Piezoelectric Coatings on Implants

Sample preparation and construction of test-equipment for in vitro experiments

Annakarin Olsson

LITH-IFM-EX--05/1408--SE
Diploma Work

Piezoelectric Coatings on Implants

Sample preparation and construction of test-equipment for in vitro experiments

Annakarin Olsson

LITH-IFM-EX--05/1408--SE

Supervisors: Prof. Ulf Helmersson, Plasma and Coatings Physics, IFM
Prof. Tommy Sundqvist, Medical Microbiology, IMK
Doc. Ola Wahlström, Orthopaedics and Sports Medicine; INR

Examiner: Ulf Helmersson

Linköping 1 June, 2005
Abstract
Implants are commonly used for orthopaedic and dental applications. There is however a problem with implants; they have a tendency to get loose after 10-15 years of usage. Bone that is not used will get weaker; this can be concluded from studies of people being immobilised or in microgravity. When an implant is put into bone, the surrounding bone does not experience any deformation and it will resorb. This is called stress shielding. Finally the implant will get loose. To avoid this problem we want to give electrical stimulation to the bone surrounding the implant. Electricity has been used before to stimulate bone, and it has been shown that immobilised bone can almost be maintained by using electric stimulation.

Piezoelectricity is a property of certain materials that make them generate electricity when they are deformed. When an implant is coated with a piezoelectric material, electrical stimulation can be achieved for the surrounding bone that is stress shielded.

In this diploma work, a test-equipment is built to stimulate cells. Cells will be grown on a piezoelectric plate that is bent by the test-equipment. Thus, the cells will be stimulated by both mechanical stress and electric potential since the piezoelectric material generates electricity when it is deformed. Piezoelectric samples and culture wells suitable for bending applications are prepared and tested in the equipment.

Some initial cell growth experiments have been performed to see that the material is suitable for cell growth.

Keywords
Piezoelectricity, Stress shielding, Bone growth, Implant, Coating
Abstract

Implants are commonly used for orthopaedic and dental applications. There is however a problem with implants; they have a tendency to get loose after 10-15 years of usage. Bone that is not used will get weaker; this can be concluded from studies of people being immobilised or in microgravity. When an implant is put into bone, the surrounding bone does not experience any deformation and it will resorb. This is called stress shielding. Finally the implant will get loose. To avoid this problem we want to give electrical stimulation to the bone surrounding the implant. Electricity has been used before to stimulate bone, and it has been shown that immobilised bone can almost be maintained by using electric stimulation.

Piezoelectricity is a property of certain materials that make them generate electricity when they are deformed. When an implant is coated with a piezoelectric material, electrical stimulation can be achieved for the surrounding bone that is stress shielded.

In this diploma work, a test-equipment is built to stimulate cells. Cells will be grown on a piezoelectric plate that is bent by the test-equipment. Thus, the cells will be stimulated by both mechanical stress and electric potential since the piezoelectric material generates electricity when it is deformed. Piezoelectric samples and culture wells suitable for bending applications are prepared and tested in the equipment.

Some initial cell growth experiments have been performed to see that the material is suitable for cell growth.
Acknowledgements

I want to thank my supervisors Prof. Ulf Helmersson, Prof. Tommy Sundqvist, and Doc. Ola Wahlström for answering all my questions and for interesting discussions.

I also want to thank technician Rolf Rohback for advice on the drawings for the construction and for producing the parts for the construction, and technicians Kalle Brolin and Thomas Lingefelt for good advice and help to find things and people.

The diploma work has been financed by the Materials in Medicine strategy field.

I am personally much appreciative to:

My mentor Åsa Dahl for advises on how to organise my work and the presentation, and all support and self confidence you have given me during the last year as my mentor.

My family and friends for supporting and believing in me at all times.

My partner Lars for your encouragement whenever needed, and for always being there for me.
# Table of contents

Chapter 1  Introduction........................................................................................................1
  1.1  Background.............................................................................................................1
  1.2  Purpose ..................................................................................................................3
  1.3  Delimitations .........................................................................................................3
  1.4  Conventions used in this thesis .............................................................................3
  1.5  Important words ....................................................................................................3

Chapter 2  Theory.............................................................................................................5
  2.1  Bone growth ..........................................................................................................5
    2.1.1  Bone’s constituents .......................................................................................6
    2.1.2  Cells ..............................................................................................................7
    2.1.3  Bone remodelling .........................................................................................7
  2.2  Stimulation of cell growth ...................................................................................8
    2.2.1  Mechanical stimulation ................................................................................9
    2.2.2  Electrical stimulation .................................................................................13
  2.3  Piezoelectric theory .............................................................................................16
    2.3.1  History .........................................................................................................16
    2.3.2  Piezoelectric response to deformation .......................................................16
    2.3.3  Materials .......................................................................................................18
    2.3.4  Poling ............................................................................................................20
    2.3.5  Piezoelectric equations ..............................................................................22
  2.4  Piezoelectricity and bone ....................................................................................27

Chapter 3  Design of bending equipment and culture dish samples ........................................29
  3.1  Four-point bend ....................................................................................................29
  3.2  Material requirements ..........................................................................................31
  3.3  Dimensions .........................................................................................................33
  3.4  Motor ..................................................................................................................33
  3.5  Culture dish samples ............................................................................................34

Chapter 4  Experimental setup .........................................................................................37
  4.1  Using the equipment .............................................................................................38

Chapter 5  Experiments and results ..................................................................................41
  5.1  Preparatory experiments ......................................................................................41
    5.1.1  Bending of microscope slides .....................................................................41
    5.1.2  Strain measurement ....................................................................................41
    5.1.3  Attachment of piezoelectric material .........................................................42
    5.1.4  Piezoelectric response to bending ...............................................................42
    5.1.5  Attachment of culture wells ........................................................................44
  5.2  Cell growth experiments .....................................................................................45
    5.2.1  Cell growth on un-stimulated piezoelectric material ................................45
    5.2.2  Cell growth on stimulated piezoelectric material ........................................46

Chapter 6  Discussion ......................................................................................................47
  6.1  Suggestions for further work .................................................................................47

References .........................................................................................................................49
Appendices ........................................................................................................................53
Disposition of report

Chapter 1 gives an introduction to the problem. The background to and the purpose of this diploma work are given.

Chapter 2 gives the theory on bone growth and how to stimulate it and theory about the piezoelectric phenomenon.

Chapter 3 describes the design and construction of the test-equipment and cell growth samples.

Chapter 4 gives a description on how to use the equipment and how to prepare the samples.

Chapter 5 describes the experiments performed and the results achieved.

Chapter 6 concludes the results and further work is discussed.
Chapter 1

Introduction

This diploma work is done at the Plasma and Coating Physics division at the Department of Physics and Measurement Technology in cooperation with the Faculty of Health Sciences at Linköping University. The outline is to use piezoelectric coatings on implants to give electrical stimulation to surrounding cells.

1.1 Background

Implants are becoming more and more general in orthopaedic and dental applications. During the year 2003, 12 693 hip arthroplastic operations were performed in Sweden. The total number of operations from 1979 to 2003 in Sweden is 229 031 [1].

One problem with implants is that after a long time of use; normally 10-15 years, the implant has a tendency to get loose. When this happen a complicated surgical operation with bone transplantation must be performed. After 10 years are 5 percent of the hip implant patients re-operated. The amount of people that need to be re-operated is, however, higher than 5 percent, but many people are too old or not healthy enough to go through this complicated operation. Another issue of the implant loosening problem is that young people that need a hip implant can be rejected operation, since it is most likely that they will have to replace the implant later in life. So, implant loosening is a problem that concerns both people that have an implant and people who need one.

Implants loosen because the implant itself supports almost all forces acting on the bone, with the result that the remaining bone around the implant does not experience any deformation. This phenomenon is called stress shielding; the bone is shielded from stress (deformation) by the presence of the implant. Wolff’s law, which can be summarised as “form follows function”, means that bone that is not used will get weaker and bone that is used will get stronger. This has been seen in studies of people that has been in space for a long time [2,3] or from people that has been in bed for a long time due to illness. Stress shielding will then, according to Wolff’s law, lead to bone resorption and finally implant loosening. Figure 1.1 shows x-ray pictures of hips, one with an implant and one without.

---

1 One other reason for implant loosening is possible; that is, that wear particles from the polyethylene (plastic) of the implant may contribute to a reaction of the bone tissue resulting in bone resorption and implant loosening. This aspect is not further considered here.
To avoid the implant problem that arises in the long term, some stimulation of the bone around the implant is required.

Bone is normally stimulated by mechanical deformation, which occurs naturally in bone whenever it is used. When an implant is present in the bone, the neighbouring bone cells cannot be stimulated by deformation, since the implant shields the bone from stress.

It is known that electrical potential also stimulates bone growth [4], and it is known from *in vivo* studies that bone itself generates potentials when it is deformed [5]. By making the implant piezoelectric\(^2\) an electrical stimulation will be present for the stress shielded bone cells, which might stop the resorption of bone around the implant. The electrical potential will vary with the deformation, meaning that high deformation of the implant will lead to high potential to stimulate the cells in that region. This potential shall mimic the natural electricity in bone.

