Improvement of an experimental setup to perform relaxation tests on articular cartilage

Graduation report
D. van Doorn
BMTE04.26
June, 2004

Author:
D. van Doorn

Supervisors:
Ir. W. Wilson
Dr. C.C. v. Donkelaar
Ir. J.M.M.P. Rutten

Technical University Eindhoven
Eindhoven, June 2004
Graduation project at Fontys Hogeschoolen
Eindhoven
Improvement of an experimental setup to perform relaxation tests on articular cartilage

Author:
D. van Doorn

Supervisors:
Ir. W. Wilson
Dr. C.C. v. Donkelaar
Ir. J.M.M.P. Rutten

Technical University Eindhoven
Eindhoven, June 2004
Graduation project at Fontys Hogescholen
Eindhoven
Summary

Osteoarthritis is a joint disease, resulting in articular cartilage degeneration and thickening of the underlying subchondral bone. Since osteoarthritis is a disorder of the whole synovial joint organ, it is necessary to understand the normal behaviour of each tissue of the joint.

The goal of this project was to realise a relaxation test. To investigate the articular cartilage a Finite Element Analysis model (FEA) of this tissue based on a theory that includes the arcade-like collagen structure characteristic for articular cartilage has been developed by Wilson et al. (2003). The unknown material properties for this FEA model had to be acquired by performing relaxation tests. The problems in these tests had to be solved during this project.

The in vitro construction used in the experiments consisted of a custom-made indentation device that uses a material testing machine to apply a constant displacement. The displacement is measured by a digital extensometer and steers the material testing device. A 20N force cell registers force, caused by relaxation of the collagen fibers in the extra cellular matrix (ECM) and exudation of water from the ECM. By performing a relaxation test before and after applying damage to the articular cartilage, a possible change in mechanical properties can be determined. The tissue samples used in the experiments are osteochondral plugs of young bovine tibia plateaus.

According to Janssen (2004) to be able to perform a relaxation test the construction stiffness should be decreased. The stiffness was decreased by placing a spring between the materials testing device and the construction. The crosshead travel was increased and this had to result in more accuracy. The results didn’t meet the expectations, an accurate relaxation test still wasn’t possible.

The control parameters for the experiments were further examined without using the spring. After performing different kind of tests it could be concluded that under a position controlled experiment, a relaxation test was possible. The results were comparable with the relaxation test theory of DiSilvestro and Suh (2001). More tests have to be performed to test the reproducibility. Relaxation experiments before and after applying excessive overloading can be tested then.
Preface

This is a report about my graduation project of the study Medical Technology, a sub course of Mechanical Engineering at Fontys Hogescholen Eindhoven. This project is performed at the department of Biomedical Engineering TU/e. It was my task to gather experimental data about cartilage in order to verify a numerical model of articular cartilage that was designed by my supervisor Wouter Wilson, a PhD student at TU/e. The described subject is a follow-up on the work performed by a previous graduate student also coming from Fontys Hogescholen. I had to improve the method to measure the relaxation behaviour of articular cartilage. If there was enough time the method of damaging the cartilage and measure this influence on the relaxation behaviour of articular cartilage had to improve.

After four months of deepening my knowledge about a little aspect at this interesting and surprising tissue the testing construction has been totally examined. Relaxation tests are possible but still more research has to be done.

I want to thank all the people who made it possible for me to do this project. Especially I want to thank my supervisor Wouter Wilson who was willing to help me or tell me his critical view. I want to thank Rob van de Berg for his advices about the testing construction and realising this for the project. I want to thank my supervisor Dhr. Rutten for counselling me. Also I want to thank Tom Engels, a graduating student of Mechanical Engineering, for advising me with Matlab. Last but not least I want to thank my girlfriend Brechje Swüste for her support.
Index

