel was stiffer than the PAMPS/PAAm gel in spite of having a higher water content (94% v. 90.9%) and correspondingly containing only approximately 66% as much polymer. This suggests that the gel stiffness parameters are more strongly influenced by differences in structure related to the use of different second-network components (PDMAAm v. PAAm) than by gel water content.

Surgical Fixation Stability

As mentioned in the Introduction, there are clinical situations in which cartilage defects are not perfectly contained. It is thus an advantage if a repair material can be secured with sutures. The suture tear-out strength experiments showed that both of these DN-gels were tear resistant enough to be secured with fine surgical sutures. The suture tear out of PAMPS/PDMAAm was comparable to nasal cartilage pull out of sutures. Farhadi et al. [3] showed for nasal cartilage a suture pull-out force of 4.5 N/mm normalized to the thickness of the specimen. The value for PAMPS/PDMAAm normalized to the thickness is 3.5 N/mm. Perhaps in the suture tear-out tests reported here, the somewhat lower water content (higher polymer content) of the PAMPS/PAAm gel also contributed to increased toughness compared to the PAMPS/PDMAAm gel. The tear-out strength for this DN-gel should be seen as truly remarkable considering its 90.9%-water content. The high suture tearout strength stems from the exceptional fracture energy of acrylamide DN-gels, as high as 103 J/m^2. In studies of PAMPS/PAAm, it has been shown that achieving such fracture energies depends critically on several factors, and it is believed that the molecular weight of the second-network component is the most important one. If it is above a certain value, the increase in chain entanglement greatly increases the work to fracture [23].

The tissue adhesive pull-off strength tests also showed promising high results for both of the two DN-gels to either inorganic (silicate glass) or tissue cartilage) surfaces. The results thus suggest that pull-off strength is more closely related to bonding phenomena between the primary DN-gel component (PAMPS in both cases) and the glass or cartilage surface than to any effects related to the second component. Overall, bonding strength of the gels to glass was somewhat higher than bonding to cartilage (0.23 and 0.18 N/mm^2 v. 0.20 and 0.15 N/mm^2). The values were variable but of the same order of magnitude. The variability of the results may be explained by the relatively big impact of small changes of the contact areas between the DN-gels and the surface they were glued to. Also, a small amount of not perfect perpendicularity between glass or cartilage and the DN-gel might have influenced the forces measured.

These preliminary results for the adhesion of DN-gels to cartilage with tissue adhesive are certainly promising. The force-to-failure values are of the same order of magnitude as for single-suture tear out. On the one hand, multiple sutures might provide even greater tear-out strength, although practically speaking, each suture has the disadvantage of damaging the normal surrounding cartilage [6]. On the other hand, no effort was made here to optimize the adhesion of gel to cartilage; for example, the actual area of cartilage-gel apposition may have been less than the apparent area, and no effort was made to prepare either surface in any special way. It should also be noted that attachment was to a mechanically cut gel surface. Thus, one can concludethat this pull-off strength in this test was due to bonding with the internal gel bulk structure, not just to a bond with as-prepared surface structures. However, the asprepared surface of DN-gels is known to be covered with the second-network component, and thus, adhesion to this surface might well be different. However, it should be noted that retention of water in these DN-gels does not depend on a special surface structure. The gels are not observed to leak water either when they are cut or subjected to substantial mechanical deformation for short times. It is certainly a weakness of our study that no more than three measurements per DN-gel type were recorded, but we felt that this was sufficient data to give a good impression that DN-gels can either be glued or sutured to surrounding cartilage and bone.

Concerning the future of PAMPS/PAAm hydrogels for clinical cartilage repair, it may be possible to alter their structure to render them anisotropic and thus further mimic the structure and properties of articular cartilage. Also, as yet unpublished research suggests that cell infiltration is possible. In addition, PAMPS/PAAm hydrogels can be produced in desired shapes and/or trimmed with surgical instruments in the operating room. Finally, sterilization can be accomplished by conventional methods without altering structure and properties. These statements are based on works in progress, and the details of methods and results are reserved for future reports.

CONCLUSIONS

The previous work of Gong and her colleagues has already shown that acrylamide-based DN-gels have intriguing and promising potential for use in the repair of skeletal system soft tissues. This study was performed to further investigate several mechanical properties related to clinical implant use. The results further support the potential of acrylamidebased DN-gels for such use. In spite of their very high water content, >90%, the gels studied exhibited stiffness (E*) values approaching that of swine meniscal tissue.

Suture tear-out strength values approached those for natural cartilage, again in spite of the extremely high water content. Equally intriguing was the finding that the strength of attachment of a cut gel surface to natural cartilage with an acrylic tissue adhesive approached single-suture tear-out strength.

Finally, the double-network structure has obvious parallels to the double-network strategies employed by the body in creating cartilage and other load-bearing soft tissues. Further laboratory mechanical property studies are underway with acrylamide DN-gels of much lower water content, similar to articular cartilage.