\(^2\) Piezoelectricity is a property of certain materials that when they are deformed an electric potential is generated, see further section 2.3.
Cell growth studies will be done in parallel on three plates under different stimulation-conditions; these are:
- Mechanical and electrical stimulation
- Mechanical stimulation
- No stimulation
In this way the cell growth due to electrical stimulation can be separated from that caused by mechanical stimulation.

1.2 Purpose
The main purpose is to stimulate cells both mechanically and electrically, and to distinguish the contribution of each stimulation method. This is done by growing cells on a piezoelectric material that is exposed to deformation, thus causing both mechanical and electrical stimulation.

The aim of this diploma work is twofold; first to construct a test-equipment suitable to generate mechanical and electrical stimulation to cells, and secondly to prepare the piezoelectric material that the cells will grow on.

The research objective is to coat implants with a piezoelectric ceramic. This gives electric stimulation to the neighbouring bone, which can reduce the bone resorption caused by stress shielding.

1.3 Delimitations
This report will not deal with any aspects of how to use the implant or how to perform cell growth.

In section 5.1.3, where different glues are tested, only one test for each glue has been performed so there is no statistical certainty in the choice of glue. The same limitation is valid for the cell growth experiments discussed in section 5.2.1.

1.4 Conventions used in this thesis
Throughout this report references are given as numbers in square brackets. The reference list that starts at page 49 is sorted in order of appearance in the text.

Footnotes are given as exponents and the footnote text is at the bottom of the current page. Footnotes are used mainly for explaining complicated words and expressions, but also to give technical data on equipment used.

Appendices are used for long derivations etc and are located at the end of the report.

1.5 Important words
Some important words and abbreviations that are used throughout this report are pointed out and explained here.
In vivo – Latin for (with)in the living. This term normally refers to research performed on whole organisms, e.g. a human or an animal
In vitro – Latin for within glass. Research performed outside the living organism, e.g. cell growth
Trabecular bone – the spongious, inner part of bone
Cortical bone – the hard outer surface of bone
Stress – the ratio of force applied to a material and the area it is applied to
Strain – the ration of the length difference caused by the stress and the un-deformed length
Piezoelectric effect – application of a mechanical deformation gives an electric potential
Converse piezoelectric effect – application of an electric field gives a deformation
e.g. - for example
i.e. – that is
Chapter 2

Theory

The theory of bone growth and how to stimulate bone is given to get an understanding of the problem that is outlined in this diploma work. The piezoelectric theory is given to get some knowledge on how this kind of material works, and to understand why a piezoelectric coating can be a solution to the implant loosening problem.

2.1 Bone growth

Adult skeleton consists of two types of bone, cortical bone$^3$ and trabecular bone$^4$. In long bones, such as the femur, both types of bone are present; see Figure 2.1. Cortical bone forms the wall of the shaft, and trabecular bone is concentrated at each end.

![Figure 2.1 a) Illustration of cortical and trabecular bone in femur. b) Close-up of trabecular bone, c) close-up of cortical bone. [6]](image_url)

---

$^3$ Dense bone that forms the surface of bones

$^4$ Spongy bone that forms the bulk of the interior of most bones
Bone is a complex material that is continuously renewing. The renewing process and the constituent of bone, down to the cellular level, are described here and possible reasons for weakness in bone are discussed.

### 2.1.1 Bone’s constituents

Bone is a two-phase material. 65-70 percent of bone is composed of inorganic substances; almost all of this is hydroxyapatite, which is the mineral phase of bone. The chemical composition of hydroxyapatite is $\text{Ca}_10(\text{PO}_4)_6(\text{OH})_2$. The remaining 30-35 percent is organic and almost 95 percent of this part is a substance called type-1 collagen. Collagen is the crystalline matrix of bone, see Figure 2.2, and is composed from strongly aligned polar organic protein molecules. Collagen has a very high tensile strength, and is the major fibrous component of skin, bone, tendon, cartilage, blood vessels and teeth. Hydroxyapatite forms in the gaps of the collagen matrix, and the matrix mineralises.

![Collagen diagram](image)

Figure 2.2 a) Collagen is built up by chains of protein molecules. b) Collagen fibres form a matrix. c) I) Collagen molecule, II) Hydroxyapatite can form in the gaps, and mineralise the matrix. [7]
2.1.2 Cells

Cells are the structural and functional unit of all living organisms. This is a short overview of the cells responsible for bone remodelling; that is, osteoblasts, osteocytes, osteoclasts, and to some extent fibroblasts.

- **Osteoblasts**
  An osteoblast is a cell that produces osteoid [6]. Osteoid is a protein mixture, mainly type-1 collagen, which becomes bone when it crystallises. Osteoblasts arise when osteoprogenitor cells\(^5\), that are located near all bony surfaces, differentiate under the influence of different growth factors.

- **Osteocytes**
  Osteocytes are mature bone cells and are the most common cells in bone. It is a star-shaped cell that is formed as osteoblasts become trapped in the mineralised matrix. Their main function involves maintaining the bone tissue.

- **Osteoclasts**
  An osteoclast is a multinucleated cell that resorbes the mineralised bone matrix. Osteoclasts have two functions; they secrete acid, which dissolves calcified material, and they secrete hydrolytic enzymes, which digest the collagen matrix [3].

- **Fibroblasts**
  The fibroblast is the most common cell in the body. It exists in connective tissue, and can produce collagen. Fibroblasts are normally not involved in bone growth, but after a damage of bone there can temporarily be a large amount of fibroblasts in the bone.

2.1.3 Bone remodelling

Bone remodelling is a natural life-long process by which mature bone is renewed through the continual processes of bone resorption and formation. Different cells are responsible for resorption and formation as described in section 2.1.2. Observations have been done where a small single trabeculum\(^6\) of bone has been totally destroyed and replaced in a new orientation within 24 hours [5]. The normal remodelling rate is 2 to 10 percent of skeletal mass per year [8]. If this natural process of bone resorption and bone formation is disturbed it can lead to osteoporosis.

2.1.3.1 Osteoporosis

Osteoporosis is a bone condition in which the amount of bone is decreased, the strength of trabecular bone is reduced, and cortical bone becomes thin and porous. The result of this is that osteoporotic bones are very susceptible to fracture. Figure 2.3 shows a normal and an osteoporotic bone.

---

\(^5\) Mesenchymal cells; that is, adult stemcells that can differentiate into osteoblasts, chondrocytes, myocytes and adipocytes

\(^6\) A trabeculum is one beam of the spongious bone (trabecular bone).
Osteoporosis can be caused by long bed-rest due to illness or immobilisation, weightlessness, or from the use of plaster bandage after bone fracture. In these cases the bone does not experience much physical stimulation from deformation, which according to Wolff’s law\(^7\) is necessary for bone formation. This type of osteoporosis is called disuse osteoporosis.

Lack of hormones, such as oestrogen and testosterone, can affect osteoporosis. Osteoporosis is known to increase with age. The exact reason is not known, but it is probably a combination of too little exercise (deformation) and lack of hormones. Osteoporosis is especially common among women older than 50, probably due to the reduced hormone production caused by the menopause. For men the hormone reduction starts later.

The remodelling process can also be disturbed by implants since the implant will, just as a plaster bandage, act as a shield for the natural stress subjected to bone. The implant will support all forces, and the surrounding bone will not experience any deformation. Implants can therefore lead to osteoporosis in the surrounding bone.

Bone remodelling is further discussed by Sikavitsas et al [10].

### 2.2 Stimulation of cell growth

When considering the aspect of needs; bone can be compared to any manufactured product. Both bone and products require [11]:

- raw material; e.g. for bone: calcium, phosphate, amino acids, vitamins C and D
- functional machinery; e.g. for bone: mesenchymal cells and osteoblasts
- energy supply; e.g. for bone: mechanical, electrical, chemical

If any of these factors is absent or inadequate, the final product is non-existent or restricted in function.

\(^7\) Wolff’s law will be described in section 2.2.1.1.
In this diploma work the aspect of energy supply is considered; the other needs can be read about elsewhere [5]. The mechanical and electrical stimulations are the energy supplies considered here.

2.2.1 Mechanical stimulation

Stressing of cells by deforming the cell growth surface mimics the in vivo environment, since bone (and cells) in vivo experience stress from forces acting on the bone as it is used. It is known that bed rest for long time, immobilisation, weightlessness etc. will result in a decrease of bone mass. This is due to the reduction of deformation of the bone under these conditions. Stimulation by mechanical strain is therefore interesting and much research has been done in this field.

2.2.1.1 Wolff's law

In 1892 Wolff [12] proposed that trabecular bone is oriented to best resist the extrinsic forces acting on it. This can be expressed simpler as “form follows function” and is known as Wolff’s law. It means that the bone will change its architecture depending on the magnitude and direction of the forces exerted upon it. Bone that is not used will get weaker and vice versa. Figure 2.4 shows two cross sections of bone from dog, one that has been used as normal, and one that is from a limb that has been immobilised for 40 weeks.

Figure 2.4 The left bone sample has been used as normal, and the one to the right has been immobilised for 40 weeks. Big difference in the amount of trabecular bone can be seen. [6]

Wolff’s law can also be demonstrated from studies on bone that has not healed correctly, see Figure 2.5a. The forces acting on this mal-aligned bone, see Figure 2.5b, make new bone form on the concave side, and bone resorp on the convex side, resulting in a straightening of the bone. For children the bone can eventually straighten totally, as illustrated in Figure 2.5c.
Figure 2.5 Bone remodelling in response to stress. a) A bone that has not healed correctly. b) Bone resorbes on the convex side and forms on the concave side. c) Bone will eventually straighten. [6]

Wolff’s law is also valid for other tissues such as tendon, ligaments, fascia, cartilage, and arteries. It has been shown that it is the collagen in bone that responds to forces. In 1951 MacConaill said: “As iron filings are to a magnetic field, so are collagen fibres to a tension field” [5].

