Influence of excessive loading on the mechanical properties of articular cartilage
Graduation report
P.J.F.M. Janssen
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Summary

Osteoarthritis is a joint disease; during which wear of articular cartilage (AC) occurs, eventually resulting in cartilage destruction. Our hypothesis is that cartilage damage caused by excessive loading starts with destruction of the collagen network. Initial collagen failure is assumed to initiate just below the cartilage surface before any crack formation will be visible. This study focuses on the experimental measurements performed to determine the influence of excessive loading on the mechanical properties of AC.

The goal of this study is to determine the influence of excessive loading on the mechanical properties of AC. In order to reach this goal the creep behavior of AC was measured. This was done by performing indentation experiments, before and after application of a destructive cyclic load in an in vitro setup. The influence of this cyclic load was visualized. To get more knowledge on the mechanical properties of healthy AC, unconfined compression creep experiments where performed.

The tissue samples used in the experiments are osteochondral plugs of young bovine tibia plateaus. The in vitro setup used in the experiments consisted of a custom-made indentation device that uses a materials testing machine to apply a constant force. A digital length gauge registers displacement, caused by relaxation of the collagen fibers in the ECM and exudation of water from the ECM.

Converting all displacements, caused by the application of a constant load, to strain allows us to compare date between samples. Resulting from the first indentations is a mean force-strain picture that represents the mechanical properties of healthy AC. Comparing the results of creep experiments preformed before and after application of a destructive cyclic load, is expected to show a decrease in stiffness of the AC.

The results of the performed creep experiments did not meet the expectations, due to side effects caused by membrane springs applied in the setup. These membrane springs act as an axial bearing for the indenter to ensure linear movement perpendicular to the cartilage surface. It seems that resulting from this side effect it is not possible to apply a constant force to the articular cartilage. This means that both strain and force are different in each experiment, which makes it impossible to compare data between experiments. Even though the force was not held constant the obtained data is still useable for it represents the creep behavior under a known load.

Comparing the data between healthy and damaged AC creep experiments was not possible for there are only two successful measurements of damaged AC. A second problem with comparison of data is that the used setup in its current form does not produce a clear picture of change in stiffness of the AC.

The results from the unconfined compression experiments show a large deviation between the individual experiments. This large deviation is assumed to be caused by the non-level cartilage surface of the osteochondral plugs used in the experiments.

The overall performance of the setup did not meet our expectations, though it is proven that the individual components of the setup have a sufficient accuracy. Operating this setup in relaxation experiments, after some minor modifications, is assumed to be successful. Indications are that relaxation experiments can in fact give a clear picture on the change in mechanical properties of AC resulting from excessive loading.
Preface

This report is the result of a mechanical engineering graduation project executed at the department of Biomechanical Engineering of Eindhoven University of Technology. As the title suggests the subject of this report concerns damage development in cartilage. The described subject is a follow-up on the work performed by a previous graduate student also coming from Fontys University of professional education.

The initial purpose of this assignment was to obtain data from damaged cartilage. This data would be compared to healthy cartilage in a finite element model, made by my supervisor Wouter Wilson. Setbacks during the tuning process of a new setup did not allow for this assignment to proceed exactly as planned. Eventually some data was obtained from experiments by performing an alternative way of measurements. Other results consist of recommendations for future experiments.

I would like to thank my supervisor Wouter Wilson for his advice and support during the project and writing of this report. My thanks also go out to Kees Meesters, Leon Govaert and Rob van de Berg for their advice and support on fine-tuning the experiments. I would also like to thank Wim van Grieken, my Fontys supervisor. Most of all I would like to thank my parents and my girlfriend Simone for making the past months as comfortable as possible.
Chapter 1 INTRODUCTION

Articular cartilage (AC) is a material found in joints, its main function is decreasing friction and distribution of the load on the joints. A multi-phase structure made of a collagen fiber network, proteoglycans and water, forms the base of the material.

Arthritis is a joint disease, during which articular cartilage wear occurs. The most common form of arthritis is osteoarthritis, which eventually results in cartilage destruction and changes in the subchondral bone. By the age of 55-65, up to 85% of people will have some degree of arthritis, in one or more joints, that results in pain and disability.

Literature shows that damage to articular cartilage results in decrease of proteoglycans and collagen damage. Other changes are increasing articular cartilage thickness, decreasing elastic stiffness and increasing permeability. It is not known what causes these changes, and what the sequence of these changes is because the history prior to damage of the articular cartilage is not known.

A numerical model may provide insight into the internal mechanics and damage of tissue. For this purpose Wilson et al. (2003) made a finite element model (FEM) of articular cartilage. This model is the first model that includes a realistic collagen structure and proteoglycan levels.

Articular cartilage is a biphasic material that consists of a solid and a fluid phase. As a result of this biphasic structure articular cartilage is viscoelastic. Viscoelasticity is a combination of viscous and elastic, which results in time dependant force-strain behavior.

By creating an in vitro setup capable of measuring creep or relaxation, before and after applying excessive loading, the influence of damaged cartilage can be determined. This data can then be used to fit the FEM; by comparison of the data the numerical model will show the changes in proteoglycans and collagen.

The goal of this study is to determine the influence of articular cartilage damage on the mechanical properties of articular cartilage. This will be done by performing indentation experiments, before and after applying excessive loading. Experiments are performed on osteochondral plugs of articular cartilage taken from a young bovine tibia plateau.

The applied excessive loading is intended to cause damage in the superficial layer of the cartilage without causing cracks in the cartilage surface. This is done so the initiation of cartilage damage can be ascertained.