ACKNOWLEDGMENTS AND FUNDING

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5

Double network acrylamide hydrogel compositions adapted to achieve cartilage-like dynamic stiffness

Since articular cartilage has a limited potential for spontaneous healing, various techniques are employed to repair cartilage lesions. Acrylate-based double-network (DN) hydrogels containing ~90% water have shown promising properties as repair materials for skeletal system soft tissues. Although their mechanical properties approach those of native cartilage, the critical factor -stiffness- of DN-gels does not equal the stiffness of articular cartilage. This study investigated whether revised PAMPS/PAAm compositions with lower water content result in stiffness parameters closer to cartilage. DN-gels containing 61, 86 and 90% water were evaluated using two non-destructive, mm-scale indentation test-modes: fast-impact (FI) and slow-sinusoidal (SS) deformation. Deformation resistance (dynamic modulus) and energy handling (loss angle) were determined. The dynamic modulus increased with decreasing water content in both testing modes. In the 61% water DN-gel the modulus resembled that of cartilage (FI-mode: DN-gel=12, cartilage = 17; SS-mode: DN-gel =4, cartilage = 1.7 MPa). Loss angle increased with decreasing water content in fast-impact, but not in slow-sinusoidal deformation. However, loss angle was still much lower than cartilage (FI: DN-gel = 5, cartilage = 11; SS: DN-gel = 10, cartilage = 32°), indicating somewhat less ability to dissipate energy. Overall, results show that it is possible to adapt DN-gel composition to produce dynamic stiffness properties close to normal articular cartilage.

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INTRODUCTION

Articular cartilage is an extraordinary tissue because of its ability to tolerate a tremendous amount of intensive and repetitive physical stress, and to frequently do so for a lifetime [17]. However, the greatest limitation of articular cartilage is its poor capacity to heal spontaneously [5, 9]. Consequently, unhealed damage to articular cartilage in the knee is a common clinical problem. Widuchowski et al. [27] examined 25,124 knees from patients with acute knee injuries or unexplained knee pain and dysfunction from 1989 to 2004. Arthroscopy revealed that 60% of the patients had chondral lesions. Also after anterior cruciate ligament injuries, the risk of cartilage lesions is very high [24]. These unhealed injuries can lead to chronic pain, markedly restricted mobility and further degeneration of the articular cartilage, such as secondary osteoarthritis. In many cases the situation must finally be ameliorated by major surgery - i.e. total joint arthroplasty.

In recent years several techniques have been devised and used to repair cartilage lesions and stave off further degeneration. These include microfracture, autologous chondrocyte transplantation, mosaicplasty and most recently tissue-engineered constructs [15, 19, 20, 22]. These repair techniques focus on repairing the cartilage structure. Unfortunately most of these methods do not result in cartilage with initial local mechanical properties (e.g., stiffness, strength) at the time of implantation that are even remotely similar to normal articular cartilage. Another drawback of most of these methods is that the rehabilitation period takes several months up to 1-2 years in order to establish repaired tissue capable of bearing cyclic impact loads of the knee of the magnitude and frequency associated with normal daily activity [9, 11, 12, 23]. From a patient's point of view, these repairing techniques still need improvement.

Therefore our research focuses on the replacement of cartilage function so energy distribution and dissipation in the surrounding cartilage will remain similar and further degeneration of cartilage is avoided. The repair material studied here is an example of a double-network hydrogel (DN-gels) developed by Gong et al. [2, 4, 13, 14, 29]. It is based on a highly crosslinked first polymer, PAMPS—i.e. poly(2-acrylamido-2-methylpropane sulfonic acid) with a second slightly crosslinked polymer, PAAm -- i.e. poly(acrylamide) then created within the first structure. PAMPS/PAAm is a tough, tear resistant, non cytotoxic, non-absorbable DN-gel. The surprising result is a material with impact and tear resistance approaching an elastomer in spite of the high water-content. Structurally, they also resemble cartilage and other skeletal system soft tissues, which are also high water-content materials or structures with a double-network strategy to achieve their mechanical properties. Cartilage for instance can be seen as a high water content double network material or structure comprised of highly crosslinked collagen fibres interspersed with proteoglycan gel.

Importantly, a closely related formulation, PAMPS/PDMAAm DN-gel, has previously been shown to support cartilage formation. In a rabbit study [30] a DN-gel plug inserted in a large osteochondral defect induced substantial spontaneous cartilage formation in vivo by 4 weeks. In contrast, this was rarely found for empty defects or defects filled with either a polyvinylacrylate gel or an ultra-high molecular weight polyethylene plug.

It also has been previously shown [1] that a 90%-water PAMPS/PAAm DN-gel approaches the stiffness of articular cartilage. It also has attractive surgery-related attachment properties—e.g. it can be sutured or can be attached to tissue with cyanoacrylate tissue adhesives. However, this 90% water PAMPS/PAAm DN-gel is still not as stiff as native articular cartilage. Accordingly, to improve the possibility of using DN-gels as a cartilage repair material, PAMPS/PAAm DN-gel water content was adapted in this study to approach dynamic stiffness properties of normal articular cartilage.

MATERIALS

PAMPS/PAAm DN-gels with lower water content: In order to fine-tune the biomechanical properties of the DN-gels, the molecular ratios of both the first and the second network had to be varied accordingly in order to produce gels with different water contents. The three PAMPS/PAAm DN-gels produced were determined to have water contents of 90.9%, 86.5%, and 61.3% (see Table 5.1). The dimensions of the DN-gel specimens

Table 5.1: Preparing ratios and water content of the three double-network hydrogels

DN-gel	Components: first network			Components: second network			Water
	Monomer	Cross-linker	UVI	Monomer	Cross-linker	UVI	
PAMPS/ PAAm 90%	AMPS 1 mol/l	MBAA 4 mol%	0.1 mol%	AAm 2 mol/l	MBAA 0.01 mol%	0.03 mol%	90.9%
PAMPS/ PAAm 86%	AMPS 1 mol/l	MBAA 4 mol%	0.6 mol%	AAm 2 mol/l	- 0.01 mol%	0.01 mol%	86.5%
PAMPS/ PAAm 61%	AMPS 1 mol/l	MBAA 4 mol%	0.1 mol%	AAm 2 mol/l	MBAA 0.01 mol%	0.01 mol%	90.0%

Note: Prepared from the following: AMPS: 2-acrylamido-2-methylpropanesulfonic acid; AAM: acrylamide; MBBA: N,N`-methylenebisacrylamide; UVI: ultraviolet light initiator

were 20x10x3 mm. The method used to produce these various DN-gels is described elsewhere [4]. After producing the DN-gels, they were shipped to Basel in normal saline and stored at 4 to 6 °C before testing at room temperature.