The load-induced bone formation is due to the fact that osteoblasts and osteocytes, as many other cell types, are able to induce a “load response” when subjected to even low levels of mechanical deformation. Osteoblasts and osteocytes have been shown to respond to deformation by releasing signalling molecules or by direct increase of bone formation and remodelling [13]. There are both short- and long-term changes that occur when cells are mechanically deformed; some examples are alterations in signalling, the rate and amount of protein synthesis, and the rate of cell division [14]. Mechanical deformation of bone is according to these results known as one “energy supply” for bone growth.

Since it is to complex to perform systematic in vivo studies of mechanical deformation of cells, it is relied on the use of in vitro methods. In this project will cells be mechanically stimulated by growing them on a plate that is exposed to bending. The bending method used is called four-point bend and is described in section 3.1. Some previous results from mechanical deformation of cells are given next.

2.2.1.2 Previous experiments with mechanical stimulation

Many types of experimental apparatus have been developed to study cells exposed to mechanical deformation. In many of these experiments the same deformation method as in this experiment is used, namely the four-point bend. Other methods used are cantilever bend, three-point bend, biaxial bend of a circular membrane, or hydrostatic pressurisation. This is not a detailed review of other experiments, but rather an attempt to get an overview of what that has been done, some available methods, and the approximate size of the stimulating parameters. Comparisons between studies are difficult due to differences in cell types, loading mechanisms, applied strain magnitude, frequency etc. Some of the given examples has not reported any cell growth results, and are just included on the basis of the construction of the
test equipment. For detailed information on each experiment, see the stated references.

Labat et al. [15] applied stress to a circular disc by cyclically pushing the centre of the disc. It produced a strain of about 400 microstrain in the centre of the disc. The strain cycle had a frequency of 0.5 Hz and it lasted for 6 hours. The materials used to grow cells on are porous alumina (Al$_2$O$_3$), porous hydroxyapatite (Ca$_{10}$[PO$_4$]$_6$[OH]$_2$) and a duplex hydroxyapatite-coated porous alumina ceramic (HA/Al$_2$O$_3$). The effects of strains on short-term cell viability, cell growth, ALP-activity\(^8\), and collagen biosynthesis were studied.

In the work by Sotoudeh et al [16] a collagen coated silicon membrane was used. It was subjected to sinusoidal biaxial strain with a magnitude corresponding to a 0 to 35 percent area change of the membrane. The frequency was 1 Hz and was applied for 16 h. Area changes from 15 to 35 percent demonstrated a change in a gene expression\(^9\).

Peake et al [13] has studied the influence of Ca\(^{2+}\) signalling in the upregulation of the early mechanical response gene c-fos. c-fos has been demonstrated earlier to be involved in bone remodelling from mechanical deformation. The culture is mechanically loaded at a strain of ~1000 microstrain and frequency of 1 Hz for 1800 cycles, equivalent to 30 minutes. It was shown that the Ca\(^{2+}\) ion gave a higher c-fos response after just one hour.

Brighton et al [17] exposed bone cells from newborn rats to cyclic biaxial strain. Strains of 200, 400 and 1000 microstrain at a frequency of 1 Hz were used. The duration time varied from 15 minutes to 72 hours. The results indicated that bone cells exposed to 400 microstrain proliferated, but cellular integrity may be compromised\(^10\). These results could be seen after only 15 minutes of applied strain.

Three dimensional collagen gel blocks was deformed at a magnitude of 200-40000 microstrain, and frequency 0-100 Hz as reported by Tanaka [18]. A piezoelectric actuator was used to control the deformation, which was done by compression or tension of the collagen gel. Different strain patterns are possible, such as sine and square wave and also arbitrary strain waveforms synthesized on a computer to mimic in vivo strain waveforms.

Mitton et al [19] has investigated the stretch response of cultured human eye trabecular meshwork cells by using simple uniaxial distension of rectangular silicon substrates. The cells were grown on the substrates and stretched to 10 % static strain (100000 microstrain).

Bottlang et al [20] used the four-point bend technique to bend silicon dishes with cells. It delivered strain levels in the range encountered by bone in vivo, typically several hundred to about 3000 microstrain, and frequencies from 0 to 30 Hz. Other very similar experiments have been performed by Pitsillides et al [21] and Jones et al [22].

Another experiment with deformation caused by four-point bend was conducted by Kaspar et al [23]. Strain was applied over two days for 30 minutes per day with a frequency of 1 Hz and a strain magnitude of 1000 microstrain. Intrinsic studies had been done before on different

---

8 Alkaline Phosphatase Activity – an indicator of osteoblast function

9 Gene expression is the process by which a gene’s information is converted into the structures and functions of a cell. Gene expression is a multi-step process that begins with transcription and translation and is followed by folding, post-translational modification and targeting. The amount of protein that a cell expresses depends on the tissue, the developmental stage of the organism and the metabolic or physiologic state of the cell. [24]

10 Some cells may be damaged by this procedure
strain magnitudes: 10000 microstrain was shown to stimulate proliferation of osteoblasts. From 40000 to 880000 microstrain there was no influence or a decrease in proliferation. The use of 1000 microstrain gave enhanced cell proliferation.

Implants can be made of titanium, and in the work by Winter et al [25] cells are grown on titanium plates that are bent. A four-point bend equipment is used and strains from ~200 to ~1000 microstrain are applied to the cell culture. Cells strained at ~1000 microstrain exhibited 21%-24% more protein but 45%-49% less ALP activity than cells strained at ~200 or ~400 microstrain. The bending took place for 4 hours with a 20 hours rest or for 24 h without rest, at a frequency of 1.5 Hz.

There are commercial equipments available to expose cells to strain. One example is Flexcell® [14] that has been available since the late 1980’s and was developed by Al Banes. This equipment applies strain to cells grown on flexible membrane plates that are bent by vacuum pressure.

2.2.1.3 Stimulation parameters

As can be seen from section 2.2.1.2 there are a number of parameters to control when stimulating something by bending. Many articles have been written on how to control these parameters to best stimulate cell proliferation. The influence of the parameters are difficult to separate from each other, e.g. frequency and number of cycles [26], since a change in one of them will give an other optimal value for the other. Some concluding guidelines are given next.

- **Deformation method**
  - Four-point bend, three-point bend, cantilever bend, biaxial bend of membrane, hydrostatic pressurisation etc.

- **Magnitude of strain**
  - Frost [27] has described a window of mechanical strain for bone in the range of 50 to 1500 microstrain. The lower limit is chosen from the fact that bone resorption occurs beneath 50 microstrain. The upper limit is the minimum effective strain above which bone undergoes modelling and changes its structure in order to reduce the local strains. The range from 50 to 1500 microstrain is therefore the adequate values for these experiments. It has been shown that only periostal osteoblasts\(^{11}\) are sensitive to strains within the physiological range. Osteoblasts from the haversian system (=osteocytes) do not respond except at higher, un-physiological strains [22].

- **Frequency**
  - *In vivo* animal studies have shown that the response of bone cells is dependent on frequency. In most of the previous experiments performed a frequency of 1 Hz is used. It has also been assumed that bone reacts to very high frequencies that are caused by physiologic tremor due to postural muscle activity [28]. It has been shown that high frequencies (~30Hz) combined with low levels of deformation gives a more effective stimulus to bone than low frequencies and high level of deformation [29].

\(^{11}\) Osteoblasts at the bone surface
• **Number of applied cycles**
  An *in vivo* animal experiment has shown that as few as four cycles of loading a day with physiological strain magnitudes were enough to maintain cortical bone mass, while 36 or more cycles increased bone mass [30].

• **Strain duration**
  Duration times from minutes to days have been tested, but no conclusions can be drawn on which time is better.

• **Strain pattern**
  For the previous experiments performed it is almost always the sinus pattern that is used. Strain patterns mimicking *in vivo* conditions can be simulated by computers.

### 2.2.2 Electrical stimulation

Another “energy supply”, the electrical energy, is evident knowing that bone can convert mechanical energy to an electrical signal [5]. In 1912, Gayda [5] demonstrated electrical properties of cartilage, tendon and bone. Natural electricity is generated in tissue due to electrochemical generation of potential difference by segregation of ions. External forces acting on the bone can obtain such ion-segregation. This phenomenon will be referred to as stress potentials\(^{12}\) and will be discussed in section 2.2.2.3. Figure 2.6 shows that electric potentials are generated when bone is deformed. The convex side develops a positive potential and the concave side a negative potential.

![Figure 2.6 Compressed areas develop a negative potential, tensed areas develop a positive potential.](image)

\(^{12}\) The expression stress potentials is taken from [5]
2.2.2.1 Previous experiments with electrical stimulation

In 1964 it was demonstrated that continuous direct currents of 2-3µA for 2-3 weeks caused a massive increase in osteogenesis about the cathode (negative pole) and, possibly, a suppression of bone formation at the anode (positive pole) [4]. These experiments were conducted by implanting battery packs in dog femurs.