The second chapter will give a more detailed description of the theory behind AC, creep and relaxation behavior. Chapter 3 will describe the methods used in this project. The results will be presented in chapter 4 and the discussion will follow in chapter 5. Recommendations for future experiments are presented in chapter 6.
Chapter 2 THEORY

2.1 Articular Cartilage

Articular cartilage is a thin layer of connective tissue covering the articular surfaces of the bones in the synovial joints. In freely moveable synovial joints articular cartilage is the bearing surface that permits smooth motion between adjoining bone segments, as shown in figure 2.1. The entire joint is enclosed in a fibrous tissue capsule, the inner surface of which is lined with the synovial membrane that secretes a fluid known as synovial fluid. A small amount of synovial fluid is present in a normal joint: less than 1 ml. Mature articular cartilage is aneural, avascular, and alymphatic. It receives most of its nutrition and oxygen from the synovial fluid by a process of diffusion and or convection. (Maroudas, 1975)

![Fig. 2.1 Anatomy of the knee joint and the articular cartilage surfaces](image)

In the human tibial plateau, the thickness of the cartilage layer will range from 1 to 2 mm. The function of the articular cartilage is to transmit loads from one bone to another, distribute stresses on the subchondral bones during peak loading, and to provide, in combination with the synovial fluid, a low-friction articulation. Efficient lubrication enables cartilage to minimize friction and wear (Mow et al., 1992) and to function under a wide range of loading conditions.

The ability of articular cartilage to perform its physiological function under these loading conditions depends critically on the structure, composition, and integrity of its extracellular matrix (ECM). The cyclic stresses and strains generated during repetitive loads in joint motion may cause fatigue failure within the tissue. With time, the internal failures may extend to the cartilage surface, causing cracks and fissures. (Mow et al., 1992, Broom, 1986) This tissue damage may lead to wear of the articular surfaces, known as osteoarthrosis, associated with pain and eventually with loss of joint function.

2.1.1 Composition

Articular cartilage consists of a sparse distribution of cells (chondrocytes) embedded in an extracellular matrix (ECM), hydrated with water. The extracellular matrix consists mainly of collagen fibrils, proteoglycans and proteins.

![Fig. 2.2 The Benninghoff arcade model representing the orientation of the collagen fibers.](image)
Proteoglycans
Proteoglycans (PGs) consists of a protein core to which glycosaminoglycans are attached to form a bottlebrush-like structure. The PGs are responsible for the compressive strength of the cartilage. Approximately 10 - 15% of the wet weight of articular cartilage is made up of PGs. Due to fixed charges ion concentration inside the tissue is higher than in the surrounding synovial fluid. This excess of ion particles within the matrix creates pressure referred to as Donnan osmotic pressure, which is the driving force for fluid flow. PG concentration and water content vary through the depth of the tissue. Near the articular surface, PG concentration is relatively low, and the water content is highest in the tissue. In the deeper regions of the cartilage, near the subchondral bone, the PG concentration is greatest, and water content is the lowest (Maroudas et al., 1979).

Collagen
Collagen is a fibrous protein that makes up 20% of the wet weight of the tissue; it forms the cartilaginous framework and is responsible for the tensile strength of cartilage. It also provides a resistance against swelling of the matrix. Disruption of the collagen network is a key factor in the development of OA.

Type II is the predominant collagen in articular cartilage, type IX lies on the surface of type 2, acting as interfibrillary glue. The orientation of collagen fibers varies through the depth of the tissue as described in the model of Benninghoff (1925). Bundles of primary fibrils extend perpendicular from the subchondral bone and splitting up close to the cartilage surface. Each vertical bundle is assumed to split up in four different fibril directions, curving in radial and circumferential directions (Fig. 2.2).

Chondrocytes
Chondrocytes are metabolic active cells that are responsible for the matrix production, maintenance and gradual turnover. Chondrocytes contribute 10% of the volume of cartilage.

Water
Water is the most abundant component of cartilage accounting for 60 - 80% of the wet-weight. The amount of water present in cartilage depends on the fixed charge density associated with the PGs, the organization of the collagen network and the strength and stiffness of this network. Shifting in and out in response to stress it allows deformation of the surface. The water content largely determines the mechanical properties of the tissue. Increased water content causes an increase in the permeability, decreased strength and decreased Young’s modulus (less stiff).

2.2 Osteoarthrosis
The main pathological characteristic of osteoarthritis is progressive destruction of the articular cartilage and subchondral eburnation, usually in combination with synovial inflammation (Huch et al., 1997). The causes of osteoarthritis are suggested to be biomechanical, biochemical and genetic factors. However, the initial event that triggers the pathological process is unclear and it is still not evident whether the initial changes occur in the cartilage, the subchondral bone or even in the synovium (Dieppe, 1999). Patients with a severe form of OA feel pain and stiffness in their joints, mostly in their finger, knee and hip joints. Sex differences become more evident with increasing age. Before 50 years of age, the prevalence of OA in most joints is higher in men than in women. Women after the age of 50 are more often affected then men of that age (Felson et al., 2000). Many people with OA are not severely affected, nor do they progress to severe joint damage.
It is generally assumed that mechanical overloading causes OA; resulting in a disorder of the whole synovial joint organ. Beside the changes in cartilage, the following changes have also been observed in OA patients: The zone of calcified cartilage will be changed by fatigue micro damage (Mori et al., 1993, Sokoloff et al., 1993). The subchondral bone becomes thicker and its stiffness decreases (14 %). This will be compensated by the increasing thickness. Totally the stiffness will increase. Both the stiffness and density of the cancellous bone, underlying the subchondral bone, increase.

![Fig. 2.3 a) The lateral tibia plateau of a nearly healthy knee. b) The lateral tibia plateau of a knee with severe OA.](image)

### 2.3 Hypothesis on cartilage damage

The amount of collagen damage is proportional to swelling of cartilage (Bank et al., 2000) and swelling is the initial event of cartilage degeneration (Maroudas et al., 1976). This indicates that the development of OA starts with failure of the collagen network (Verzijl et al., 2002).

The hypothesis for OA development is formulated as: after initial damage to the collagen network, this network can no longer prevent the tissue from swelling. Because of the failure of the network, the small PGs inside the cartilage can flow out, which decreases the swelling. Because of this outflow the ECM looses its stiffness and the excessive loading causes a larger deformation. This deformation leads to more stress of the fibers, which causes damage to the collagen network. This damage will cause swelling. Because of the reduction of PGs the chondrocytes will up regulate the PG production. However, these are predominantly small PGs, which may also be lost. The proposed schematic pathway of cartilage damage is indicated in figure 2.4.