Swine cartilage specimens: Cylindrical osteochondral plugs of 7.6 mm in diameter were harvested from the knee of 10-month-old swine using a standard diamond core-drill designed for mosaicplasty (Synthes, Oberdorf, Switzerland). Plugs were harvested from the lateral condyles and kept wet with phosphate-buffered saline (PBS) prior to and during testing.

METHODS

Mechanical testing

Two micro-indentation methods were used as previously described [1, 21] to determine the dynamic stiffness parameters (dynamic modulus E* and loss angle δ) of cartilage, meniscus and possible implant materials. The dynamic modulus is a measure of the deformation resistance of a material. The loss angle is a measure of the energy dissipation. If a cyclic load is applied to a viscoelastic material, the time to maximum strain will lag the time to maximum stress [8].

Articular cartilage is a structure, but as a first approximation it can be treated as a material for purposes of assessing mechanical properties and comparing them with those of true materials. The dynamic stiffness parameters of poroviscoelastic materials, e.g. cartilage, are even more strain rate dependent since the extent to which water is forced from the material also depends on strain rate and time. This is seen in the behaviour of cartilage under two different loading regimes—(a) the sudden transient deformations which occur during gait and are too brief to force water out of the tissue due to its low permeability, and (b) the slow quasi-cyclic deformations which cause fluid to move in and out of cartilage and thus provide a means for nutrition. Therefore, both a Fast Impact Mode and a Slow Sinusoidal Mode test method were developed and are used by the authors working in Basel. For both test modes, the dynamic modulus can be calculated as described by Wirz et al. [28] and Kren et al. [7]. The loss angle can be calculated directly from the time lag of the displacement curve relative to the load curve.

Fast impact (FI) mode

To simulate the impact velocity in normal human gait, a fast impact micro-indentation instrument was used. This is a modified version of an instrument developed at the Minsk Institute of Physics [7]. A pendulum-mounted spherical indenter (diameter: 1.0 mm; 1.9 g) falls down on the specimen under gravitational force. The motion of the indenter is captured electromagnetically during indentation and rebound. The duration of impact was 1-2 ms and the initial impact velocity ~0.3 m/s. On each specimen, 10 replicate FI measurements were performed

on the same spot at ~20 s time intervals. Resultant E^* and δ were calculated for each impact and then each set was averaged to get one set of specimen values.

Slow sinusoidal (SS) mode

To simulate more static loading patterns of human cartilage, a Synergie 100 MTS mechanical testing instrument was used to perform slow sinusoidal micro-indentations. A spherical indenter (diameter 1.0 mm) was moved sinusoidally with a frequency of 0.1Hz under computer software control of displacement. Indentation was performed to a depth of ~0.05 mm (SS-0.05) and ~0.1 mm (SS-0.1), with a maximum speed of ~0.015 and ~0.03 m/s. The same specimens were measured as in FI mode. On each specimen, 3 replicate SS measurements were performed at intervals of ~1 min on the same spot. Resultant E^* and δ were averaged to get one set of specimen values.

Statistics

A Lilliefors test was used to determine whether the E* and δ data sets were normally distributed. If data were normally distributed, a two-sample t-test was performed with $\alpha=0.05$, otherwise a Wilcoxon rank sum test would be performed. Statistical analysis was accomplished using R (R Foundation for Statistical Computing, Austria).

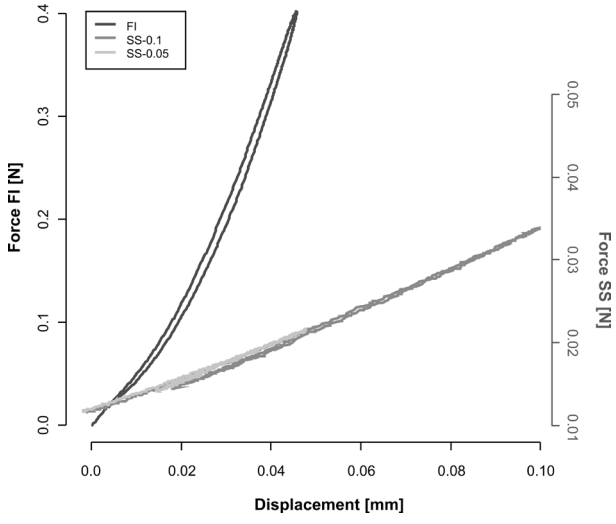

Figure 5.1: Typical Force-displacement curves of the PAMPS/PAAm 90.9% in FI-mode (black), in SS-0.1-mode (dark grey) and in SS-0.05-mode (light grey)