Deformation of bone gives a biphasic electric pulse, as illustrated in Figure 2.7

![Electric response from bone, when deformed.](image)

In vivo studies were performed with pulses such as the left ones in Figure 2.7 externally applied [4]. It showed that symmetric biphasic pulses do not stimulate bone formation, but unsymmetrical pulses do. Alternating current is therefore not an effective stimulus. This indicates that electrical stimulation by piezoelectric material is good since the electrical pulses look just like the electric response of bone and are the pulses are not equal in size; see Figure 2.8.

Electrical stimulation was applied to immobilised bone of rabbit. Both square wave pulses of potential 10 mV, pulse duration 0.1 seconds, and frequency 5 Hz; and constant voltage of 0.65 V were used to stimulate two groups of rabbits. Results indicate that both methods are good to maintain bone when immobilised, but constant voltage indicates slightly better results [11].

A number of experiments with electrical stimulation of bone growth have also been performed on humans. Non-union of long bones has been healed in at least 70 percent of the cases by applying electricity to the bone. The gaps between the bone parts in these cases were at least 5 mm [31].

It is known that electrical stimulation can enhance bone formation, but it is not so easy to apply the electricity to bone in a controlled manor. Experiments have been done on humans

---

13 Bone generation
with electrodes going inside the body, but since electrodes inside the body can cause infections and other long term problems, it is not viable to use this method. Extramagnetic fields have also been used, but since it cannot be ruled out that the electromagnetic field can be harmful to some organs this is not a good method either. One solution to this is to use a material that generates electricity inside the body without any connection to the outside. A piezoelectric coating on the implant can fulfil this requirement since it will generate electricity as soon as the implant is deformed, i.e. as soon as bone is used. The amount of electrical stimulation will be automatically controlled; since areas exposed to a high degree of stress will generate more electricity to the surrounding bone, and vice versa. This will make implants mimic the natural electricity of bone to maintain the electrical stimulation of cells that is lost when an ordinary implant is used.

2.2.2.2 Stimulation parameters
The parameters to control when using electrical stimulation are magnitude of voltage, and frequency. Many experiments have been done on this field, but no conclusions on the parameter values can be drawn from these experiments, due to many different ways to apply the voltage.

2.2.2.3 Stress potentials
Stress potentials are based on the principle of mechanically induced charge separation. The stress potentials can arise as intramolecular or extramolecular transduction of mechanical to electrical energy. These two properties can explain why bone gives an electric response to deformation.

- **Intramolecular stress potentials**
  Intramolecular stress potential is the intrinsic charge separation caused by mechanical deformation of the atomic, ionic or molecular structure of crystalline materials. Stress potentials may arise intrinsically in materials with any of the following properties:
  - Piezoelectric
  - Pyroelectric
  - Ferroelectric-ferroelastic
  - Semi-conductor
  - Electrets.
  Bone has been reported to possess all these properties, but stress potentials in bone have generally been ascribed to piezoelectricity [5], but it is evident that all of the mentioned properties can contribute to the electrical response.

  Piezoelectricity is defined as the production of an electric potential in a material by applying mechanical stress onto it. Only materials with a lack of symmetry centre are piezoelectric. The deformation makes the positive and negative charges in the material separate and a potential is thereby generated. The physical piezoelectric phenomenon will be further discussed in section 2.3, and the issue of piezoelectricity in bone will be discussed in section 2.4.

- **Extra molecular stress potentials**
  Extramolecular stress potential is the result of electrostatic repulsion or attraction occurring at the surfaces of charged structures. It can be seen as a streaming potential that arises when ions, dipoles or charged bodies flow past sites of fixed charge. Streaming potentials occur in both vessels and tissue. When bone is deformed the charged liquid is
squeezed through charged pores in the tissue, and a potential change arises.

There is no doubt that both piezoelectricity and streaming potentials exists in bone. The actual source of electricity in bone is not important for this diploma work; it is enough to know that bone generates electric potentials and that bone growth is stimulated by electricity.

### 2.3 Piezoelectric theory

Piezoelectricity is defined as electricity generated by applying stress to a material with certain properties. The voltage generated is proportional to the stress applied, and the sign of the voltage changes as the deformation changes direction (compression vs. tension). There is also the converse piezoelectric effect that will make the material deform when a voltage is applied to it. For a material to be piezoelectric, it has to be:

- Non-centro symmetric
- Polar

Non-centro symmetric means that the material has no symmetry axis, and polar means that the material can be polarised; that is, the dipoles in the material can be rearranged.

#### 2.3.1 History

The piezoelectric phenomenon was first discovered in 1880 by Jacques and Pierre Curie. They discovered the unusual property of certain crystalline minerals, such as quartz, that when they were subjected to mechanical force the crystals became electrically polarised. The voltage generated was proportional to the applied force, and compression and tension of the crystal gave voltages of opposite sign. This was called the piezoelectric effect; the word comes from the Greek *piezein* which means to squeeze or press. The so-called converse piezoelectric effect, were the application of an electric field results in deformation of the crystal, was mathematically deduced from fundamental thermodynamic principles in 1881 by Lippman and confirmed experimentally by the Curies.

Real applications of piezoelectric materials came during World War 1, where quartz was used in sonars to detect the echo from the sound waves.

#### 2.3.2 Piezoelectric response to deformation

When a piezoelectric material is deformed a voltage pulse is immediately generated. The deformation gives a dislocation of charges from its equilibrium in the material, which generates an electric potential. If the material is maintained in the deformed state the voltage decreases (approximately exponentially) since the charges reorganise to a new equilibrium. When the deformation force is released, the displaced charges return to their initial “undeformed positions” and a pulse with opposite sign from the first is generated. The magnitude of the second pulse is less than for the first pulse, due to charge leakage. Figure 2.8 illustrates this behaviour.
Figure 2.8 The upper picture shows the deformation pattern that is applied to the material. The lower picture shows the schematic electrical response to deformation. The second pulse is always smaller than the first.

Figure 2.9 describes the resultant voltage-change due to application of compressive or tensile stress, parallel or perpendicular to the poling direction.

Figure 2.9 Electric response to deformation.

Figure 2.10 shows the result of applying voltage in the same direction as the polarisation or in opposite direction.

Figure 2.10 Mechanical response to electricity
The amplitude of the electrical potential generated in piezoelectric materials is dependent upon the rate and magnitude of the deformation. The polarity of the potential is determined by the direction of deformation. Piezoelectric materials undergoing compression/tension develop negative/positive potentials.

### 2.3.3 Materials

Piezoelectric crystals, such as quartz, can be found in nature and has been known to be piezoelectric since 1880. Nowadays it is also possible to manufacture piezoelectric ceramics by a poling process.

#### 2.3.3.1 Piezoelectric crystals

There are thirty-two crystal classes in nature, each built up by monoaxial and biaxial combinations of individual symmetry elements. Twenty of these thirty-two classes possess the piezoelectric requirements; that is, they are non-centrosymmetric and polar. The requirement of non-centrosymmetry can be understood by thinking of a symmetric crystal subjected to symmetric stress; Figure 2.11 illustrates this situation. If this system is rotated 180 degrees the crystal will still have its symmetry and so will the stress. The polarisation on the other hand will be opposite in direction. Since the rest of the system is as it was before the polarisation should also be as before, thus the polarisation must be zero. In the non-symmetric case the crystal is not identical after rotation, and thereby the polarisation is not zero.

![Symmetric crystal](image-a)

![Non-symmetric crystal](image-c)

![Symmetric crystal](image-b)

![Non-symmetric crystal](image-d)

Figure 2.11 The system consists of the crystal (A-B), the forces (F) acting upon it, and the polarisation (P) of the crystal. In b) the crystal has been rotated 180 degrees compared to a), the same is done with d) and c). The forces are the same in figure a) and b), that is $F_1 = F_2$. The crystal is also the same since it is symmetric, that is $A=B$. This means that the system in a) is the same as in b) except for the direction of the polarisation, which is why the polarisation must be zero ($P=0$). In c) and d) the crystal is not identical, which is why there is a polarisation present in this case.

Examples of piezoelectric crystals are: quartz$^{14}$, Rochelle salt$^{15}$, tourmaline$^{16}$, and rutile$^{17}$.

---

$^{14}$ SiO$_2$

$^{15}$ KNa$_2$C$_4$H$_6$O$_6$·4H$_2$O

$^{16}$ Na(Al,Fc,Li,Mg,Mn)M$_3$Al(Si$_6$O$_{18}$)(BO$_3$)$_3$·(OH,F)$_4$

$^{17}$ TiO$_2$
2.3.3.2 Piezoelectric ceramics

For many years piezoelectricity was presumed to be only a branch of crystal physics. In 1944 it was discovered that there are ceramics that can be made piezoelectric by the application of a strong electric field [32]. The process, called poling, is comparable to the magnetisation of a magnet. Since the piezoelectric effect in natural materials such as quartz, tourmaline etc is quite small; much effort was put into finding piezoelectric materials with improved properties. The first ceramic was Barium oxide-Titanium oxide and this showed a dielectric constant as high as 1100 [32]. This was a huge value since rutile (TiO₂) with a dielectric constant of 100 was the highest value known so far.