![Fig. 2.4 The schematic pathway proposed for cartilage damage](image)

According to this hypothesis, cartilage damage caused by excessive loading starts with collagen damage.
2.4 Mechanical experiments
Two examples of mechanical tests to determine the material properties of articular cartilage are unconfined compression and indentation test. During an indentation experiment, the fibrils, especially of the superficial zone, will be loaded and therefore stretched. This means that the behavior of the fibrils themselves can be observed. If the cartilage is indented totally, the total ECM will be loaded. The fibrils in the radial zone relax and the behavior of the cartilage is depending on the fluid exudation. The equilibrium strain and lateral expansion are controlled by the elastic modulus and Poisson’s ratio of the solid matrix. This can be done during unconfined compression tests.

2.4.1 Viscoelasticity
Articular cartilage is a viscoelastic material that. Its viscoelastic behavior is a result from the biphasic structure and the viscoelasticity of the fibrils (Mow et al., 1980). The actual viscoelastic reaction under compression results from exudation of water from the structure. Figure 2.5 shows the response of a viscoelastic material to a static force excitation.

Applying stress initially will result in an elastic response: strain will increase instantaneously. In contrast to elastic substances, the material will keep on deforming in time. This phenomenon is known as creep. Viscoelastic materials show partial energy dissipation. This means the material partially stores energy, like and elastic material, while the rest is dissipated like in fluids. Combination of this part elastic, part viscous behavior results in time dependant behavior. At high loading rates articular cartilage acts stiffer, for there is less time for relaxation of the collagen fibers and exudation of fluid.

![Excitation and Response](image)

*Fig. 2.5 The viscoelastic response to a constant force excitation.*

Some phenomena in viscoelastic materials are:
- If the stress is held constant, the strain increases with time (creep);
- If the strain is held constant, the stress decreases with time (relaxation);
- The effective stiffness depends on the rate of application of the load;
- If cyclic loading is applied, hysteresis (a phase lag) occurs, leading to a dissipation of mechanical energy;
- Acoustic waves experience attenuation;
- Rebound of an object following an impact is less than 100%;
- During rolling, frictional resistance occurs.
2.4.2 Creep
During creep experiments a force is applied to the articular cartilage layer of osteochondral plug and kept constant for a period of time. As a result of this force application the cartilage will start to deform until equilibrium is reached between the applied force and the fully relaxed ECM. The deformation is measured by recording the path of the indenter under the constant force, obtained from these experiments are plots similar to those shown in figure 2.6. First an initial force is applied and left to equilibrate after which an additional force is added and left to equilibrate, this ensures a more linear measuring region.

![Fig. 2.6 Creep response of a viscoelastic material on a 2-step constant force excitation.](image)

2.4.3 Stress relaxation
During stress relaxation experiments a displacement relative to the cartilage thickness is applied and held constant for a period of time. As a result the cartilage initially will have an increase in stress, after reaching maximum strain and holding this, stress will start to decrease in time. The force cell records this decrease in stress until equilibrium is reached between strain and internal stress. The principle of a 2-step stress relaxation experiment is shown in figure 2.7.

![Fig. 2.7 The stress relaxation response of a viscoelastic material on a 2-step constant displacement excitation.](image)
Chapter 3 METHODS

The procedure used to determine the effect of overloading on the viscoelastic behavior of articular cartilage, is to perform indentation and unconfined compression experiments on cartilage samples before and after overloading. Osteochondral plugs are prepared out of a young bovine tibia-plateau. After measuring the thickness of the cartilage layer, the samples are preconditioned. The next phase is to determine the creep behavior using an indenter, apply overloading, and measure the creep behavior again. A second test using unconfined compression is performed to determine the creep behavior of undamaged samples only.

3.1 Sample preparation

In our experiments we used young bovine tibia-plateaus obtained from a local abattoir. Samples are prepared as described in Meessen (2003). After removing the menisci and other excessive tissue, the tibia-plateau is fixed in a custom made clamp that enables it to be positioned under a column drill. Using a diamond trephine with an inner diameter of 8.5mm, a 15mm cylinder is drilled out of the cartilage covered bone perpendicular to the cartilage surface. Experiments show that the correct rotational speed for this particular diamond trephine is 2000 rpm. By cutting of the upper 10-15mm of the plateau with an oscillating saw, the cylinder is released. This cylinder consists of bone and has one end covered with a cartilage layer. Using a diamond circular saw the part covered with cartilage is cut off at 4-6mm parallel to the cartilage surface. The prepared osteochondral plugs (Fig. 3.1) are stored in a saline solution (EBS) for a maximum of 2,5 days before performing experiments.

3.2 Thickness measurement

When performing creep or relaxation experiments it is necessary to know the thickness of the cartilage layer. In creep experiments the initial thickness is needed to calculate the strain resulting from the applied force. Relaxation experiments require the initial thickness to calculate the displacement that is to be applied. There are various ways of measuring cartilage thickness e.g. Optical, ultrasound or MRI, as described in a previous report by Meessen (2003) the only applicable method at TUE is optical.

3.2.1 Stereo microscopy

The first method used for measuring the cartilage layer’s thickness was to prepare a 1,0mm thick osteochondral slice taken from next to the osteochondral plug. By drilling 2 columns shifted 1mm from each other a half moon like shape is obtained (Fig. 3.2). Using a stereomicroscope equipped with a calibrated measuring eyepiece (10x, Carl Zeiss), the thickness of this slice can be measured (DiSilvestro et al., 2000; Bachrach et al., 1998). This method allows a theoretic precision of 0,02mm, depending on lighting conditions and moon shape thickness the maximum effective precision is 0,04mm. A disadvantage of this test is that it cannot measure the plug directly, but just the surroundings of the plug. This means that the actual thickness of the plug could be different than the thickness of the slice.
Another disadvantage is that the fibrous structure is ruptured during specimen preparation, which can cause swelling of the tissue. If the cartilage does not seem to be swollen looking by the eye, the estimation of the deviation is 8% of the real thickness of the cartilage layer (Meessen, 2003).