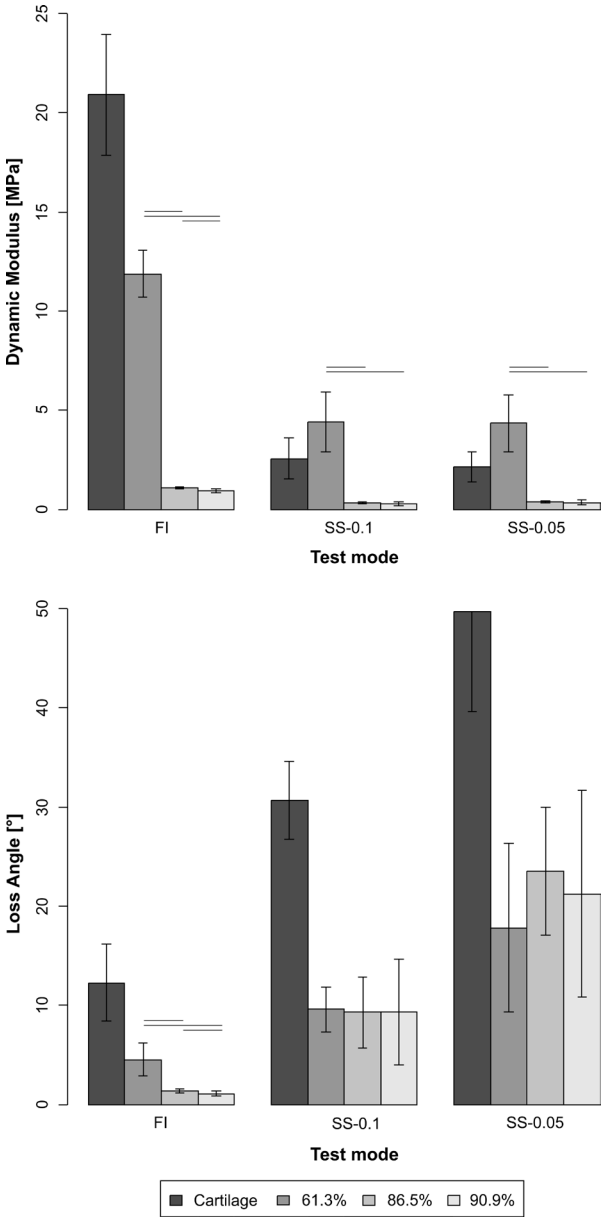

Figure 5.2: Calculated dynamic modulus (E*; up) and loss angle (δ; bottom) of swine cartilage, PAMPS/PAAm61%, PAMPS/PAAm87% and PAMPS/PAAm91% in the different test modes (FI, SS-0.1 and SS-0.05). Dashed horizontal lines are average swine cartilage data values added for comparison. Solid horizontal lines indicate a significant difference with $p<0.05$. E* is significantly higher in FI-mode compared to both SS-modes in all gels. In all gels there is a significant difference between all test modes in δ.

RESULTS

In FI-mode, force rose more rapidly with displacement than in SS-mode (Figure 5.1). This indicates a higher stiffness of the DN-gels in FI-mode. The force-displacement slopes in SS-0.05 and SS-0.1 modes were comparable, which indicates a similar stiffness in these test methods.

The data sets were all normally distrib-

uted and therefore a two-sample t-test was used. The calculated E* was significantly higher in FI-mode compared to SS-mode for all three DN-gels tested. PAMPS/PAAm 61% had a higher E* in all test modes compared to PAMPS/PAAm 86% and PAMPS/PAAm 90%. In FI-mode PAMPS/PAAm 86% had a higher E* than PAMPS/PAAm 90% (Figure 5.2).

The δ was significantly higher in SS-mode in all gels compared to FI-mode. In SS-0.1-mode the δ was lower than in SS-0.05-mode in all gels. In SS-mode no difference was found in δ among the three different gels. However, in FI-mode the δ was higher in PAMPS/PAAm 61% compared to PAMPS/PAAm 86% and PAMPS/PAAm 90%, and higher in PAMPS/PAAm 86% than in PAMPS/PAAm 90%.

Cartilage had a higher E* in FI-mode compared to SS-mode. In FI-mode E* values of cartilage were higher compared to all DN-gels (Figure 5.2). In SS-mode E* of cartilage was significantly higher compared to PAMPS/PAAm 86% and PAMPS/PAAm 90% and lower than PAMPS/PAAm 61%.

The δ of cartilage was lower in FI-mode compared to SS-mode. In SS-0.1-mode the δ was lower than in SS-0.05-mode. In all test modes the δ of cartilage was higher than all DN-gel tested.

DISCUSSION

DN-gel water content effects

The dynamic modulus, E*, increased as the water content decreased in all test modes. A possible explanation is that if the concentration of polymer is higher, there is more structure per unit volume to resist deformation. Conversely, the DN-gel δ did not change as a function of water content in SS mode. In our previous work we suggest that the δ in SS-mode is mainly due to water movement within the structure [21]. The results presented here imply that the water movement is similar in all PAMPS/PAAm DN-gels and the polymer-water-ratio does not change the ability of a given deformation to move water within the structure. But in addition, in FI-mode, the δ increases with decreasing water content. This means that an increase in polymer concentration increases the δ at high deformation rates. Since the cross-linked polymer structures are themselves viscoelastic, a higher concentration of polymer could be expected to dissipate more energy. However, the ratio between the two polymers was necessarily different among the three DN-gels, and this perhaps makes trying to explain the results on a basis of water content alone too simplistic.