SUMMARY ................................................................................................................................. 3
PREFACE .................................................................................................................................... 4
INDEX ......................................................................................................................................... 5
CHAPTER 1  INTRODUCTION ...................................................................................................... 7
CHAPTER 2  THEORY .................................................................................................................. 8
  2.1 ARTICULAR CARTILAGE ................................................................................................... 8
  2.2 COMPONENTS OF CARTILAGE ........................................................................................ 8
  2.2.1 Proteoglycans ............................................................................................................. 8
  2.2.2 Collagen .................................................................................................................... 8
  2.3 STRUCTURE OF ARTICULAR CARTILAGE ...................................................................... 9
    2.3.1 Superficial zone ...................................................................................................... 9
    2.3.2 Intermediate or transitional zone .......................................................................... 9
    2.3.3 Radial zone .......................................................................................................... 9
    2.3.4 Calcified zone ...................................................................................................... 9
  2.4 VISCOELASTICITY .......................................................................................................... 9
CHAPTER 3  METHODS ............................................................................................................. 15
  3.1 SAMPLE PREPARATION .................................................................................................. 11
  3.2 THICKNESS MEASUREMENT ....................................................................................... 11
  3.3 TEST CONSTRUCTION ................................................................................................... 11
    3.3.1 Materials Testing Device ....................................................................................... 11
    3.3.2 Construction .......................................................................................................... 12
    3.3.3 Displacement sensor ............................................................................................. 12
    3.3.4 Spring-part ............................................................................................................ 12
  3.4 PRECONDITIONING ........................................................................................................ 14
  3.5 INDENTATION TEST ....................................................................................................... 14
CHAPTER 4  RELAXATION TEST .............................................................................................. 15
  4.1 INTRODUCTION .............................................................................................................. 15
  4.2 TEST CONSTRUCTION WITH A SPRING ....................................................................... 15
    4.2.1 Testing with spring-part ........................................................................................ 15
    4.2.2 Testing construction without a sample ................................................................. 16
    4.2.3 Examination of the extensometer ....................................................................... 17
  4.3 THE SOFTWARE PARAMETERS ...................................................................................... 17
    4.3.1 Position controlled testing without sample ......................................................... 17
    4.3.2 Position controlled testing with sample ............................................................... 17
    4.3.3 Strain controlled testing without sample ............................................................ 18
    4.3.4 Strain controlled testing with sample ................................................................. 19
  4.4 CONCLUSION ................................................................................................................. 19
CHAPTER 5  RELAXATION TEST WITH THE ORIGINAL TEST CONSTRUCTION ...................... 15
  5.1 CALIBRATION ............................................................................................................... 20
  5.2 STRAIN CONTROLLED TESTING WITHOUT SAMPLE ................................................ 20
    5.2.1 Strain controlled testing with sample .................................................................. 20
  5.3 RELAXATION TEST ARTICULAR CARTILAGE ............................................................. 21
    5.3.1 Calibration and Measuring ................................................................................... 21
    5.3.2 Extensometer values compared to the crosshead travel ...................................... 22
  5.4 CONCLUSION ................................................................................................................. 23
CHAPTER 6  IMPACT LOADING .................................................................................................. 24
  6.1 PRELOADED SPRINGS ................................................................................................. 24
  6.2 SPIRE AXIS .................................................................................................................... 25
  6.3 A MASS ON THE VERTICAL ROD ................................................................................. 25
Chapter 1  Introduction

Articular cartilage (AC) forms a thin layer that covers the ends of long bones and is crucial for the functioning of normal synovial joints. It enables frictionless motion between the articular surfaces, transmits loads from one articular surface to the other, distributes loads within the joint and provides shock absorption. Articular cartilage is a biphasic material that consists of a solid and a fluid phase. The tissue consists of a collagen network, proteoglycans and water. As a result of this structure articular cartilage is viscoelastic. Viscoelasticity is a combination of viscous and elastic which results in time dependant force-strain behaviour. Once damaged, articular cartilage has limited or no ability to heal and often undergoes degeneration. A change in the mechanical properties of the articular cartilage is the first detectable sign of cartilage degeneration.

A numerical model may provide insight into the internal mechanics of articular cartilage. For this purpose Wilson et al. (2004) made a finite element model (FEM) of articular cartilage. This model is the first model that includes a realistic collagen structure and proteoglycan levels.

Relaxation tests have to be performed to get experimental data for validation of the FEM. To examine if a change in mechanical properties of articular cartilage occurs, a relaxation test before and after applying overloading has to be performed. Excessive overloading is used to damage the cartilage. In the project of Janssen (2004) these experiments were performed by a materials testing device. The forces weren’t reproducible and the loading time was too slow.

An in vitro test construction is developed by Meessen (2003) and Janssen (2004) to perform a relaxation test on articular cartilage. Unknown material parameters of the FEM-model had to be determined with this test. The construction in its current form could not be used for its initial purpose. According to Janssen (2004) modifications to the stiffness of the construction are needed to meet accuracy of movements needed in a relaxation experiment.

The first goal of this graduation project is to improve the current construction, so that it enables us to perform an accurate relaxation test. To reach this goal tests are done to examine parameters. To lower the construction stiffness a ‘spring-part’ is developed and tested, this will be described in chapter 3 and 4. Also additional experiments are performed with the current construction (Chapter 5). Experiments are performed on osteochondral plugs of articular cartilage taken from a young bovine tibia plateau.

Another goal was to improve the excessive overloading to damage the articular cartilage. There was no time left to improve this method. In chapter 7, recommendations, a few improvement options are described.

The theory about cartilage and the methods to investigate this tissue are described in chapter 2 and 3. This information provides knowledge to understand the performed experiments better.
Chapter 2  Theory

2.1 Articular cartilage
Articular cartilage forms a thin layer that covers the ends of long bones and is crucial for the functioning of normal synovial joints. It enables frictionless motion between the articular surfaces, transmits loads from one articular surface to the other, distributes loads within the joint and provides shock absorption. In large human joints as the knee and the hip the thickness of the cartilage is about 2-4 mm, but it can be as thin as 0.02 mm in the ankle joint of the mouse. In the next paragraphs an overview will be given of the composition, structure, mechanical properties and degeneration process of articular cartilage.