This inaccuracy was the reason to look for an alternative way to measure the cartilage thickness. The idea was to use a coaxial illuminated stereomicroscope (Stemi 2000-c, Carl Zeiss). This technique can be used to measure the direct visible thickness of the cartilage layer. Resulting in a higher accuracy of measured data because the average outer thickness can be determined by measuring multiple points on the outline of the osteochondral plug, instead of measuring the thickness on a moon like shape that will have a relative large amount of damage sustained from preparation.

The used technique for measuring the thickness of the cartilage layer, was to place the plug perpendicular under the coaxial illuminated microscope. This should make the border between the subchondral bone and cartilage layer visible. Using a measuring eyepiece (10x, Carl Zeiss) enhancing the view 5x with the co-axial illuminated stereomicroscope, a distinctive border is visible. This procedure was repeated 3 times over the outline of the osteochondral plug. The actual thickness in mm is 0,2 times the average measured.

### 3.3 Setup description

The setup used in the experiments is a custom made construction that is placed in a standard materials testing device. Providing a sample holder and equipped with an external displacement sensor this construction allows creep and relaxation experiments to be conducted on cartilage samples.

#### 3.3.1 Materials testing device

The Zwick Z010 (Zwick GmbH & Co, Germany) is a materials testing device, normally used for tensile and/or compression tests on metals and synthetic materials. Its construction consists of a vertical placed frame that guides a horizontal placed traverse. Controlled by a microcomputer it can be programmed using the supplied testXpert software (Zwick GmbH & Co, Germany). This machine is used to measure force and displacement applied to a sample. As the design of the whole machine is modular it can be adjusted to specific needs for a certain experiment. Three different control modes are available: position controlled, force controlled and strain controlled.

#### 3.3.2 Digital length gauge

Because the thickness of the articular cartilage layer varies from 0,8 – 2,2mm a high-resolution displacement measuring system is needed in order to measure small deviations in inflicted strain. Displacements dealt with in creep or relaxation experiments are very small 0-500µm. The digital displacement sensor or digital length gauge system is a Heidenhain MT-12. Capable of measuring linear displacement with an accuracy of 0,5µm
this sensor replaces the length gauge system embedded in the Zwick Z010 during the actual measuring stage of the experiments. Connected to the Zwick Z010’s control box the distance ($\Delta l$) is measured between the rigid base of the setup and the cartilage surface (Fig. 3.3a). Due to limitations of the materials testing machine it is only possible to use the MT-12 to control the traverse position in strain-controlled situations.

### 3.3.3 Used force cells

Force cells are used to record any applied forces by the materials testing machine. Available in various types they each have their own advantages, the force cells used during these experiments have a maximum capacity of 20N and 2.5kN. The 20N force cell (type: KAP-S, A.S.T. Angewandte GmbH) has a high resolution and can provide a very accurate reading of the applied force during creep experiments. For applying overloading, 25N and up, the 2.5kN force cell (type: KAF-S, A.S.T. Angewandte GmbH) is used. This allows the force to be higher than the maximum force of the 20N force cell, and has the advantage to be much stiffer which allows us to apply the desired force at higher impact speeds. A disadvantage to using the 2.5kN cell is that there is a certain risk of overshoot during when applying the force at higher speeds (0.8-1 mm/s). Because the traverse of the materials testing machine cannot stop instantly there will always be a certain overshoot. This results in higher overshoot forces when using stiff, heavy load force cells.

### 3.3.4 The sample setup

The basis of the used setup (Fig. 3.3) in the experiments is an aluminum plate fitted with a sample holder and a vertical mounted rail that provides a rigid support for the linear movable parts in the setup. A vertical rod, placed in a stainless steel housing, is the moving component that performs the indentation. The digital length gauge records the travel of the vertical rod. Specific demands for this setup where that the osteochondral plug can remain in the setup during a series of experiments without shifting. Compression would have to be applied exactly concentric with the plug and perpendicular to the cartilage surface.

![A schematic representation of the sample setup used in the experiments.](image1)

![A picture of the sample setup during an actual indentation experiment.](image2)
**METHODS**

**Sample holder**
A stainless steel cup forms the base of the sample holder; it has a V-shape like receiver that holds the osteochondral plug in place in combination with spring pressure (Fig. 3.4). Resistant to the corrosive properties of saline solution it is capable of keeping the osteochondral plug hydrated to up to 8 hours of experimenting without refreshing the EBS. The V-shape receiver ensures the concentric placement of the sample with the vertical rod.

![Fig. 3.4 the sample holder](image)

**The membrane springs**
The vertical rod that holds the indenter or compression plate has to be positioned and guided concentric with the osteochondral plug and perpendicular to its surface. In order to accomplish this, 2 membrane springs (Fig. 3.5) provide the connection to the housing; they ensure concentric linear movement and block radial degrees of freedom.

![Fig. 3.5 a single membrane spring](image)

The spring characteristic of the membrane springs, in combination with the force coming from the digital length gauge, have an influence on the data resulting from the experiments. Figure 3.6 shows the forces working on the vertical rod, and the formula shows how to calculate the actual applied force on the articular cartilage. Figure 3.7 shows the characteristic of the 2 membrane springs in combination with the downward force of the digital length gauge. The mean spring constant calculated from this curve is 4,796 N/mm. As can be seen the curve is not exactly linear, but due to the small displacements dealt with in the performed experiments it is sufficient to take the mean spring characteristic of the range used in the experiments (0 – 500µm).