Ceramics can be manufactured by a firing process [33]. Once a roughly held together object (called a "green body") is made, it is baked in a kiln, where diffusion causes the green body to shrink. The pores in the object close up, resulting in a denser, stronger product. The firing is done at a temperature below the melting point of the ceramic.

A ceramic is a muddle of small, randomly placed crystals. Each individual crystal can be strongly piezoelectric, but the random orientations make the ceramic neutral as a whole. Poling must be performed to make the ceramic piezoelectric. Poling means that a strong electric field, in the order of kV/cm, is applied to the ceramic. This will make the individual crystals align in the direction of the field, and a retained polarisation will be present in the ceramic. Figure 2.12 illustrates the applied field and the alignment of the dipoles that the field causes.

![Figure 2.12 A ceramic with dipoles in an arbitrary direction is polarised by applying a strong electric field to it. The electric field is in the order of kV/cm. After the electric field is removed there is a remnant polarisation.](image)

Several ceramic materials can be made piezoelectric by poling, e.g. lead-zirconate-titanate (Pb(Zr,Ti)O₃) called PZT, lead-titanate (PbTiO₃), lead-zirconate (PbZrO₃) and barium-titanate (BaTiO₃). Some orthodox writers say that ceramics are not really piezoelectric but rather exhibit a polarised electrostrictive effect [34]. A material must be formed as a single crystal to be truly piezoelectric, but since it has the same outcome they will from now on in this report be considered as piezoelectric ceramics.

Polycrystalline ferroelectric ceramics such as barium titanate (BaTiO₃) and lead zirconate titanate Pb(Zr,Ti)O₃ are two of the most commonly used today. In Table 1 a comparison of the strengths of the piezoelectric effect between the piezoelectric crystals quartz and two ceramics can be seen. A high value means strong piezoelectric effect.
<table>
<thead>
<tr>
<th>Crystal/ceramic</th>
<th>Quartz</th>
<th>BaTiO$_3$</th>
<th>Pb(Zr,Ti)O$_3$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$k$</td>
<td>0.07-0.1</td>
<td>0.4-0.6</td>
<td>0.6-0.8</td>
</tr>
</tbody>
</table>

Table 1 Electromechanical coupling factor $k$ for different crystal and ceramics. $k$ measures the fraction of the electrical energy converted to mechanical energy when a crystal or ceramic is stressed or vice versa. Since the conversion of mechanical to electric energy is always incomplete, $k$ is always less than 1.

Ceramics manufactured from lead zirconate or lead titanate have higher sensitivity and can operate at higher temperatures compared to other ceramics. PZT materials can have very different properties depending on the proportion of zirconium and titanium in it, and depending on if any doping or impurities of other materials has been added into it. PZT is used in this diploma work, since it is a good piezoelectric material that is not too expensive and easily available. Later on, if it comes to in vivo experiments another material has to be used, since PZT contains lead and it will not be accepted to put into the body. For this purpose there is another piezoelectric material that is especially interesting, i.e. Na$_{0.5}$K$_{0.5}$NbO$_3$, which consists of biocompatible substances.

### 2.3.4 Poling

Poling is a process to make dipoles in a material align in a specified direction.

#### 2.3.4.1 Spontaneous and macroscopic polarisation

Piezoelectric materials can be built up in the perovskite structure [35]. Perovskite crystals have a chemical structure looking like ABX$_3$, where A and B are cations (positively charged ions, which have lost one or more electrons,) and X is an anion (negatively charged ion, which has gained one or more electrons).

At temperatures above a critical temperature, the Curie temperature$^{18}$, perovskite crystals often exhibit a simple cubic symmetry with no dipole moments. For temperatures below the Curie temperature crystals have been observed to have tetragonal, orthorhombic or rhombohedral symmetry; and thus a dipole moment due to displacements of the ions. The dipole moment gives a local polarisation but the directions of the dipoles are arbitrary and therefore the material has just a small or no overall polarisation. This is called a spontaneous polarisation.

A macroscopic polarisation can be comprehended by aligning all dipoles in the same direction. This is done by applying a strong direct current electric field, normally at a temperature just below the Curie temperature of the material. When the electric field is removed a number$^{19}$ of dipoles are locked into a configuration of near alignment. Now the material has an overall polarisation.

In all poling situations it is necessary to try to induce the maximum degree of domain reorientation for the lowest applied field and in the shortest time possible. This is because of

---

$^{18}$ The Curie temperature is the temperature at which the crystal structure undergoes a phase change from non-symmetric lattice (such as tetragonal) to symmetrical lattice (such as cubic). The dielectric and piezoelectric coefficients changes drastically as the phase change takes place.

$^{19}$ The number of dipoles that align depends upon the poling voltage, temperature and the time the material is in the electric field [36].
the risk of dielectric breakdown\textsuperscript{20} under high applied fields. The tendency for breakdown is accentuated by physical flaws, e.g. voids and cracks. Breakdown strength is also dependent on the thickness, shape and electron configuration of the ceramic \cite{32}.

2.3.4.2 Poling methods

Polarisation of a material can be done by putting the material in an electric field\textsuperscript{21} (dc) at a temperature below the Curie temperature $T_C$. This poling method is the most common one, but it has some drawbacks. It is limited by ohmic heating due to electronic conductivity, physical breakdown at flaws in the ceramic, and by the requirement of space charge migration within the crystal when domain rotation takes place \cite{36}.

Experiments have been done with application of both a direct and an alternating voltage \cite{36}. Results show that the poling process can be hastened, but it is not certain to have any effect on the maximum polarisation magnitude. In Figure 2.13 the experimental results on PZT can be seen with and without the ac field applied.

![Figure 2.13 The percentage of dipoles that has aligned after a certain time. Application of AC and DC field gives a faster polarisation, but it is not known if the amplitude will be higher. \cite{36}](image_url)

After the poling process the material is piezoelectric and application of a force results in a change in voltage measured over the material.

For the purpose of this diploma work, it was possible to buy piezoelectric plates from a manufacturer\textsuperscript{22}, so no poling has been performed within this project.

2.3.4.3 Depolarisation methods

Materials that already are piezoelectric can be de-poled to take away the piezoelectric effect. There are different ways to de-pole a material:

- Applying a reversed poling voltage

\textsuperscript{20} Dielectric breakdown refers to the destruction of a dielectric layer, usually as a result of excessive potential difference or voltage across it.
\textsuperscript{21} The electric field should be in the range of 1kV/cm ($= 0.1$ MV/m).
\textsuperscript{22} Ferroperm Piezoceramics A/S, www.ferroperm-piezo.com
- Increasing the temperature to above the Curie temperature of the material
- Inducing a large mechanical stress

In this diploma work it was necessary to depolarise some plates; this was done by heating the material to 250°C; this is described in section 3.5.

### 2.3.5 Piezoelectric equations

Piezoelectric equations can be derived on the basis of thermodynamic principles [37]. The equations deal with the linear relationship between stress $T$, strain $S$, electric field $E$, and electric displacement $D$. The derivation is given in Appendix A. Many piezoelectric constants are also derived there.

Piezoelectric materials are combining properties from the mechanical and electrical domain. A deformation of the material gives an electrical response, and electricity applied to the material will cause the material to deform. Some simple relationships for the mechanical domain and the electrical domain are presented next.

#### 2.3.5.1 Properties of mechanical and electrical domains

All materials are elastic to some degree. A force applied to a material will cause the material to deform to some extent. Figure 2.14 is one example of deformation caused by a force.

![Figure 2.14 Elongation of a beam. F is the applied force to elongate the beam. L is the initial length of the beam and ΔL is the elongation. A is the area of the cross section.](image)

For an arbitrary material that is elongated as in Figure 2.14 the following statements are valid for the stress and strain:

$$T = \frac{F}{A} \quad \text{and} \quad S = \frac{\Delta L}{L}$$

Within the elastic limits of a material the stress is proportional to the strain.

$$T = YS \quad (*)$$

This equation is known as Hooke’s law. The constant $Y$ is called modulus of elasticity or Young’s modulus.
For dielectric materials the following equations are stated:

\[
C = \frac{\varepsilon_0 \varepsilon_r A}{t} = \frac{\varepsilon A}{t}
\]

\[
Q = CV
\]

\[
C = \text{capacitance}
\]
\[
\varepsilon_r = \text{relative dielectric constant}
\]
\[
\varepsilon_0 = \text{dielectric constant of air} = 8.85 \times 10^{-12} \text{ F/m}
\]
\[
\varepsilon = \text{dielectric constant}
\]
\[
V = \text{voltage}
\]
\[
t = \text{thickness or plate separation}
\]
\[
Q = \text{charge}
\]

The dielectric displacement \(D\) is defined as

\[
D = \frac{Q}{A} = \frac{\varepsilon V}{t}
\]

and the electric field \(E\) is defined as

\[
E = \frac{V}{t}
\]

which gives

\[
D = \varepsilon E \hspace{1cm} (*)
\]

The *-marked equations in this section are valid for all materials that are dielectric, within the elastic range of the material. If the material is piezoelectric there is a coupling between these electrical and mechanical equations.

2.3.5.2 Direct piezoelectric effect

If a stress is applied to a piezoelectric material it will generate an electric displacement, \(D\), whose magnitude is proportional to the applied stress, that is

\[
D = dT
\]

where \(T\) is the stress applied to the material and \(d\) is a constant called piezoelectric constant.