![Figure 2.1: (a) A knee joint  (b) Articular cartilage in knee joint](image)

2.2 Components of cartilage
Articular cartilage consists of a sparse distribution of cells (chondrocytes) embedded in an extracellular matrix (ECM). It receives most of its nutrition from the synovial fluid by a process of diffusion and/or convection. The ECM of cartilage consists mainly of water with collagen molecules and negatively charged proteoglycans (PGs) (figure 2.2). The structural matrix molecules maintain and organize tissue fluid flow through the matrix. This interaction is essential for the cartilage material properties. The composition and maintenance depend on the biosynthetic activity of the chondrocytes.

![Figure 2.2: Schematic cartilage structure](image)

2.2.1 Proteoglycans
Proteoglycans (PGs) are large, complex biomolecules composed of a core protein to which one or more glycosaminoglycan (GAG) side chains are attached (figure 2.2). The proteoglycans have a considerable negative charge, this charge creates a high osmotic pressure in the matrix and binds the water compartment of the cartilage (65-80 of wet weight) which produces a hydrated gel. The swelling of this gel is restricted by the collagen network.

2.2.2 Collagen
Articular cartilage contains primarily type II collagen. Each collagen molecule is composed of three α-chains wound in a characteristic triple helical configuration. Collagen provides cartilage with its strength, and creates a framework that houses the other components of
cartilage. Collagen fibers do not offer large resistance to compression, however they are very strong in tension. The collagen fibers provide great resistance against swelling caused by the PGs and tensile stresses.

2.3 Structure of articular cartilage

Mature cartilage is an anisotropic material with four distinct zones in a vertical section and distinct matrix regions around the chondrocytes. The structure, composition and mechanical properties vary within the 4 different zones of articular cartilage (figure 2.4).

![Arcade model of Benninghoff](image)

*figure 2.4: Schematic structure of articular cartilage, the arcade model of Benninghoff*

2.3.1 Superficial zone

This zone forms the gliding surface of the joint. Collagen fibrils are densely packed, have a small diameter and are arranged parallel to the articular surface. PGs and collagens appear to be strongly interconnected, which may help to resist shear stresses produced by joint motion.

2.3.2 Intermediate or transitional zone

Collagen fibrils have a larger diameter and are more randomly arranged compared with the superficial zone. PG content is highest in this zone.

2.3.3 Radial zone

The collagen fibrils have their largest diameter and appear to be perpendicular to the subchondral bone. This zone has the highest PG content and the lowest water content.

2.3.4 Calcified zone

The cartilage is mineralized with crystals of calcium salts and has a low PG content. The collagens fibrils from the deep (radial) zone cross the tidemark and insert into the calcified zone providing a strong anchoring system for the tissue on the subchondral bone.

In human femoral cartilage, the superficial and the calcified zone each occupy about 5-10% of the total thickness and the intermediate and deep zones each about 40-45%.

2.4 Viscoelasticity

The definition of viscoelasticity is to respond to stress as if the cartilage is a combination of elastic solids and viscous fluids. There are two distinct mechanisms responsible for the viscoelastic behaviour of articular cartilage. The frictional drag force of interstitial fluid flow through the solid matrix and the time-dependant deformations of the solid matrix are causing the behaviour. The cartilage configuration prevents the free flow of water within the matrix and thereby provides the viscoelastic properties of the cartilage. Loading of the tissue, forces the water from the molecular domain driving the negatively charged chains closer together.
This action increases the local charge density, which obstruct a further water flow. Combined this leads to increased resistance to deformation by higher loading. Viscoelasticity has two distinct types of behaviour: relaxation and creep.

### 2.4.1 Relaxation
Cartilage indentation is a commonly used method to determine the mechanical properties of articular cartilage. A load is perpendicular applied to the cartilage surface through a solid indenter. A typical experimental technique for indentation testing is stress relaxation. In an ideal stress relaxation test, a constant displacement is imposed on the cartilage. The stress required to maintain the displacement decreases with time until a new equilibrium stress is obtained (figure 2.7).

![figure 2.5: The relaxation response of a viscoelastic material on a 2-step displacement excitation](image)

### 2.4.2 Creep
During creep experiments a force is applied to the articular cartilage and kept constant for a period of time. As a result of this force, 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 (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.

![figure 2.6: The creep response of a viscoelastic material on a 2-step displacement excitation](image)
Chapter 3  Methods

To determine the influence of damage on the relaxation behaviour of articular cartilage different kind of tests can be used. Some tests are able to create a displacement and force curve. Osteochondral plugs are prepared out of a calf tibia-plateau. After measuring the thickness of the cartilage layer, the samples (plugs) are loaded. Starting with a preconditioning, this is followed by an indentation test to determine the force curve at a constant displacement. After that the articular cartilage has to be damaged by overloading and then the same indentation test has to be done.