![Fig. 3.6 Forces working on the vertical rod](image)

\[
F_{RS} = F_{FC} - k_{SPRING} \times f
\]

- \(F_{RS}\) = Reaction force sample
- \(F_{FC}\) = Force recorded by force cell
- \(k_{SPRING}\) = Spring constant
- \(f\) = Displacement

![Equation](image)
Linear stage
When performing a creep or relaxation experiment, it is necessary to have the indenter placed on the cartilage surface of the osteochondral plug, before applying a certain force or strain. For this reason the setup is equipped with a linear stage (Newport UMR 3.5) that is mounted between the rail carrier and the membrane spring housing. The linear stage allows accurate linear travel driven by a micrometer or a fine thread set screw. First the rail carrier is moved downward positioning the indenter 1-2mm from the cartilage surface. By gently turning the setscrew of the linear stage the exact positioning of the indenter is accomplished.

Mounting point of the digital length gauge
In the original design of the setup that was finished at the start of this research, the mounting point for the digital length gauge was connected to the rail carrier (Newport CXL48-50) as seen in figure 3.8a. By doing this the distance of the indenter relative to the steady base can be measured. Accurate positioning of the indenter or compression plate was impossible because there was only visual confirmation of when the indenter touched the cartilage surface. This was reason to relocate the mounting point of the digital length gauge to the membrane spring housing as seen in figure 3.8b. This resulted in a situation in which the indenter can be positioned on the cartilage surface by moving the linear stage. When the indenter comes in contact with the cartilage surface, the digital length gauge registers the displacement of the vertical rod.

Fig. 3.7 A plot of the membrane spring characteristic

Fig. 3.8: a) Mounting of the digital length gauge in the original situation. b) Mounting of the digital length gauge in the current situation.
3.4 Preconditioning
Preconditioning is a procedure in which a compression load is applied in a non-destructive manner on the osteochondral plug. By doing this the reproducibility increases for the cartilage is relieved of the stresses that remain in the structure resulting from the preparation of the osteochondral plugs. In this condition the cartilage is weaker which results in a larger deformation. Every sample used in experiments was subjected to an unconfined compression of 5x2N. This load has to be applied within 20 s or so. After preconditioning the sample was allowed to equilibrate for 15 – 30 min without any surface contact.

3.5 Indentation
During an indentation experiment (Fig. 3.9), the articular cartilage is compressed locally by an impermeable indenter with a radius of 2mm. Fluid is free to exude from the ECM in any direction. After loading is applied the cartilage deforms resulting in stresses and strains in the ECM. In time fluid will flow and out allows the strain in the ECM to increase. At the same time relaxation of the collagen fibrils occurs, which will result in a decreased stiffness of these fibrils. This also makes it possible for strains to increase. Strain will become constant when equilibrium is reached between the external load and the fully relaxed matrix.

By performing indentation before and after applying excessive loading, a change in maximum strain should be noticeable, due to the fact that articular cartilage gets weaker as a result of local damage to the ECM.

Each of the osteochondral plugs was preconditioned at 5x2N, loaded with an initial force of 0.8N in 20s and left to equilibrate for 1200s, and an additional force of 0.4N was applied in 10s and left to equilibrate for 1200s. An exact description of the used protocol can be found in appendix A.

3.6 Unconfined compression
During an unconfined compression experiment (Fig 3.10), the articular cartilage is compressed by an impermeable plate and left to equilibrate. Fluid is free to exude from the side of the articular cartilage. Unconfined compression takes much longer to equilibrate after applying a force, compared to indentation, due to the fact that water can’t flow out as fast as in the indentation experiments. The restricting factor in fluid exudation is that water can only flow out through the sides of the sample.

The information needed from unconfined compression experiments was the material properties of healthy articular cartilage. Hence, to save time unconfined compression experiments were only performed before applying damage. Furthermore damage applied by an indenter is approx 2% on the cross-sectional area of a sample. It is assumed that it is...
very hard to see, or cannot be seen at all in data coming from unconfined compression experiments.

Each of the osteochondral plugs was preconditioned at 5x2N, loaded with a force of 3N in 20s and left to equilibrate for 240min, and an additional force of 1.5N was applied in 10s and left to equilibrate for 240min. An exact description of the used protocol can be found in appendix A.

![Image of compression experiment](image.png)

**Fig. 3.10 A schematic representation of an unconfined compression experiment.**

### 3.7 Applying overloading

Since the 20N force cell was found not able to apply a sufficient high force to cause cartilage damage, the 2.5kN force cell was used during the overloading stage of the experiments. An advantage of this heavier force cell is that it allows the overloading to be applied at a much higher rate. The fact that the 2.5kN force cell is stiffer it needs less displacement to reach a certain force. This higher loading rate will make the damage more resembling to impact damage.

Overloading that intended to result in cartilage damage without crack formation, was applied with an indenter (r=2mm) using the 2.5kN force cell. Cartilage damage is suggested to occur using 5 cyclic loads of 25 to 27N.

### 3.8 Operating the setup

Problems encountered during relaxation experiments made it impossible to continue with these without modification of the setup. The main reason that relaxation could not work was the materials testing machine’s limitation in correcting the traverse with the accuracy needed in this type of experiment. Operating in a strain controlled situation the materials testing device has to be able to correct the movements recorded by the digital length gauge with an accuracy of 0.5µm. But caused by the high overall stiffness the slightest movement of the traverse resulted in an unacceptable high deviation of the measured force. With no solution for this problem at hand, and the high need for experimental data, there was no other option but to start with force controlled creep experiments.
Chapter 4 RESULTS

4.1 Reproducibility
To determine the accuracy of the setup, a creep indentation experiment was performed on twice on a single osteochondral plug. The same was done on a different sample using a creep unconfined compression experiment. The performed experiments are carried out on the same day to prevent any influence caused by aging. During these experiments the osteochondral plugs are subjected to the same forces that will be used during actual experiments.

![Fig. 4.1: a) Results from the indentation reproducibility experiment. b) Results from the unconfined compression reproducibility experiment.](image)

The indentation experiment (fig. 4.1a) reaches equilibrium after approximately 600s. It has a deviation of 0.14% strain at t=1500s and 0.49% at t=3000s. The unconfined compression experiment (fig. 4.1b) does not reach equilibrium. It has a deviation of 0.28% strain at t=1500s and 0.17% at t=3000s. Between the unconfined compression experiments the osteochondral plug came out of the sample holder. Placing it back in the sample holder resulted in a vertical shift of the second plot. Hence, it was decided to put the end points of both plots together.