Dynamic stiffness of DN-gels compared to cartilage

The results show that it was possible to bring the dynamic stiffness of the PAMPS/PAAm DN-gels closer to normal cartilage by modifying the structures in a way which allowed lower water content. As shown in Figure 5.2, the PAMPS/PAAm 61% was about 1.5-2 times stiffer than cartilage in SS-mode, but ~30% less stiff in FI-mode. Compared to tissue engineered constructs, which are only up to 10% of cartilage stiffness [22] and autologous chondrocyte transplantation, which is about 60% of cartilage stiffness a year after surgery [19] initial repair stiffness is closer to native cartilage. On the other hand, the δ of PAMPS/PAAm 61% in FI-mode was ~60% lower than that of cartilage and ~70% lower in SS-mode. PAMPS/PAAm 86% and 90% had a lower E* and a lower δ compared to cartilage in all test modes.

The crucial dynamic mechanical difference between all three of the PAMPS/PAAm DN-gels and normal cartilage is that all three had much lower loss angles (δ) than cartilage in both test modes. This means that compared to cartilage, these gels are less able to dissipate energy. Also, due to its higher δ, the E* of cartilage is more strain rate dependent than that of DN-gels. Therefore, by adjusting water content in the manner done here, the low loss angles of PAMPS/PAAm DN-gels means that their E* values could not be made similar to cartilage at both strain rates—i.e. during both fast impact (FI) and slow sinusoidal (SS) testing. For example, if one "tunes" the DN-gel value of E* to be similar to cartilage in FI, one is left with a gel which has a higher E* in SS mode. The consequence of this difference in mechanical properties with the surrounding tissue can only be speculated upon. However, the difference would be much lower compared to the same properties produced using tissue repair techniques already

in use.

One possible structural reason that the δ of all the PAMPS/PAAm DN-gels is low compared to cartilage may be because both components of the polymer structure are highly chemically crosslinked. This crosslinking reduces the possibility of sliding between the polymer chains during deformation and thus reduces the frictional dissipation of energy. Another possible cause of lower energy dissipation compared to cartilage might be less movement of water either within the DN-gel structure or out of the structure during deformation. Water can be forced out of cartilage by static loads [10] but similar loads do not result in forcing water out of PAMPS/PAAm DN-gels. Gong et al. [4] showed that after deformation to ~20% of the original thickness; still no water is squeezed out of the structure. In other words, the water within the DN-gel-structure is more highly trapped compared to cartilage.

These results show that cartilage-like dynamic stiffness can be achieved by these compositional changes; however, it is unknown how it affects other important mechanical properties. Therefore the authors plan to investigate other mechanical properties, such as strength, fatigue and tear resistance in further study. These DN-gels have already been shown to be superior to conventional gels in simulated use friction and wear tests [29].

These DN-gels have potential for clinical use. They are easy to sterilise since autoclaving has been shown not to affect their structures. They can be trimmed or produced in desired shapes and the surgical fixation, e.g. with sutures or tissue adhesive, capability has proven to be substantial. Besides this, if the gel is created with pore size are large enough, cell infiltration is likely possible, to assure integration to the surrounding tissue. Also, as previously mentioned [30] non-porous plugs of a highly similar DN-gel have been shown to foster cartilage formation in a rabbit osteochondral defect model.

Although these DN-gels look promising as a cartilage repair material, in this study only their dynamic stiffness was investigated. Before these DN-gels can be used in clinic other aspects should be investigated mainly focussed on the biocompatibility, such as immunological reactions, absorption and integration to the surrounding tissues.

CONCLUSIONS

In all three of the PAMPS/PAAm DN-gels, the δ increases with decreasing deformation rate in SS mode compared to FI mode. This is what is expected for viscoelastic materials [8, 18]. Although the DN-gels thus show normal viscoelastic behaviour with respect strain rate and δ, they do not do so with respect to E*. For normal viscoelastic materials, E* increases with increasing strain rate [8, 18] - and is thus higher in FI-mode compared to SS-mode. However, for the DN-gels no difference in E* was found between the two SS-modes, even though the deformation rate was doubled for SS-0.1 compared to SS-0.05 (~ 0.03 vs. 0.015 m/s).

Biomechanically these DN-gels look promising as potential cartilage repair materials. However, other properties, such as fixation stability and mechanical performance in vivo have to be explored.

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6

Discussion and Outlook

DISCUSSION

Economic and social impact of OA

Last decade was chosen by the World Health Organisation (WHO) as the Bone and Joint decade to get more attention for musculoskeletal deceases. One of the most common forms of joint diseases is osteoarthritis (OA). As the risk of getting OA increases with age (70% of >65 year old people suffer from OA) and the age of the world population increases the prevalence of OA will rise in the next years [4, 10, 21].

Large untreated cartilage lesions, e.g. due to traumatic accidents, will inevitably lead to secondary OA [3]. Therefore an optimal treatment is needed to prevent or delay OA. Several techniques to treat cartilage lesions are already applied in clinic [7, 14, 17, 22]. However, there is no satisfying treatment found yet. If a large portion of the cartilage is damaged, these cartilage repair techniques are insufficient and the only possibility is to implant a total or partial joint replacement prosthesis. Such an implant costs a lot of money, with the operation and planning, the stay in the hospital and rehabilitation afterwards. However, if those people would not get a prosthesis and therefore would be disabled and would not be able to live independently due to their disability, the costs of health care, help at home or living in an (elderly) home would after a few months already be more expensive than the joint replacement.

Due to the higher demand of people who want to be able to be active without suffering from pain also when they are getting older, the number of people getting a prosthesis increases and the average age when they get it decreases. The life time of a prosthesis is about 10-15 years, depending on the joint in which it is implanted. As the age of people getting a prosthesis decreases, more people will possibly need a replacement. People suffering from early OA get informed about risk factors and go a physical therapist. If the OA is worsened nothing can be done until it has become so bad that total or partial joint replacement is the only option. If a good cartilage repair or treatment of OA would be available or possible, the prevalence of a prosthesis and thus also a replacement prosthesis would decrease and the age when people get one would increase. This would influence health costs positively and have a benefit for the quality of life for many people around the world.