3.1 Sample preparation
The first step in the sample preparation is to get a bovine tibia-plateau from a local abattoir. For a good fixation the unnecessary tissue has to be removed. In between the steps the cartilage is drowned in 85%-saltsolution. Then the plateau is fixed into a precision device. By using a mini-level the straight and most level spots are determined.

With a column drill the samples can be drilled perpendicular to the cartilage surface. With a diamond trephine cylinders are drilled. They are released by sawing the bone in parts with a jigsaw. Now there are cylinders of bone with on top a cartilage layer. Using a diamond circular saw the cartilage and a piece of underlying bone is sawn off. This is the requested plug with the upper and lower planes parallel. The prepared plugs (figure 3.1) are stored in a saline solution for a maximum of 2,5 days before performing the experiments.

3.2 Thickness measurement
When performing relaxation experiments it is necessary to know the thickness of the cartilage layer. The initial thickness is required to calculate the displacement that will be applied. There are different methods to measure the cartilage thickness. According to Janssen (2004) the best current method is to use a coaxial illuminated stereomicroscope (Stemi 2000-c, Carl Zeiss). With this method the articular cartilage sample is placed perpendicular under a coaxial illuminated microscope. The border between cartilage layer and subchondral bone will be visible. The layer thickness is measured by using an eyepiece (10x, Carl Zeiss) enhancing the view 5 times with the coaxial illuminated stereomicroscope. This procedure was repeated 3 times over the outline of the osteochondral plug. The actual thickness can be calculated through correcting the enlarged view by 0.2 times the average measured values, units in mm.

3.3 Test construction
The test equipment used in the experiments consist of a custom made construction that is placed in a standard materials testing device. The construction is equipped with a sample holder and an external displacement sensor.

3.3.1 Materials Testing Device
The Zwick Z010 (Zwick GmbH & Co, Germany) is a material testing device, normally used for tensile and/or compression tests on metals and synthetic materials. The device consists of
a vertical frame that guides a horizontal crosshead. Controlled by a microcomputer it can be programmed using the supplied testXpert software. It measures force and displacement applied to a sample. Three different control modes are available: position controlled, force controlled and strain controlled.

### 3.3.2 Construction

It isn’t possible to perform a relaxation test on cartilage with only a materials testing device. A construction (figure 3.2) is placed in the device to make these experiments possible. The basis of the construction is an aluminium plate fitted with a sample holder and a vertical mounted rail that provides a rigid support for the linear moveable parts. On the vertical rail a moveable membrane spring housing is placed. This part ensures the straight loading on the articular cartilage sample. The vertical rod that holds the indenter has to be positioned and guided concentric with the osteochondral plug and perpendicular to its surface. To accomplish this, two membrane springs ensure concentric linear movement and block radial degrees of freedom. The sample holder has a V-shape receiver with a spring to keep the sample in place.

![figure 3.2: (a) A schematic representation of the test construction. (b) Picture of the test construction](image)

### 3.3.3 Displacement sensor

The displacements that are applied for the relaxation experiments are very small, 0 - 500µm. The material testing device cannot measure that small displacements very accurate and the crosshead travels far above the cartilage surface (figure 3.2). For the experiments an extensometer (Heidenhain MT-12) with an accuracy of 0,5µm is used. This extensometer replaces the length gauge system embedded in the material testing device during the measurements. 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 (figure 3.2).

### 3.3.4 Spring-part

According to Janssen (2004) the test construction was too stiff. The travel of the crosshead had to be longer, to increase the loading accuracy. To increase the travel a spring was put between the force cell and the membrane springs housing. After setting the free spring, a relaxation test was performed. The result was inaccurate.

During the test the spring was free to turn and bend. Incorrect results because of torque were the consequence. To check the spring behaviour, only the spring is compressed (figure 3.4a). A hysteresis (decrease because of torque) curve is shown.
This problem was solved by fixating the spring ends in cups. The spring together with the two cups will be referred to as ‘spring-part’ (figure 3.3). The lower cup can be attached on the vertical rod (figure 3.2) and isn’t able to move. To make sure that the force cell will compress the spring right in the middle, a centre hole was made in the upper cup. It is assured that the spring has no friction and has a very small loss through bending force.

**figure 3.3: The spring fixated in cups, the spring-part**

To check whether the hysteresis has disappeared or is negligible the spring-part is loaded. Only the spring-part is loaded and the displacement was measured by the crosshead travel. After the fixation in the cups the loss of force is negligible (figure 3.4b).

**figure 3.4: Behaviour of a spring before (a) and after (b) fixation in cups.**

To get a smooth displacement curve the stiffness of the spring had to be determined.

*Testing spring-part on relaxation*

It is tested if any relaxation occurs in the spring-part. This was done by compressing only the spring-part and measuring the force and the displacement of the crosshead (figure 3.5).