4.2 Indentation experiments
Out of the 13 indentation experiments performed on healthy articular cartilage 7 came out as reliable. The maximum standard deviation of the strain plot is 0.376% at t=1000s and 0.594% at t=2000s (Fig. 4.2a). The maximum standard deviation of the force is 0.0179N at t=1000s and 0.0309N at t=2000s (Fig. 4.2b). The average time to reach equilibrium in the performed creep indentation experiments is approximately 600s for each load application. The drop in force after load application is 0.28N or 80.4% for the first step, and 0.168N or 46.9% for the second step. Note the large drop in the force-time plot (Fig. 4.2b) resulting from the influence of the membrane springs; this indicates that it’s not a true creep experiment because the force is not held constant.

![Fig. 4.2: a) Mean strain + standard deviation of indentation experiments on healthy cartilage. b) Mean force + standard deviation of indentation experiments on healthy cartilage.](image)
4.3 Unconfined compression experiments
A total of 5 unconfined compression experiments were performed. The standard deviation of the unconfined compression experiments was very high (Fig. 4.3). Most samples did not reach equilibrium after left to creep for 240 min, though strains rates are very low after approximately 120 min.

Fig. 4.3: Results from the unconfined compression experiments.

4.4 Applied overloading
The intended process of overloading consisted of 5 indentations where force of 27 N is applied in 0.3-0.5 s (Fig. 4.4a). Actual applied forces varied from having minor overshoot (Fig. 4.4b), to minor overshoot and some instability (Fig. 4.4c). Some of the cases showed major instability (Fig. 4.4d) of the materials testing machine. This would cause the materials testing machine to stop the experiment due to excessive deviation from the desired path. Experimental data that encountered overloading like in figure 4.4d where excluded from further processing.

Fig. 4.4: a) The desired load application to cause cartilage damage as programmed, b) The resulting force as actually applied having minor overshoot, c) Resulting force as actual applied showing minor instability at the 4th step, d) Failure during cyclic load application at the 2nd load application step causing the materials testing device to halt.
4.5 Damage results

The mean of the seven healthy cartilage indentation results are put out against two damaged cartilage indentation results. The deviation between the healthy and damaged indentation experiments is 0.326% at t=1000s for the first step. For the second step there is a deviation of 0.577% at t=2000s. As can be seen there is a difference between the two plots, but there is no indication for cartilage damage with any certainty.

![Graph showing damage results](image)

**Fig. 4.5** Results from healthy and damaged indentation experiments, representing the mean + standard deviation of 7 healthy indentation experiments, and the mean + standard deviation of two damaged indentation experiments.

So far 2 out of 10 experiments show change in creep behavior without crack formation in the cartilage surface resulting from overloading, the remaining 8 had cracks in the indentation pit.
Chapter 5 DISCUSSION

The influence of cartilage damage on the mechanical properties of articular cartilage was investigated in an in vitro setup. Tests consisted of indentation creep experiments before and after overloading. Unconfined compression creep experiments where performed to determine the mechanical properties of healthy articular cartilage.

Performed reproducibility experiments show the accuracy of the setup in an indentation and unconfined compression experiment. It has to be taken in account that there was only one experiment performed for each method. To increase the reliability and accuracy more experiments would have to be carried out.

The setup in its current form could not be used for its initial purpose of relaxation experiments. Modifications to the stiffness of the setup are needed to be able to meet accuracy of movements needed in a relaxation experiment. As a result creep experiments where performed. The first results where promising although it was known that they would be less comparable than relaxation experiments. The reason for this is that the force was not applied related to the cartilage thickness.

Experiments also show a high influence of the membrane springs, their negative force increases with increase of strain. This effect makes it impossible to apply a constant force on a viscoelastic material. Relaxation experiments are not bothered by this effect. In a relaxation experiment the displacement is constant; hence, the resulting negative force of the membrane springs is constant.

The accuracy of the 20N force cell and digital length gauge was proven sufficient. Reproducibility of overloading is very poor using the setup in its current form in combination with the 2,5kN force cell.

The sample holder did not align the osteochondral plug with the indenter. Its current design is too vulnerable for splinter formation resulting from cutting the subchondral bone. Therefore, the sample holder needs some modification to allow accurate placing of the osteochondral plug.

Preparation of the osteochondral plugs could use some improvements. During the drilling process there is a need for internal cooling of the diamond trephine. Cooling only the outside of the trephine is not enough. The inside of the cutting edge on the trephine gets cluttered with. Selection of a level surface of the articular cartilage was inaccurate and based on visual identification only. Thickness measurement using the coaxial illuminated microscope still isn’t ideal, though its accuracy is higher compared to previous used techniques. Another advantage of the current method is that each osteochondral plug can be measured individually. A problem with the coaxial illuminated microscope technique is to identify a distinctive border between the cartilage layer and the subchondral bone. Perhaps using color filters to increase the contrast between the cartilage and the subchondral bone this problem can be solved.

Remarkably an unconfined compression creep experiment can take up to over 4 hours to equilibrate, while an unconfined compression relaxation experiment takes only 1000s to equilibrate (DiSilvestro et. al., 2001). The reason for this long equilibration time could be after a displacement is applied in a relaxation experiment; reaction forces start high and equilibrate to the end value. After a while the decrease in force is non-existing or just can’t be measured.

After loading is applied in a creep experiment the cartilage deforms resulting in stresses and strains in the ECM. In time fluid will flow out allowing the strain in the ECM to increase. At the same time relaxation of the collagen fibrils occurs, which will result in a
decreased stiffness of these fibrils. This also makes it possible for strains to increase. Not until there is equilibrium between the external load and the fully relaxed matrix will the strains become constant.