Mathematical models

Different types of models are used in order to describe cartilage behaviour. None of these models describe cartilage perfectly; they all have their advantages and disadvantages. All of them are partly based on (wrong) assumptions and only an approach of real cartilage. One should be aware of the disadvantages of the model used so no wrong conclusions are drawn when using a certain model. However, when a perfect model for cartilage would exist, the question remains how valuable it would be. Cartilage is a living tissue, its structure and ECM components vary between different locations and needs of the human body. The model would most probably not be appropriate anymore when testing OA cartilage, since in OA cartilage the quantity of ECM components and the structure have changed. Also replacement materials do not necessarily have a similar structure and thus a model describing cartilage will most likely not apply to these materials. Here the question rises whether the model still can be used and, if not, whether results from two different models can still be compared.

Mathematical models are developed in order to being able to calculate cartilage stiffness out of indentation data. This is important for the understanding of cartilage behaviour in different situations and for evaluation of stiffness distribution on a joint. To do so there is the need for a relative stiffness value. Thus a model which can discriminate between cartilage stiffness on different locations and between healthy and degenerated cartilage will be prefered.

Cartilage stiffness and energy dissipation

Although cartilage has a low capacity for self-repair it has the capability to adapt to its needs. When cartilage is unused, i.e. no loading is present, the chondrocytes will not get nutrition anymore and eventually the cartilage will degenerate and disappear. But it also works the other way around: at locations where a lot of (shear) loading is present, a cartilage layer can form. This is seen in people with a sesamoid bone, where a piece of bone with a cartilage layer is formed in

a tendon.

Articular cartilage has varying thickness and stiffness, even within one joint. It is adapted to the need of a specific location and has developed into the ultimate design for a specific person. At locations with high compressive forces the cartilage layer can, and maybe has to, be thicker compared to locations where mainly shear forces act, since fluid flow is more apparent in compressive loading situations. The thickness, stiffness and energy dissipation of the cartilage plays a major role in stress distribution and transmission to the bone. Besides this, also the strain rate-dependency defines how much energy is stored or dissipated. In chapter 3, we have shown that differences in mechanical behaviour might be more apparent in certain loading frequencies and that not only stiffness of cartilage is important but also energy dissipation. In order to fully understand cartilage behaviour it should always be tested in more than one loading frequency and not only stiffness, but also the energy loss should be determined.

Osteoarthritis diagnosis

The mechanical properties of cartilage change when it degrades. In an advanced degradation stage this can be measured, whereas in an early stage these differences may lie within the normal variation of mechanical properties. Brown et al. [1] found a decrease in indentation stiffness for degraded cartilage, but only 17% lay outside normal variation and therefore it cannot be used for detection. Stolz et al. [20] found a difference in nano-stiffness between different grades of OA. However, due to the small number of patients and the increase in stiffness with age and decrease in OA it is uncertain whether this could be applied in the clinic. The advantage of nano-stiffness measurements is that the properties of the collagen matrix and the proteoglycan gel can be determined separately [9]. A change in one of the two components (collagen matrix or proteoglycan gel) might be detected earlier when not determined simultaneously.

The proteoglycan content increases in OA, which changes the capability of the cartilage to hold water. These (small) differences might be detected in certain loading frequencies and not in others. When the capacity of holding the water in the collagen changes, this is more likely detected earlier in tests using slow deformation rates compared to fast deformations, since water movement is more apparent in slow deformation. Besides this, since the energy dissipation of the cartilage is more uniform, small changes might be detected earlier when looking at energy dissipation.

Once, when we are able to diagnose OA in early stages, we will be able to apply tissue engineered cartilage methods like autologous chondrocyte transplantation [5, 13] in earlier stages and better result may be expected. With the possibility of early detection of OA we will learn more about the natural history of OA. We also will be able to treat persons, where early OA is detected accidentally during an arthroscopic intervention e.g. because of a little meniscal tear. Of course, it is not feasible to test everyone every few years in order to be able to detect early OA, but it may be expected that the results of non-interventional examinations like CT or MRI will show a correlation to mechanically detected early OA [16]. A positive correlation is found in bone mineralization measured with CT and bone strength [12, 24]. In a preliminary study we found an inverse correlation between cartilage stiffness and bone strength [18]. Further, it is known that there is a strong correlation between OA and trauma in the knee and ankle joint and obesity [2, 6, 11]. It also has been shown that with MRI it is possible to predict how high the chances are to develop severe OA if early OA is already diagnosed [15]. When it is known which people are developing OA, treatment should become possible to slow down or stop cartilage degradation or preferably even recover the damages which already took place. Of course this works vice versa as well: does it make sense to find a treatment for (early) OA if it is not possible to detect it before it is too late? Therefore it is crucial that early detection of OA is made possible.

Replacement materials

Several cartilage repair strategies are being used or developed to treat cartilage lesions. The overall goal of these strategies is to restore the function of the joint, in order to make pain-free and non-restricted movement possible. To achieve normal joint function, different strategies are applied. Most of them are based on restoring the cartilage structure with its original components.

In osteochondral allografting and mosaicplasty, plugs are inserted in the defects ensuring that at least part of the defect is filled with cartilage. In chondrocyte transplantation and microfracturing, the defect is filled with cells and liquid, which should regenerate a cartilage layer. However, a hyaline cartilage layer is not always regenerated. Also the approach of tissue-engineered constructs is based on making a tissue which has cartilage-like structure and components.