**figure 3.5: Relaxation test of the spring-part, displacement (left) and force (right) are displayed.**

The graphs show a constant displacement and a constant force. Hence, there is no relaxation.
3.4 Preconditioning

Preconditioning is a procedure in which a compressive load is applied in a non-destructive way on the cartilage sample. Because the cartilage is relieved of the stresses that remain in the structure as a result of the sample preparation the reproducibility increases. 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 was applied within 20 seconds. After preconditioning the sample was allowed to equilibrate for 15 – 30 minutes.

3.5 Indentation test

To be able to compare different samples, the strain is constant during a relaxation experiment. A standard percentage of the cartilage thickness will be indented. In that way samples can be compared. A spherical impermeable indenter with a radius of 2 mm indents the cartilage surface locally (figure 3.6). Fluid is free to exudate 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 out and the stress in the ECM decreases. At the same time the collagen fibrils relax, which will result in a decreased stiffness of these fibrils. Force will become constant when equilibrium is reached between the external load and the fully relaxed matrix.

\[ \Delta l \]

\[ l_0 \]

\[ \text{Indenter} \]

\[ \text{Osteochondral plug} \]

*figure 3.6: Indentation test on a osteochondral plug*
Chapter 4  Relaxation test

4.1 Introduction

In chapter 3 the test construction and the testing methods have been described. In this chapter the improvements to the current setup, to make a relaxation test possible, are discussed. First the most important parameters that influence the test will be discussed.

Preload
To ensure that all parts of the setup are in contact, a preload is applied. The influence of the preload magnitude and the travel speed will be examined.

Strain and Position controlled testing
Relaxation tests can be controlled by position or strain. According to Janssen (2004) position controlled testing won’t work because of incorrect displacement tuning. Both options will be tested.

Displacement
The relaxation test is based on the tests described by Disilvestro and Suh (2001). In that relaxation test first a 10% strain was applied and after reaching equilibrium an additional 5% strain was applied. For testing the method a displacement of 0.1mm is used, because the average articular cartilage thickness from the tibia-plateau is about 1.0mm.

Cycle speed
This parameter determines the loading speed. In most tests the cycle speed is set to reach the programmed displacement in 20 seconds.

Loop amplification
This parameter is the same as the P-value in a P-tuner. The higher the loop amplification, the faster the system responds. The stability will decrease with increasing loop amplification.

4.2 Test construction with a spring

According to Janssen (2004) the test construction was to stiff. The travel of the crosshead should be longer to increase accuracy. To increase the travel a spring-part was designed. In paragraph 3.3.4 has been described how the spring is fixated in cups. A relaxation test on articular cartilage was performed to examine the new test construction.

4.2.1 Testing with spring-part
The construction with spring-part was used to conduct the relaxation test described by DiSilvestro and Suh (2001). In figure 4.1 the results of the test are displayed.
The displacement shows an overshoot and has constantly been corrected. The loop amplification determines the system speed and therefore the correction speed. This all result in the unusable force curve. The force curve decreases linear, the cause of this decrease will be examined.

4.2.2 Testing construction without a sample
To determine if the force decrease is caused by the cartilage sample or the membrane springs housing a test is done without a sample. The membrane spring housing is clamped far above the sample holder (figure 3.2) and is then loaded like a normal relaxation test. In the graphs of figure 4.2 it can be seen that displacement is incorrect and a similar incorrect force curve appears.

Hence, the sample does not cause the incorrect results, it is likely that the problems are caused by the membrane-springs, extensometer or the beam that is used for measuring the displacement. Janssen (2004) has shown that the membrane springs does not cause relaxation or other strange effects. Another cause could be the influence of the programming software.
4.2.3 Examination of the extensometer

To examine if problems are caused by the extensometer, the functioning of this part is tested. The extensometer is placed on the beam under the membrane spring housing (figure 3.2), it functions with a little spring that causes a force (0.6 – 0.8 N). The influence of this force was examined by clamping the extensometer at different heights and repeating the same test. The curves were similar, only the magnitude of the forces changed as a result of the little force. Although the extensometer causes a little displacement of the membrane springs, it cannot be the problem.

4.3 The software parameters

Based on the results of the previous paragraph it could be concluded that the problem is in the software. Therefore different parameters will be tested.

In this paragraph the difference between position controlled and strain controlled testing will be investigated. The effects of different loop amplification values are tested in strain and position controlled tests. The new test construction is used without a sample.

4.3.1 Position controlled testing without sample

The cycle speed was programmed to reach 0.2 mm displacement in 40 seconds.

![Position controlled test without sample](image)

It is shown that the displacement and the force are both constant (figure 4.4). The displacement is reached in almost 150 seconds (figure 4.4). If the test is controlled by the crosshead instead of the extensometer this could be the cause of that problem. The extensometer should control it according the software. The crosshead travel wasn’t measured in these tests, in paragraph 5.3 the control will be examined.

4.3.2 Position controlled testing with sample

The same test is performed on an articular cartilage sample (figure 4.5).
Reaching the prescribed displacement again takes too much time. After reaching the programmed displacement the value still increases. Although the displacement increases the force is constant, it is creep instead of relaxation. This is an unusable result, it can be concluded that the software in combination with the new test construction cannot use position controlled testing for the relaxation test.