The main reason for the large deviation in the unconfined compression experiments is most likely the non-level cartilage surface. This causes the compression plate not to be in contact with the entire cartilage surface as shown in figure 5.1. The best way to avoid this is to prepare samples out of the most level part of the tibia plateau. Generally this is the part covered by the menisci. A better way of identifying flat and level surfaces on the tibia plateau could also provide more useable osteochondral plugs, and reduce the deviation.

![Fig. 5.1 Non-level cartilage surface in unconfined compression](image)

No difference could be found in the results of indentation experiments performed before and after overloading that would indicate cartilage damage. The main reason for this is a lack of successful damaged cartilage indentation experiments. Exclusion of experiments had various reasons e.g. crack formation resulting from excessive overloading, aging of the cartilage or faulty sample preparation. Appendix B gives a summary of indications that suggest unusable cartilage samples.

The current setup does not produce a clear picture of change in creep behavior resulting from cartilage damage. Generally a weakened ECM suggests an increase in strain resulting from the same force as applied on the healthy cartilage. Comparison of individual creep experiments show this is not always true, as shown in figure 5.2a. The second indentation experiment shows a decrease of strain compared to the first indentation. There can be two causes for this. The first explanation would be that a pit remains in the cartilage surface resulting from the overloading. Incase there is a pit that remains from the overloading; the initial thickness of the cartilage will be less at the start of the second experiment. A decrease in initial thickness means there is less fluid in the matrix and relative more fibers per volume-unit. Hence, less water can be expelled from the ECM and so the ECM would behave stiffer than before damaging (Meessen, 2003).

A second explanation can be found in the influence of the membrane springs. The loading stage of the plot shows a decrease in stiffness of the cartilage (Fig. 5.2a). This increase in deformation speed tells us that the membrane springs in the setup are stretched faster. This means their negative reaction force increases at a higher rate. These negative reaction forces are subtracted from the force recorded by the force cell (Fig. 5.2b). Having done this it is clear that the true applied force at the point of maximum deformation is lower in the damaged cartilage indentation experiment. Lower true force means an increase in deformation of the articular cartilage. Hence it is likely that in fact the articular cartilage did get weaker resulting from the overloading.
**Fig. 5.2.** The resulting creep (a) and actual applied forces (b) resulting from a single indentation – damage – indentation experiment. The plot shows a decrease of 0.3 – 0.4% in strain and 0.004 – 0.01N in force for the damaged cartilage.

**Conclusions**

It can be concluded that:

- The accuracy of the setup in creep experiments is ± 0.2 - 0.5%.
- The accuracy of thickness measurement has increased but it is recommended to keep looking for more accurate methods.
- There is no distinctive change in shape of the strain-time plot of damaged articular cartilage relative to healthy articular cartilage using the current setup.
- Strain dependant reaction force resulting from the membrane springs make creep experiments impossible using a constant force.
- The current method for application of overloading is not accurate enough and has poor reproducibility.
- The sample holder could use improvements on alignment of osteochondral plugs that show splinter formation resulting from cutting.
- The preparation process for the osteochondral plugs is satisfactory but could use improvement on cooling during the drilling process, and identification of level surfaces on the articular cartilage.

Limitations of the setup that are of influence on creep experiments are of none concern when performing relaxation experiments. Hence, it is important to think of a way to bring down the stiffness of the setup and get the relaxation experiments to work properly. Suggestions and recommendations for improvements on methods are made in chapter 6.
Chapter 6 RECOMMENDATIONS

6.1 Reducing the stiffness of the setup
Reducing the stiffness of the setup will result in more travel of the crosshead of the materials testing device to reach a desired displacement on the articular cartilage. This way the accuracy of the moment is increased and relaxation experiments become possible. The overall stiffness can be reduced by application of an additional spring between the force cell and the vertical rod (Fig. 6.1). A detailed explanation of how the stiffness of the setup is reduced by application of an additional spring can be found in appendix C.

![Fig. 6.1 A schematic representation of the modified setup suitable for relaxation experiments](image1)

6.2 Accurate application of overloading
Accurate and reproducible application of an excessive load will increase the number of successful damage experiments. An alternative method is to apply the weight of a large metal block instantly on the cartilage surface (Fig. 6.2). This method is used and described by Meessen (2003) and presumed usable for impact loading. The essence of this method is that the block’s weight is carried either by the spindle or the cartilage. During the excessive load the digital length gauge can still record application the displacement.

![Fig 6.2 A schematic representation of the setup modified for impact loading.](image2)
6.3 Thickness measurement using surface a roughness scan

A new alternative method for measuring the cartilage thickness might be found in using a surface roughness scanner. The idea is to scan the contour of the osteochondral plug (Fig. 6.3a) and record the distance between the transition of bone to cartilage and the cartilage surface. The cut surface of bone has a much higher roughness than the smooth cartilage (Fig. 6.3b). Hence, the difference in roughness should be measurable. The very low pressure of the scanning needle is not likely to damage the cartilage in any way.

Using this method it should be able to measure the cartilage thickness with great accuracy, especially when multiple scans are made over the outline of each osteochondral plug.

![Fig. 6.3: a) A schematic drawing of what a surface roughness thickness measurement could look like. b) An example of what data obtained from the surface scanner could look like.](image)

6.4 Modifications to the receiver of the sample holder

A simple but effective way of modifying the sample holder to allow better positioning of the osteochondral plug is to modify the receiver (Fig. 6.4a). By chamfering the lower part of the receiving planes, space is created for the splinters that result from cutting the subchondral bone (Fig 6.4b).

![Fig. 6.4: a) The modified receiver of the sample holder showing the chamfered receiving planes. b) A representation of the osteochondral plug, with splinter formation, in the modified sample holder.](image)
6.5 Internal cooling of the diamond trephine
Cooling the inside of the diamond trephine might prove to be difficult. No modifications to the trephine and column drill can be made so a converter would have to be applied. Using a hollow converter that has a series of holes over the outline, in combination with a drag coupling might work (Fig 6.5).