Since the piezoelectric potential is dependent on the direction of the applied force, it is

---

\(^{23}\) For a material to be dielectric it has to be insulating and polarisable.

\(^{24}\) The electric displacement field is sometimes known as the "macroscopic electric field," in contrast to the electric field \(E\), which is analogously the "microscopic electric field." The difference is that the macroscopic field "averages out" the jumble of electric fields from charged particles that make up otherwise electrically neutral material.
necessary to use tensors. When a general stress, \( T_{jk} \), is acting on a piezoelectric crystal, each component of the dielectric displacement, \( D_i \), is linearly related to all the components of \( T_{jk} \), i.e.

\[
D_i = d_{i11}T_{11} + d_{i12}T_{12} + d_{i13}T_{13} + \\
+ d_{i21}T_{21} + d_{i22}T_{22} + d_{i23}T_{23} + \\
+ d_{i31}T_{31} + d_{i32}T_{32} + d_{i33}T_{33}
\]

By using the Einstein summation convention\(^{25}\) this can be written as

\[
D_i = d_{i,j}T_{jk}
\]

The general relationship is then

\[
D_i = d_{jk}T_{jk}
\]

The indices \( i, j \) and \( k \) each take the value 1, 2 and 3. This gives 27 piezoelectric coefficients that relate \( D_i \) to the nine possible \( T_{jk} \). Symmetry conditions in \( j \) and \( k \) gives only 18 independent coefficients. Hence the equation can be rewritten into

\[
D_i = d_{i,j}T_{j}
\]

where \( i=1,2,3 \) and \( j=1,...,6 \). \( T_1, T_2 \) and \( T_3 \) are the tensile or compressive stresses, and \( T_4, T_5 \) and \( T_6 \) are the shear stresses. The lattice structure of the material decides the piezoelectric constants. For centrosymmetric materials all \( d_{ij} \) components are zero, and the dependence of electrical polarisation upon mechanical stresses disappears, i.e. they are not piezoelectric.

2.3.5.3 Converse piezoelectric effect

Application of an electric field to a piezoelectric material will result in a stretch or a compression of the material. Stretch and compression can be referred to as strain (positive strain=stretch, negative strain=compression). For a piezoelectric material the strain, \( S \), is proportional to the electric field, \( E \); that is

\[
S = dE
\]

The same procedure as for the direct piezoelectric effect gives the tensor relation:

\[
S_i = d_{im}E_m
\]

where \( i=1,...,6 \) and \( m=1,2,3 \).

\(^{25}\) The Einstein summation convention means that when an index occurs more than once in the same expression, the expression is implicitly summed over all possible values for that index, e.g. \( M_{ij}v_{ik} = \sum_{i} M_{ij}v_{ik} \)
2.3.5.4 Piezoelectric constants

There are a large number of constants to consider when describing the properties of piezoelectric materials. Only the most common constants are given here.

**Piezoelectric constant, \( d \)**

\[
d = \begin{cases} 
\text{electric displacement generated per unit of mechanical stress applied} \\
\text{or mechanical strain experienced per unit of electric field applied}
\end{cases}
\]

**Tensor \( d_{ij} \)**

\[
i = \begin{cases} 
\text{direction of electric displacement generated in the material when the electric field is zero} \\
\text{or direction of the applied electric field}
\end{cases}
\]

\[
j = \begin{cases} 
\text{direction of applied stress} \\
\text{or direction of induced strain}
\end{cases}
\]

\[
D = dT \\
S = dE
\]

**Piezoelectric voltage constant, \( g \)**

\[
g = \begin{cases} 
\text{electric field generated per unit of mechanical stress applied} \\
\text{or mechanical strain experienced per unit of electric displacement applied}
\end{cases}
\]

**Tensor \( g_{ij} \)**

\[
i = \begin{cases} 
\text{direction of the electric field generated in the material} \\
\text{or direction of the applied electric displacement}
\end{cases}
\]

\[
j = \begin{cases} 
\text{direction of applied stress} \\
\text{or direction of the induced strain}
\end{cases}
\]

\[
E = -gT \\
S = gD
\]
Permittivity, $\varepsilon$

$\varepsilon = \text{dielectric displacement per unit of electric field}$

$\varepsilon^T = \text{permittivity at constant stress}$, $\varepsilon^S = \text{permittivity at constant strain}$

Tensor $\varepsilon_{ij}$

$i = \text{direction of the dielectric displacement}$

$j = \text{direction of electric field}$

$D = \varepsilon E$

---

Dielectric constant, $K$

$$K = \frac{\varepsilon'}{\varepsilon_0} = \frac{\text{charge stored on an electroded slab of material brought to a given voltage}}{\text{charge stored on a set of identical electrodes separated by vacuum}}$$

$K^T = \text{“free” dielectric constant} – \text{measured at constant (zero) stress}$

$K^S = \text{“clamped” dielectric constant} – \text{measured at constant (zero) strain}$

The free and clamped dielectric constants may differ greatly for strong piezoelectric materials. The difference is related to the electromechanical coupling factor $k$ by

$$K^S = K^T (1 - k^2)$$

For organic material the $K$ value is normally under 5 and for most inorganic material it is under 20. Piezoelectric ceramics however generally have much higher dielectric constants, typically several hundred to several thousand.

---

Elastic compliance

$s = \text{the strain produced per unit of applied stress}$

$s^E = \text{elastic compliance at constant electric field}$,

$s^D = \text{elastic compliance at constant electric displacement}$

Tensor $s_{ij}$

$i = \text{direction of strain}$

$j = \text{direction of stress}$

$s_{ij}$ and $s_{jj}$ are the reciprocals of the modulus of elasticity (Young's modulus)

---

Young's modulus, $Y$

$Y = \text{the stress applied to the material divided by the resulting strain in the same direction}$

$T = YS \quad (\text{Hooke's law})$
2.4 Piezoelectricity and bone

It has been known for a long time that bone is piezoelectric. In 1957 Fukada and Yasuda [4] demonstrated that bone exposed to deformation develops an electric potential. They found a direct linear relationship between polarisation and stress. This is the direct piezoelectric effect. They also demonstrated the so-called converse piezoelectric effect, where the material deforms when it is exposed to an electric field. In the same time Bassett [38] independently found the same results, but he did not publish them. Charge separation was demonstrated both in living and dead moist bone [4] from a cantilever experiment where the concave side always developed a negative polarity and the convex side a positive polarity. Figure 2.15 illustrates the potentials that arise when bone is exposed to cantilever bend. It was demonstrated in vivo that callus\textsuperscript{26} formed at the negatively polarised regions [39]. Piezoelectricity has been demonstrated in many different materials, such as tendon, tooth [4], dry wood, rayon, silk [40] etc.

![Figure 2.15 Potentials generated when bone is bend.](image)

All materials can be divided into different point groups due to their symmetry. The main components of bone are collagen and hydroxyapatite (HA). HA-structure belongs to a centrosymmetrical point group called $P_{6_3}$. Collagen belongs to point group 3, which is asymmetric. As can be read in section 2.3, materials have to belong to an asymmetric point group to be piezoelectric.

Dry bone was early demonstrated to be piezoelectric. Collagen is most probably the source of piezoelectric effect in bone, and it has been demonstrated that dry collagen is piezoelectric [41]. When it comes to hydrated collagen, it has been shown that at a 40 percent hydration the piezoelectric effect in collagen becomes non-existent [42]. The reason is that the water molecules form hydrogen bonds with the collagen molecules and thereby increases their symmetry. The piezoelectric effect decreases as collagen, or any piezoelectric material, becomes more symmetrical. This could indicate that wet bone is not piezoelectric, but it indeed is. Bone also contains hydroxyapatite crystals (HA), which are not piezoelectric due to a centro-symmetrical structure. HA is bond to the collagen as described in section 2.1.1. These bonds reduces the number of possible sites in collagen that water can bind to, and results in the fact that bone can not have a concentration of water higher than 26 percent [42]. At this concentration collagen is still piezoelectric. Although collagen by itself is piezoelectric, it is important to consider collagen and apatite together as the source of piezoelectricity of bone.

But, as has been described in section 2.2.2.3, it is not important whether bone is piezoelectric or not for this application. The important thing is that bone itself generates electricity when it is deformed as described in section 2.2.2, and that electricity stimulates bone. The potential

\textsuperscript{26} The first step of new bone formation
generated in bone when deformed has the same characteristics as the electricity generated from a piezoelectric material. A piezoelectric coating on the implant can give electricity to the surrounding bone that is stress shielded, and therefore, hopefully maintain the bone mass to avoid implant loosening.
Chapter 3

Design of bending equipment and culture dish samples

To study the response of cells exposed to mechanical strain and electricity, a bending equipment is built using the four-point bend technique. By deforming a piezoelectric plate an electric potential is generated; thus cells grown on a bending piezoelectric plate will experience both mechanical and electrical stimulation.

There are available bending equipments as described in section 2.2.1.2. But none of these are modifiable to use here for bending of a piezoelectric plate. They are too weak to bend the material and it is not possible to attach a ceramic plate on the bottom of the flexible cell culture dishes that are available. For these reasons a new equipment is built in this diploma work.