### 4.3.3 Strain controlled testing without sample

The same test only with strain control is examined. In the graphs of figure 4.6 problems still appear.

The displacement is reached within the programmed time, with a smooth transition (sharp is better) and a constant value. After a specific period the force decreases. The cause of this decrease has to be examined.

*Adjust loop amplification*

Better results are displayed when the loop amplification is decreased. The displacement curve is the same, but the force is also constant (figure 4.7). This is probably caused by a slower response of the system, due to the lower loop amplification.
4.3.4 Strain controlled testing with sample

The same test, with a sample, is performed (figure 4.8). If the loop amplification had a great influence at the previous test, the same incorrect results should apply in this test.

A smooth transition till the programmed displacement, a constant value and after a period a decrease of the force are shown. No relaxation of the cartilage can be detected. The test shows the same result as a test without sample, only the force value is higher. Decreasing the loop amplification in this test, also resulted in a constant force curve.

4.4 Conclusion

Position controlled testing seems to be the best option. The test without a sample is promising, a solution for the loading time has to be realised. Testing with a sample results in an inaccurate displacement and is unusable. Strain controlled testing with the new test construction isn’t possible. By decreasing the responding time of the system, due to the loop amplification, the test without a sample shows a correct result. Also the smooth transition to the constant displacement is not desirable.
Chapter 5  Relaxation test with the original test construction

The conclusion of the previous chapter was that the new testing method is unusable. To know what exactly happens during a test, the original construction and the different software parameters are examined. First the method to calculate the results is described.

5.1 Calibration

In chapter 3 the test construction is discussed. The membrane springs cause a negative force, which has to be subtracted from the measured value of the force cell. Two different methods are available to do this.

The first is to determine the spring constant of the membrane springs. The force has to be measured at a given displacement of the membrane springs. The constant can be determined by dividing the measured force through the displacement.

A better method to subtract the membrane springs force from the total force is to use a calibration curve. That way the total behaviour of the membrane springs and force cell will be compensated. Every experiment has to start with a test without a sample, the resulting curve is the calibration. The calibration force is subtracted from the measurement by using Matlab, the used calculations are given in Appendix A.

5.2 Strain controlled testing without sample

The tests in paragraph 4.3 described the effect of different parameters. Strain and position controlled testing both showed incorrect results. To examine what causes the inaccuracy, first the original construction is tested with strain control (figure 5.1).

![figure 5.1](image)

*figure 5.1: Incorrect displacement (left) and force (right) at strain controlled testing without a sample*

The displacement is continuously correcting and therefore the force curve isn’t usable. The control software cannot handle the test what results in the correcting displacement.

5.2.1 Strain controlled testing with sample

Although the previous result, the same strain controlled relaxation test on a cartilage sample is examined (figure 5.2).
The displacement is again continuously correcting. The testing method is not able to create a constant displacement and therefore the test was aborted.

5.3 Relaxation test articular cartilage

Although in Janssen (2004) this option was excluded, the test is examined with the acquired knowledge of position controlled testing. To prevent problems before measurement the test without a sample is performed (figure 5.3). The reaction force is determined by subtracting the calibration curve (figure 5.4). To examine how this method is controlled, the travel of the crosshead is also measured (figure 5.5).

5.3.1 Calibration and Measuring

The test is started with a calibration. It was important that the extensometer isn’t moved, paragraph 4.2.3 showed the clamp height influence. The measuring points (force) in the first second are removed, because the test has starting problems through the preload.

The displacements are reached within the programmed time. The displacement is constant at the programmed value during the calibration. During the measurement the displacement still increases after reaching the programmed value, for about half a minute and then is constant. This inaccurate displacement will be discussed later.
The calibration force curve shows a little relaxation, most likely caused by relaxation of the 20N force cell, and is constant then. A large relaxation is displayed during the measurement.

The calibration force curve is subtracted from the measurement force curve (figure 5.4).

![Figure 5.4: Reaction force articular cartilage](image1)

It can be concluded that after reaching the displacement the cartilage relaxates to about 15% of the maximum force. The curve shows that the cartilage is totally relaxated.

5.3.2 Extensometer values compared to the crosshead travel

To examine what controls this test, the travel of the crosshead is also measured. According to the software parameters the test should be controlled by the extensometer. In figure 5.5 the displacement measured by the extensometer and the crosshead is shown.

![Figure 5.5: Travel measured by crosshead and extensometer during calibration (left) and measurement (right)](image2)

The crosshead displays a larger displacement, it is not sure the crosshead magnitudes are reliable. The curves can be compared at geometry.

During the calibration the geometries are exactly the same. During measurement the displacement measured by the extensometer increases after reaching the programmed displacement. This implies that the test is controlled by the crosshead travel.
The only difference between calibration and measurement is the sample. It’s possible that through the reaction force of the sample, the influence of the extensometer preload is changed. This will be examined.