Fig. 6.5 An assembly and an exploded view of the internal trephine cooler.
<table>
<thead>
<tr>
<th>Glossary</th>
<th>Anterior: Anterior; term uit de anatomie: naar de voorkant van het lichaam toe</th>
</tr>
</thead>
<tbody>
<tr>
<td>Articular cartilage:</td>
<td>Gewrichtskraakbeen</td>
</tr>
<tr>
<td>Bovine:</td>
<td>Van een rund</td>
</tr>
<tr>
<td>Calcify:</td>
<td>Verkalken</td>
</tr>
<tr>
<td>Cartilage:</td>
<td>Kraakbeen</td>
</tr>
<tr>
<td>Diamond trephine:</td>
<td>Diamant holboor</td>
</tr>
<tr>
<td>EBS:</td>
<td>Earle’s balanced salt solution; naast de moleculen die in een fysiologische zoutoplossing zitten bevat deze oplossing ook vitamine en mineralen.</td>
</tr>
<tr>
<td>Equilibrium:</td>
<td>Evenwicht</td>
</tr>
<tr>
<td>Exudation:</td>
<td>Uitscheiding</td>
</tr>
<tr>
<td>Indentation:</td>
<td>Indeuking</td>
</tr>
<tr>
<td>Indenter:</td>
<td>Letterlijke vertaling: indeuker. Element waarmee een materiaal samengedrukt kan worden</td>
</tr>
<tr>
<td>In vitro:</td>
<td>Methode van onderzoek buiten het levende organisme, in dit geval in het laboratorium.</td>
</tr>
<tr>
<td>Lateral:</td>
<td>Lateraal; term uit de anatomie: van het midden af, van het</td>
</tr>
<tr>
<td>Medial:</td>
<td>Mediaal; term uit de anatomie: naar het midden toe, naar het mediane vlak toe</td>
</tr>
<tr>
<td></td>
<td>Mediane vlak: vlak aangebracht door de lengte as. Deelt het lichaam bijna in gelijke helften. Ook wel symmetrievlak genoemd.</td>
</tr>
<tr>
<td>Osmotic:</td>
<td>Veroorzaakt door osmose; osmose: het verplaatsen van water door een permeabele wand van een plaats met een hoge concentratie van moleculen naar een plaats met een lage concentratie</td>
</tr>
<tr>
<td>Osteochondral:</td>
<td>Het bovenste weefsel van een gewrichtsvlak, met kraakbeen en onderliggend bot, ook wel ‘cartilage-on-bone’ genoemd.</td>
</tr>
<tr>
<td>Saline solution:</td>
<td>Zout oplossing met dezelfde zoutsamenstelling dan lichaamsvocht.</td>
</tr>
<tr>
<td>Posterior:</td>
<td>Posterior; term uit de anatomie: naar achter toe</td>
</tr>
<tr>
<td>Tibia-plateau:</td>
<td>Het gewrichtsvlak van de knie aan de bovenkant van het onderbeen.</td>
</tr>
<tr>
<td>Unconfined compression:</td>
<td>Onbegrenste samendrukking, het samendrukken van een materiaal tussen twee platen.</td>
</tr>
</tbody>
</table>
Literature


Bank, R., Soudry, M., Maroudas, A., Mizrahi, J., Tekoppele, J., *The increased swelling and instantaneous deformation of osteoarthritic cartilage is highly correlated with collagen degradation*, Arthritis Rheum., **43**: 2202-2210, 2000


Appendix A Creep measurement protocol

**Preconditioning**
1. Surface contact
2. Apply a cyclic load of 5x2N at 0.05 N/s
3. Remove surface contact
4. Re-equilibrate in EBS for 30 min

**Healthy cartilage indentation experiment**
1. Preconditioning
2. Surface contact
3. Apply a force of 0.8 N at 0.05 N/s
4. Equilibrate for 1200s.
5. Additional force of 0.4 N at 0.05 N/s
6. Equilibrate for 1200s
7. Remove force
8. Re-equilibrate in EBS for 45 min

**Excessive loading**
9. Replace 20N force cell with 2,5kN cell
10. Surface contact
11. Apply cyclic load of 5x27N at 0.8 mm/s
12. Remove surface contact
13. Re-equilibrate in EBS for 90 min

**Damaged cartilage indentation experiment**
14. Surface contact
15. Apply a force of 0.8 N at 0.05 N/s
16. Equilibrate for 1200s.
17. Additional force of 0.4 N at 0.05 N/s
18. Equilibrate for 1200s
19. Remove force

**Unconfined compression**
1. Preconditioning
2. Surface contact
3. Apply a force of 3 N at 0.05 N/s?
4. Equilibrate for 240 min.
5. Additional force of 1.5 N at 0.05 N/s
6. Equilibrate for 240 min.
7. Remove force
Appendix B Indications for unusable samples

The following images and represent indications for unusable samples and data resulting from experiments.

Cartilage swelling caused by faulty drilling speed or insufficient cooling during the drilling process

Crack formation resulting from excessive loading
APPENDIX B

Reduced creep behavior, indicating weak AC.

Large displacement during excessive loading (+/- 0.5mm is normal)

A relative long time to reach the load of 27N and that there is no overshoot, indicating weak AC.
Appendix C Detailed description of setup stiffness

Sample stiffness

Total setup stiffness:

\[ K_{\text{total}} = \left[ \frac{1}{K_{AC}} + \frac{1}{K_{\text{setup}}} + \frac{1}{K_{\text{force cell}}} + \frac{1}{K_{\text{bench}}} \right]^{-1} \]

Simplified:

\[ K_{\text{total}} = \left[ \frac{1}{K_{AC}} + \frac{1}{K_{\text{force cell}}} \right]^{-1} \]

*The stiffness of the setup and bench are left out because they are near incompressible and neglect able compared to the other components.

Adding an additional spring to reduce the overall stiffness results in:

\[ K_{\text{total}} = \left[ \frac{1}{K_{AC}} + \frac{1}{K_{\text{spring}}} + \frac{1}{K_{\text{force cell}}} \right]^{-1} \]

A very weak spring compared to the rest of the components will have a dramatic influence on the total stiffness.