When constructing the equipment there are some criteria that are necessary to fulfil for this particular application:

- Dynamic and static applications of uniform, homogenous strain
- Control of the magnitude of strain, frequency of bending, etc.
- Operation inside an incubator
- Simultaneous stimulation of two samples; one piezoelectric and one non-piezoelectric
- Bending of a bulk piezoelectric material requires a stiff bottom plate; the equipment must be strong enough to bend it and precise enough not to break it
- Electric fields and heat radiation must be avoided not to influence the cell cultivation
- Growing cells require a well. This well must be able to resist (not break) the bending of the plate

The following sections will describe the solutions used in this work to fulfil these criteria.

3.1 Four-point bend

Four-point bend is a commonly used technique for fatigue tests in solid mechanics. Figure 3.1 shows a schematic drawing of the bending mechanism.
Figure 3.1 The principle of four-point bend. A beam is resting on two supports allocated at a distance \( a \) from each end. Forces, \( F \), are applied at the edges of the beam.

One advantage of four-point bend compared to other bending methods (e.g. three-point-bend, cantilever bend) is that it delivers a uniform strain between the two inner supports. The uniform strain is due to the fact that between the inner supports the bending momentum is constant, as can be seen in Figure 3.2; and the momentum is proportional to the strain, that is

\[ M = CS \]

as derived in Appendix B. Figure 3.2 illustrates the constant momentum in a bending-momentum diagram.

Figure 3.2 Shear force diagram and bending-moment diagram for four-point bend. The momentum is constant between the two supports.
A uniform strain is of big importance in this diploma work, since it is desirable to have the same magnitude of strain throughout the whole cell culture. This is obtained by making sure that the whole culture dish is aligned in the region in-between the inner supports. Another advantage is that the shear stress is negligible [43].

### 3.2 Material requirements

Since the piezoelectric material will generate electrical potentials no metallic materials is used in the close surrounding of the bending plate; this is to avoid induced potentials that might affect the cell growth. The possible problem with heat sinking, that arises when a metal construction is taken out of the warm incubator into the colder surrounding, is also avoided by using plastic. The material used for the construction is called Delrin and is a Polyoximetylen-plastic (POM) purchased from Industriplast AB. Delrin is a suitable plastic for this purpose since it has a high level of electrical insulation, it is stable and rigid, and has a low friction. The screws used for parts that are in contact with the bending part are made of nylon, while stainless steal screws are used in other parts of the construction.

One drawback with the use of plastic for the construction is that the possible precision is lower for plastic than for metal. With the method used to produce the parts for the test-equipment, the precision for plastic is about 1/10 mm, and for steal 1/20 mm.

In Figure 3.3 a schematic drawing of the bending equipment is shown and in Figure 3.4 a photo of it can be seen.

![Figure 3.3 Schematic draw of the bending equipment.](image-url)
Figure 3.4 Photo of bending equipment. A microscope slide is used as bending device.

The base of the device is made in stainless steel since it is a stable material, and this part is located “far” from the culture dish area. The motor is attached under the top plate of the base segment, as can be seen in Figure 3.5

Figure 3.5 Photo of the bending equipment and the motor. When the motor rotates the arm attached to the motor axel is dragged down or pushed up and causes the microscope slide to bend.
3.3 Dimensions

The equipment is supposed to operate inside a normal incubator, therefore the total size is limited to approximately 30×30×30cm (width×depth×height).

The bending part of the equipment has a depth big enough for two samples to be bent at the same time. This is very important, so that parallel studies can be performed. The bending plates are regular microscope slides, and the piezoelectric material is glued onto the slide. By putting in one sample with a piezoelectric plate and one with a non-piezoelectric plate and bending both, the response from mechanical stimuli versus both mechanical and electrical stimulation can be studied as described in Chapter 4.

The distance between the sample holders and the holding gap are adjusted to fit the microscope slides. The distance between the supports has been chosen from calculations on how much the plate must bend to achieve a certain strain. The calculations are presented in Appendix C. It should be noted that the equations used are valid for beams and will not be totally accurate for a plate. The size of the error due to the use of beam theory is in the order of 0.1 percent [44]. This is considered as a minor affect in this work since it is not the actual size of the strain that is important, rather the homogeneity of the strain. The most important thing is that both the piezoelectric and the non-piezoelectric plate exhibit the same strain-pattern, so that all cells experience the same strain.

3.4 Motor

A stepper motor27 controls the bending equipment using a step size of 0.9 degrees per step. It is programmable and can auto-execute loops on start-up. The axle of the engine rotates clockwise or counter clockwise and makes the sample holders move up and down causing the samples to bend. The frequency can be controlled by software and the strain magnitude is controlled by changing the number of steps. The motor is controlled by a control box28 that is connected to a computer via the COM-port. Simple instructions such as move (MA), stop (ST) and set velocity (SV) is used to control the motors movements. Windows HyperTerminal is used to communicate with the control box. Figure 3.6 shows a photo of the motor and the control box that is connected to a computer.

---

27 McLennan Servo Supplies, model 34HS 209
28 McLennan PM301 Stepper Controller
3.5 Culture dish samples

The plates used for bending are regular microscope slides of glass. Microscope slides are stiff and cannot be bent much without breaking, but the stiffness is necessary in this experiment. A piece of a piezoelectric material (top plate) is glued onto the microscope slide (the bottom plate). To be able to bend the top plate, the bottom plate must be stiffer than the top plate. If the top plate is stiffer than the bottom plate it is attached to, only the edges of the bottom plate will bend. The top plate will then not experience any strain, and in this case, no electric potential will be generated.

The piezoelectric material used in this work was delivered already polarised and coated with silver electrodes. Before the cell growth experiments the piezoelectric plates are polished to get the electrodes off, since silver is poisonous and it is not known how it will affect the cells. Since the structure of the growth-surface affects the cell growth, the non-piezoelectric plate is produced from the same material but it has been depolarised. The depolarisation is done by heating the material to above the Curie temperature. An oven was heated to 250º C, and the samples were put in when the temperature was reached. After 15-20 minutes the oven was turned off and the samples were left to cool inside the oven until room temperature was reached. The plates were afterwards tested to see that they were depolarised by bending them with an oscilloscope connected to each side. No voltage was generated, so the depolarisation...
was successful. See section 2.3.4 for more information on depolarisation.

The piezoelectric plates are attached to the microscope slides by using different kinds of glue. The glue must be able to stick the ceramic plate to the glass during the bend and it must also be compatible with cell growth. It is necessary that the glue can keep the plates together during the whole cell growth, which takes approximately three to five days. Long time experiments were performed as described in section 5.1.3. Cell compatibility is tested as described in section 5.2.1.

Culture dishes are available in many different shapes and materials, but they are all stiff. In this experiment the culture dishes must be able to bend to follow the glass plate, which they are attached to. Experiments with different types of hand-made wells were performed as described in section 5.1.5.

Before the cell growth experiments started, characterisation of the bending and piezoelectric response to bending has been studied. This is discussed in chapter 5.1.
Chapter 4

Experimental setup

Two stimulation methods are used for the cells in this experiment; mechanical and electrical. The mechanical stimuli come from a strain caused by bending of the cell growth surface, and the electrical stimulation develops from having the bending plate made of a piezoelectric material. In this case the electrical stimulation can be seen as an indirect effect of the mechanical load.

Many *in vitro* studies have been done on how the deformation caused by a strain will affect the cell growth, and it is possible to achieve an enhanced proliferation [20, 26], see section 2.2.1.2. This is not surprising considering that this is the *in vitro* counterpart to the strengthening of bone when used, as described by Wolff’s law, see section 2.2.1.1.

There are three samples in each measurement. One microscope slide has a piezoelectric plate attached onto it. The other two slides also have a plate attached of the same piezoelectric material, but these plates have been depolarised before attachment. Figure 4.1 illustrates the design of the three samples. The piezoelectric and depolarised plates are together referred to as ceramic plates.

![Diagram of three samples](image)

Figure 4.1 Illustration of the three samples that are used in each experiment.

Around each of the three plates a cell culture dish is attached with a silicon stripe to make it resist bending, see Figure 4.2.
Cells are grown inside the wells on the ceramic materials. The slide with the piezoelectric material (sample A) is put into the bending equipment together with one of the depolarised samples (sample B). Bending of the sample B will expose the cells to strain, and bending of sample A will expose the cells to both the strain and a piezoelectric potential. The third slide (sample C) is used as a reference, and is not stimulated by either strain or electricity. It is put next to the equipment inside the incubator.

Strain is already known to enhance cell growth as discussed in chapter 2.2.1. This is why all three samples are necessary to be able to draw any conclusions about the contribution of the electric potential from these experiments. By comparing the cell growth on sample B and C, the effect of mechanical stimulation can be seen. By comparing the cell growth on sample A and B, and keeping the result from comparison of B and C in mind, the cell growth due to electrical stimulation is achieved.

Putting the two samples (A and B) parallel into the bending equipment at the same time assures that both samples are exposed to exactly the same strain-pattern. The cell cultivation is done in parallel on the three plates, so that any perturbation in the environment affects all samples to the same degree, and cells from the same culture is used for all experiments.

The bending equipment and the three samples are placed in an incubator where the normal procedure of cell growth takes place. How to perform the cell growth procedure is not discussed in this diploma work, but can be read about elsewhere [45].

4.1 Using the equipment

Tensile or compressive strains, or alternation of both, can be placed upon the cell culture samples by using different motor sequences. In Figure 4.3 the bending patterns are shown.
Figure 4.3 Possible bending patterns are: straight to convex, straight to concave, or convex to concave. This will expose cells to tensile strain, compressive strain, or alternating strain.