The strategies applied in the clinic are based on filling of the defect with blood, cells or osteochondral plugs. However, the question rises whether filling of the defect is necessary. Yasuda et al. [23] showed that it was possible to regenerate cartilage on top of a DN-gel implanted in an osteochondral defect in rabbits. However, the result was dependent on how much space was left on top of the implanted DN-gel. No hyaline cartilage was regenerated when the vacant space was deeper than 3.5 mm and the best results were found when the vacant space was 1.5 - 2.4 mm deep.

What about the mechanical properties of the repair? Isn't it more important that the function of cartilage is restored? Cartilage absorbs and distributes loads which reduce peak loads in the bone. If the mechanical properties of the repair are different, loads will be distributed differently in the joint. If the material is too stiff, the load will become focused in a small region, whereas when stiffness is too low, load will be transmitted to the bone. If the energy dissipation is too low, too much energy will be stored, which might increase peak loads, whereas when too much energy is dissipated, recovery is slower which might increase loads in the surrounding cartilage. So the mechanical properties are an important aspect of a possible cartilage repair. However, up to now it is not known how similar the mechanics of a repair should be compared to cartilage, especially since there is large variation between individuals and even within one joint. The replacement material should not change loading too much in the surrounding cartilage nor in the underlying bone, since that might induce cartilage degradation, bone loss, or bone fracture.

Another aspect which should be bore in mind is cartilage nutrition. Cartilage receives its nutrition from fluid flowing through the tissue and this should still be possible also when part of the cartilage is replaced. Otherwise the chondrocytes in the cartilage will not survive and the cartilage will eventually degrade.

Patients heterogeneity also plays a role in cartilage repair. It has been seen that microfracture leads to better results in young patients compared to older patients [19]. Besides this, the demand of the patients might vary. Other qualifications have to be met in young patients who want to continue playing sports, compared to (older) patients who want to stay mobile for some years. It might then be worth waiting a few more months if the result afterwards is more satisfactory. A possible solution could be that young patients with still highly active cells get a cell-based repair, where cartilage regeneration is still possible, while older patients get an instant ready construct or material implanted, in order to decrease the time that they are not mobile and delay or prevent the need of a prosthesis.

In the end, the question stays: how good should the repair be? The optimal solution might be a personalized patient-specific repair. In young, healthy patients with cartilage lesions the best long-term treatment might be a cell-based or tissue engineered construct using the patient's own cells. In older patients, where it is less likely that their cells can regenerate a cartilage layer with sufficient mechanical properties, DN-gels might be the best treatment to prevent or delay joint replacement.

CONCLUSION

Cartilage is an extraordinary tissue with a complex structure. How it behaves under different loading conditions can be described by determining its stiffness and energy dissipation. Due to the complexity of the cartilage, finding a replacement material is a challenge. DN-gels have a high potential of becoming a cartilage repair, especially in older patients, whereas in young patients with cartilage defects tissue engineered constructs might be the future.

OUTLOOK

In this thesis it is shown that different indentation protocols lead to different results of mechanical properties of articular cartilage. Besides that, it is shown that not only the stiffness is a parameter, but also the energy dissipation is a key parameter which indicates cartilage behaviour. In order to understand cartilage behaviour and to make early diagnosis of osteoarthritis possible, various aspects have to be taken into account in future investigations. First of all one should be able to measure the mechanical properties in an intact joint e.g. during an arthroscopy. So results from the laboratory can be compared with results from the operation rooms and vice versa. Second, research on the changes in cartilage in OA should not only focus on cartilage stiffness, but also energy dissipation to ensure that not only one aspect of cartilage behaviour is examined. Cartilage pieces with the same stiffness, but a different capacity to dissipate energy will behave differently under the same loading conditions. This will not only affect load distribution within the cartilage, but also the load transmission to the bone. Third, cartilage stiffness and energy dissipation and their changes in OA should be determined in several loading frequencies, since cartilage behaviour is extremely strain-rate-dependent. Changes in OA cartilage might be more apparent in certain loading frequencies. Fourth, it should be determined whether changes in OA can be detected more easily by measuring just the collagen network or the proteoglycan matrix with nano-measurements. This would increase understanding of cartilage behaviour and the changes occurring in OA.

In this thesis it is shown that double-network hydrogels (DN-gels) are a promising material for cartilage repair. They can be produced with different single networks to vary their properties, such as biocompatibility and bio-absorbability. It is shown here that the dynamic stiffness values of those DN-gels can be tuned to approach those of native cartilage. However, to develop a cartilage repair material, it should be determined what key parameters are to mimick, i.e. structure, stiffness, content, fluid flow, energy dissipation and how close to native these parameters should be mimicked. Is half as stiff, stiff enough to prevent further damage? Or would half as much collagen be enough to bear loading? Do all off the cartilage ECM contents have to be replaced in the same proportions? Most probably the answers on these questions are patient specific. Young patients have a higher demand and want the repair to hold longer and withstand higher loads compared to older patients. The minimum requirements to prevent further damage should be investigated to lead to an as good as possible result.