Otherwise it’s difficult to explain, because the crosshead does not move, the force cell does not move, the vertical rod does not move, the sample does not move, what reaction does the extensometer measure.

**5.4 Conclusion**

A correct relaxation test is possible, reproducibility has to be further examined. The values for displacement and force can be corrected for the (constant) difference in displacement. The reaction force value is realistic, but some inaccuracies have little influences on it.

The test starts with a little preload of about 0.025N, the membrane springs are a little bit displaced. The values also changes when more tests are done on the same sample. The increasing displacement after reaching the programmed (measurement) value has to be examined. In these tests a total test time of 300 seconds is chosen to do many tests for examining different parameters. According to DiSivestro and Suh (2001) the test has to run 1000 seconds after reaching displacement, before the cartilage is totally relaxed.
Chapter 6  Impact loading

To determine the change in mechanical properties of articular cartilage, the tissue has to be damaged. The method as used by Janssen (2004) did not have a reproducible loading for overloading and is too slow (figure 7.1). Overload was applied with an indenter \((r=2\text{mm})\). Requirements for damaging are an accurate force (5 cycles of 25N) and a fast loading (in range of milliseconds).

![figure 7.1: Force curve of overloading, too much time and overshoot are visible](image)

In this project there was no time left to realise improvements for the method of damaging the articular cartilage. Though there is worked on a better method. Three options will be described in this chapter.

6.1 Preloaded springs

The first improvement is based on springs. The properties of a spring can be used for impact loading. With a constant displacement and an accurate stiffness the force can be determined by multiplying these parameters.

A number of springs (in figure 6.1 four), with the same stiffness, have to be clamped with a prescribed displacement, realised by cylinders. The springs have a preload because of the clamping. The indenter has to be placed almost on the vertical rod (figure 3.2). Fast impact loading is applied when the springs will be loosened. To put the indenter off the sample, preloaded springs from the bottom have to be loosened. This system is sketched in figure 6.2.

![figure 6.2: Global (left) and schematic (right) example of the overloading system based on springs](image)

The part is difficult to realise because of the small spaces between the springs, the clamps and the indenter. It is likely that a very fast and accurate loading is possible with this system.
6.2 Spire axis

A part has to be placed on the membrane springs housing that covers a spring and ensures the straight travelling of the spring. The spring has to be placed on the vertical rod (figure 3.2). The overloading force can be determined with multiplying a prescribed displacement and the spring stiffness. The displacement will be reached by indenting the spring through a spire, the spire height is the displacement. The needed spire axis (figure 6.3) is placed on the spring cover before turning it. An electric motor drives on the spire axis and provides the fast loading. It is relative easy to realise. The speed of impact loading can be determined by the rotational speed.

![figure 6.3: Left a schematic drawing of the spring displacement through a spire, right an example of a spire axis](image)

6.3 A mass on the vertical rod

The third option is probably the best and the easiest to realise. A system, which can be attached to the crosshead and can bear a mass of 25N, has to be realised (figure 6.4). The crosshead of the materials testing device travels down and places the mass on the vertical rod (figure 3.2). The impact loading is very accurate because of the given mass. Also is the loading speed fast, the moment that the mass and rod touches is the loading time. The crosshead direction will be turned, so that the mass will be lifted off the vertical rod.

![figure 6.4: Impact loading by placing a mass on the vertical rod](image)
Chapter 7 Discussion

The first goal of this graduation project was to realise an accurate relaxation test. The material properties of articular cartilage could be determined with that test. The developed in-vitro construction was tested. Also tests to determine the influence of different parameters and to investigate the behaviour of different construction parts were performed.

The stiffness of the construction had to decrease to perform a relaxation test. This is done by using a spring to increase the travel of the crosshead. First a free spring was tested, hysteresis appeared at these tests. To prevent this problem the spring ends were fixated (paragraph 3.3). After fixation the spring-part didn’t show hysteresis and wasn’t relaxing. A correct relaxation experiment could still not be performed. Decreasing the stiffness by increasing the crosshead travel didn’t result in the desirable graphs. During tests to examine the control parameters, position controlled tests showed promising results.

The current construction was used to perform position controlled experiments. Testing a healthy cartilage sample showed a relaxation to about 15% of the maximum force. The programmed displacement was 0.1mm, which is about 10% of the cartilage thickness. DiSilvestro and Suh (2001) applied 10% strain with an additional 5%, the cartilage relaxed to about 10%. That’s why it can be concluded that the relaxation is reasonable for our experiment. The cartilage thickness wasn’t measured, hence, the strain couldn’t be determined, so that it wasn’t able to compare the results.

To test the reproducibility more tests have to be performed according to the programmed parameter values of DiSilvestro and Suh (2001). Although this positive result, some inaccuracies have to be examined. The displacement increases after reaching the programmed value during measurement. The cause of this increase has yet to be determined. It’s likely that the crosshead controls the test (paragraph 5.4.2).