Not only cartilage has the ability to remodel, also bone is a dynamic tissue which adapts to the needs of the body. If the bone has to bear high stresses it will strengthen and if it is unloaded, the result is bone loss. An extreme example of bone loss due to a decrease in stress is seen in astronauts who went to outer space. Depending on cartilage thickness, stiffness and energy loss more or less load is transferred to the underlying subchondral bone. A preliminary study showed an inverse correlation between cartilage stiffness and subchondral bone strength in three human patellae [18]. No correlation was found between bone strength and cartilage energy dissipation, nor between cartilage stiffness and energy dissipation. More human patellae will be measured and also cartilage thickness and grade of OA will be determined in order to see how these factors influence subchondral bone strength. It has been shown that subchondral bone remodels in OA; bone resorption increases in early OA and bone accretes in later stages of OA [8]. To decrease the numbers of factors which play a major role in the old human patellae, research should also be performed on young patellae to be able to rule out the influence of cartilage degradation. Since young human patellae are not commonly available, animal patellae can be used for this study. Of course it would also be important to know whether the correlations found are also apparent on other locations and other joints.

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List Of Publications

Journal Publications

D. Wirz, **S. Ronken**, A.P. Kren, A.U. Daniels, Experimental verification of a non-linear model for computing cartilage modulus from microindentation data, submitted to Computational and Mathematical Methods in Medicine

S. Ronken, M.P. Arnold, H. Ardura García, A. Jeger, A.U. Daniels, D. Wirz, A comparison of healthy human and swine joint cartilage dynamic compression behaviour, Biomechanics and Modeling in Mechanobiology, 2011, DOI 10.1007/s10237-011-0338-7

M.P. Arnold, A.U. Daniels, **S. Ronken**, H. Ardura García, N.F. Friederich, T. Kurokawa, J.P. Gong, D. Wirz, Acrylamide polymer double-network hydrogels: candidate cartilage repair materials with cartilage-like dynamic stiffness and attractive surgery-related attachment mechanics, accepted for publication in Cartilage 2011

S. Ronken, D. Wirz, A.U. Daniels, T. Kurokawa, J.P. Gong, M.P. Arnold, Double network acrylamide hydrogel compositions adapted to achieve cartilage-like dynamic stiffness, Biomechanics and Modeling in Mechanobiology, 2012

I. Argatov, A.U. Daniels, G. Mishuris, **S. Ronken**, D. Wirz, Accounting for the thickness effect in dynamic spherical indentation of a viscoelastic layer: Application to non-destructive testing of articular cartilage, submitted to European Journal of Mechanics - A

Conference Abstracts

Oral Presentations:

S. Ronken, M.P. Arnold, S. Hoechel, A.U. Daniels, M. Müller-Gerbl, D. Wirz, Mapping of dynamic stiffness properties of cartilage on the human patella, 18th Congress of the European Society of Biomechanics, Lisbon, Portugal, July 1 - 4, 2012

S. Ronken, S. Hoechel, D. Wirz, M. Müller-Gerbl, Cartilage stiffness and subchondral bone strength of the human patella, 18th Congress of the European Society of Biomechanics, Lisbon, Portugal, July 1 - 4, 2012

S. Ronken, M. P. Arnold, A.U. Daniels, D. Wirz, A comparison of healthy human and swine joint cartilage dynamic compression behaviour in modes emulating joint function, International Society of Biomechanics, Brussels, Belgium, July 3 - 7, 2011

S. Ronken, M. P. Arnold, A.U. Daniels, D. Wirz, Swine joint cartilage as an ex-vivo standard of comparison for human articular cartilage, 7. Jahrestagung der Deutschen Gesellschaft für Biomechanik, Murnau, Deutschland, May 19 - 21, 2011, *nomination for the Young Investigator Award*

S. Ronken, D. Wirz, M. Stolz, A.P. Kren, A.U. Daniels, Experimental verification of a viscoelastic model for computing cartilage modulus from microindentation data, 17th Congress of the European Society of Biomechanics, Edinburgh, United Kingdom, July 5 - 8, 2010

J. Frère, **S. Ronken**, A.U. Daniels, B. Göpfert, D. Wirz, Dynamic modulus and loss angle of articular cartilage in gait- and nutrition-related test modes, 18th Conference of the European Orthopaedic Research Society, Davos, Switzerland, June 30 - July 2, 2010

Poster presentations:

S. Ronken, D. Wirz, A.U. Daniels, M. P. Arnold, Diameter of the spherical indenter markedly affects dynamic indention modulus of articular cartilage, International Society of Biomechanics, Brussels, Belgium, July 3 - 7, 2011

S. Ronken, D. Wirz, A.U. Daniels, M. P. Arnold, Dynamic indentation modulus of articular cartilage changes with spherical indenter diameter, 7. Jahrestagung der Deutschen Gesellschaft für Biomechanik, Murnau, Deutschland, May 19 - 21, 2011

S. Ronken, M. P. Arnold, A.U. Daniels, D. Wirz, Can swine joint cartilage serve as an ex-vivo substitute for human articular cartilage in mechanical tests?, 5th Basel International Knee Congress and Instructional Course, Basel, Switzerland, May 9 - 10, 2011, *best poster award*

S. Ronken, M.P. Arnold, A.U. Daniels, D. Wirz, Mechanical properties of fresh and Thiel's embalmed cartilage, 18th Conference of the European Orthopaedic Research Society, Davos, Switzerland, June 30 - July 2, 2010

S. Ronken, D. Wirz, A.U. Daniels, Mechanical properties of fresh and Thiel's embalmed cartilage, Bernd-Spiessl-Symposium, Basel, Switzerland, June 17 - 19, 2010

S. Ronken, Mechanical properties of fresh and Thiel's embalmed cartilage, 6th Instructional Course in Biomechanics, Pontresina, Switzerland, January 13 - 16, 2010, *best poster award*

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