The experiments showed that the membrane springs caused a relative large negative force. The calibration force at 0.1 mm displacement is about as high as the reaction force of the cartilage sample. The curves are in the same range, small errors can have a great influence on the results.

The increasing displacement after reaching the programmed value during measurement has to be examined. Anyhow, the consequence is a constant difference between the calibration and the measurement displacement. The increased displacement causes a higher negative force of the membrane springs. If the membrane springs behaviour is exactly linear, the difference can be compensated. Concluded from the results, it’s likely that the behaviour is linear. The percentage increased displacement can be subtracted from the reaction force curve.

In chapter 6 are three options described to improve the method for impact loading. Although it was a goal of this project, there was no time left to realise one of these solutions. In a next project this has to be done.

The performed tests have proven that a relaxation test is possible. The influences of different parameters are investigated precisely. The data from the results can be calculated to a realistic relaxation curve.
Literature


Burken van, C.G., *Collagen damage in articular cartilage*, Graduation project at Fontys Hogescholen Eindhoven and Technical University Eindhoven, January 2004


## Glossary

**Anterior**: Anterior; term uit de anatomie: naar de voorkant van het lichaam toe

**Articular cartilage**: Gewrichtskraakbeen

**Bovine**: Van een rund

**Calcify**: Verkalken

**Cartilage**: Kraakbeen

**Diamond trephine**: Diamant holboor

**Equilibrium**: Evenwicht

**Indentation**: Indeuking

**Indenter**: Letterlijke vertaling: indeuker. Element waarmee een materiaal samengedrukt kan worden

**In vitro**: Methode van onderzoek buiten het levende organisme, in dit geval in het laboratorium.

**Osmotic**: 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

**Osteochondral**: Het bovenste weefsel van een gewrichtsvlak, met kraakbeen en onderliggend bot, ook wel ‘cartilage-on-bone’ genoemd.

**Saline solution**: Zoutoplossing met dezelfde zoutsamenstelling als lichaamsvocht.

**Synovial**: Vloeistofachtige substantie in gewrichten, die voor de voedingsstoffen van het gewrichtskraakbeen zorgt.

**Tibia-plateau**: Het gewrichtsvlak van de knie aan de bovenkant van het onderbeen.
Appendices
close all

clear

files = [1 9 13 19 24 27 31];

for i = 1:size(files,2)
    eval(['[s',num2str(files(i)),'_t s',num2str(files(i)),'_f
    s',num2str(files(i)),'_x] = textread('''10-05-04 normal relaxation
test'',num2str(files(i)),'.tra''',''
    %f%f%f''',''delimiter''','''',''headerlines''',11);']);
end

load all

i       =   find(s9_t > 1);
s9_t    =   s9_t(i);
s9_x    =   s9_x(i);
s9_f    =   s9_f(i);

i       =   find(s13_t > 1);
s13_t   =   s13_t(i);
s13_x   =   s13_x(i);
s13_f   =   s13_f(i);

s9_f    =   s9_f - s9_f(1);
s13_f   =   s13_f - s13_f(1);
s9_f   =   interp1(s9_t,s9_f,s13_t);
s9_x   =   interp1(s9_t,s9_x,s13_t);
sig_f   =   s13_f-s9_f;
sig_t   =   s13_t;

figure
plot(sig_t,sig_f,'r-','linewidth',2)
xlabel ('Time [s]','fontsize',14)
ylabel ('Force [N]','fontsize',14)
title ('Reaction force sample','fontsize',16)
set(gca,'box','on','linewidth',1,'fontsize',12)
axis([0 120 0 0.1])

figure
semilogx(sig_t,sig_f,'r-','linewidth',2)
xlabel ('Time [s]','fontsize',14)
ylabel ('Force [N]','fontsize',14)
title ('Reaction force sample','fontsize',16)
set(gca,'box','on','linewidth',1,'fontsize',12)
axis([0 120 0 0.1])

figure
hold on
p1  =   plot(s13_t,s9_f,'b-','linewidth',2);
p2  =   plot(s13_t,s13_f,'r:','linewidth',2);
hold off
l1  =   legend([p1;p2],'calibration','measurement',1);
xlabel ('Time [s]','fontsize',14)
ylabel ('Force [N]','fontsize',14)
title ('Testing force calibration and measurement','fontsize',16)
set(gca,'box','on','linewidth',1,'fontsize',12)
axis([0 120 0 0.6])

figure
hold on
p1 = plot(s13_t,s9_x,'b-','linewidth',2);
p2 = plot(s13_t,s13_x,'r:','linewidth',2);
hold off
l1 = legend([p1;p2],'calibration','measurement',1);
xlabel ('Time [s]','fontsize',14)
ylabel ('Displacement [mm]','fontsize',14)
title ('Testing displacement calibration and measurement','fontsize',16)
set(gca,'box','on','linewidth',1,'fontsize',12)
axis([0 120 0 0.12])