Cell growth stimulated by the three bending patterns can be studied separately. A simple example of a motor sequence is given in Appendix D.

Two samples are put into the equipment and one is outside the equipment. The motor has a starting position that allows for the plates to be pushed in the correct position without taking of the top-cover of the equipment. When the plates are in position the equipment is powered up by pulling switch B to ON (the red light turns on). The bending starts as switch A is pulled to ON. Figure 4.4 shows the equipment and the two switches.

Figure 4.4 Switch A controls the start and stop position of the movements of the motor. Switch B is just for power supply.

Switch A is added to assure that the motor always stops in the same position, so that the glass slides are un-deformed in the stop position, and they can be pushed in and out of the equipment for analysis or change of cell culture medium. Switch A executes a start/stop loop that has been programmed for the equipment, see Appendix D. To prevent the motor from stopping in the wrong position it is necessary to:

**ALWAYS PULL SWITCH A TO OFF BEFORE PULLING SWITCH B TO OFF.**
The proliferation of cells during strain deformation is strongly dependent on the kind of strain used. The different stimulation parameters are discussed in chapter 2.2, and the parameter values for the initial experiments taking place within the time-limit of this diploma work are chosen to:

- Strain magnitude: ~200 microstrain\(^{29}\)
- Frequency: 3 Hz
- Number of applied cycles: continuous bending all the time, except for the time it takes to change the cell growth medium.
- Strain duration: since continuous bending is applied the duration of strain is not considered.
- Strain pattern: approximately triangle wave.

The strain is applied in the simplest way possible. The motor moves 60 steps\(^{30}\) counter clockwise from its start position and then returns. Each step is 0.9 degrees so the total angle here is 54 degrees. The speed is always the same, in this case 400 steps per second. This gives a strain pattern of approximately triangle shape. The engine will then move 60 steps in one direction, return to the start position and repeat this loop.

\(^{29}\) This value is measured once as described in section 5.1.2.

\(^{30}\) The number of steps is chosen from initial experiments, see section, where it is shown that if ~70 steps are used, the microscope slides will break, but at 60 steps it will not.
Chapter 5

Experiments and results

Some preparatory experiments were performed within the diploma work to characterise the bending and to see the response from the piezoelectric material when deformed. Cell growth experiments were initially not included in this diploma work, but some minor cell growth experiments have been performed.

5.1 Preparatory experiments

To assure a good function of the test-equipment before cell growth experiments start, some preparatory experiments are performed to study the bending.

5.1.1 Bending of microscope slides

Since a piezoelectric bulk material is used in this work the plate that it is attached to has to be stiffer than this plate in order to bend it. Microscope slides are stiff and also cheap and something that is used in everyday work. Microscope slides are though fragile and cannot bend much before breaking, but at least they can bend enough to get a piezoelectric response. Experiments with different deflections of the edges were performed. The maximum number of steps possible for the motor to take before the glass broke is 70, but after a few bend-cycles at that level the glass broke. When 60 steps are used, the glass did not break, not even after one week of continuous bending. The number of steps is therefore set to 60, which corresponds to a deflection of 0.81 mm of the edges of the slide.

5.1.2 Strain measurement

The strain magnitude was measured using two foil strain gauges\(^{31}\) glued onto the central part of a microscope slide on opposite sides. Strain gauges are described in Appendix E. Two gages were used in order to avoid thermal effects. Equipment\(^{32}\) was available to directly see the strain instead of just the resistance shift due to strain. The strain was only measured once, and the value when the motor had moved 60 steps was \(\sim 200\) microstrain. Of course many factors may be involved in this value and more measurements should be done to be able to trust the value. According to calculations 60 steps (deflection of the edges with 0.81mm)

\(^{31}\) Showa Measuring Instruments, type N11-FA-8-120-11

\(^{32}\) DMD20A from HBM, www.hbm.com
should have given a much higher value, approximately 700 microstrain. But since only one measurement of the strain has been done, it is not appropriate to say much about the big difference between measured and theoretical value. Possible reasons can be the that the gluing of the strain gauge onto the microscope slide was not perfect, the strain gauges used were quite old, so there can be some drift involved. The most probable reasons are though, poorly fitted holders for the microscope slides, see Figure 5.1, or a play in the motor or the connection from the motor to the bending part.

![Gap](image)

Figure 5.1 The sample holder is not tight around the microscope slide since it must be possible to push the plate into position. This gives a gap that will make the first distance the motor moves useless for bending, since the plate is not touched by the holder from the beginning.

### 5.1.3 Attachment of piezoelectric material

Piezoelectric materials were glued onto microscope slides with different types of glue. All glues are tested in a long-term run (one week) of bending at a frequency of 3 Hz. The results of the long-term test for the different glues used are given in Table 2. Only one experiment with each glue has been performed, so no statistics can be presented.

<table>
<thead>
<tr>
<th>Glue</th>
<th>Bending</th>
</tr>
</thead>
<tbody>
<tr>
<td>Super epoxy</td>
<td>OK</td>
</tr>
<tr>
<td>LocTite 401</td>
<td>OK</td>
</tr>
<tr>
<td>Araldit Rapid</td>
<td>OK</td>
</tr>
<tr>
<td>M-bond</td>
<td>Fail</td>
</tr>
<tr>
<td>Epoxilim Bostik</td>
<td>Fail</td>
</tr>
<tr>
<td>Plastic padding</td>
<td>Fail</td>
</tr>
<tr>
<td>Contact glue</td>
<td>Fail</td>
</tr>
</tbody>
</table>

Table 2 Results of gluing test. The output is only pass (OK) or fail, and each glue experiment has only been performed once.

The glues that passed this test are then tested for compatibility with cell growth as described in section 5.2.1.

### 5.1.4 Piezoelectric response to bending

The piezoelectric plates were prepared by attaching thin copper wires to each side of the piezoelectric material with silver paint glue. The silver electrodes that the material was coated

---

This value comes from the equation derived in Appendix for the relation between strain and deflection of the edges of the plate.
with are remnant for these experiments. The piezoelectric plate was glued onto the microscope slide with one copper wire in-between. The end of each wire was connected to an oscilloscope. The piezoelectric plates were for this initial experiment glued with a melt-glue, the material could then be recycled by warming the glue again. This glue also would have passed the long-term test, but it was not included since it requires extra equipment that is not commonly available. The samples were put into the bending equipment and the copper wires were connected to the oscilloscope. The plates were bent in three different sequences

1. from straight to convex and convex to straight
2. from straight to concave and concave to straight
3. from convex to concave and concave to convex

Figure 5.2 shows the results measured on the oscilloscope when the plate is bent from straight to convex. The theoretical response was described in section 2.3.2 and can also be clearly seen in these experiments. An electric response is generated immediately as the deformation is applied and it decreases as the deformation is maintained. When the deformation is released (in this case: when the bending ceases) a pulse of opposite sign arises. Figure 5.3 is the result of continuous bending from straight to convex.
The curves looks like the theoretical as described in section 2.3.

5.1.5 Attachment of culture wells

Hand made culture dishes in silicon were built directly on the microscope slides around the piezoelectric plates. These culture dishes were able to bend, but there were problems to fit the walls and the lid so that not too much air would come in to the cell culture, and it was quite time-consuming to make them by hand. These culture wells were not even tested for cell growth, since the gap between the walls and the lid was judged as too big. If too much air comes in to the cell growth well, the cell culture medium will dry in just a few hours, and it is not viable to change the medium so often.

Instead small pots with fitting lids were used. The bottom of the pot was removed and the pot was attached to the microscope slide with a silicon stripe. The silicon stripe allows the pot to resist the bending. The pots will from now on be referred to as culture wells. Long-term tests (one week) were performed with this construction with just water in the well before starting with the cell growth. This construction proved successful.
5.2 Cell growth experiments

Cell growth experiments were initially not included in the diploma work, but some basic experiments were suitable to perform.

The cells used were fibroblasts. This type of cell is used since it is easy to cultivate and it grows quickly and rapidly. The cell cultivation is analysed by colouring of the cells and studying them in microscope.

5.2.1 Cell growth on un-stimulated piezoelectric material

Cell growth is a complex procedure and cells are sensitive to what kind of surface they grow on. Surfaces are either hydrophilic (“likes water”) or hydrophobic (“dislikes water”). For cells to feel comfortable the surface should not be too hydrophilic or hydrophobic. Initial cell growth experiments with fibroblasts were performed on the ceramic material glued into ordinary cell culture dishes. Cells were grown on the material for 3 days in an incubator. The cells seemed to “like” the material immediately, which was a bit surprising. Normally it is hard to get cells to grow on new materials and some surface modifications are often needed, but here it was immediately successful. Figure 5.4 shows the results from the cell growth on some of the ceramic plates.

Figure 5.4 Pictures of the cell growth on un-stimulated piezoelectric material. These experiments were done to see if the cells could grow on the material. The bright areas are cells, and the dark are the areas between the cells. Each bright stroke is one cell.

It can be seen that the cell growth does not look the same on the different plates. This is something that has to be further investigated, but probably it is due to differences in the surface structure of the plates, such as flaws and defects.
The different kinds of glues that passed the long-term test were here tested for suitability to have among cells. Table 3 shows the results of cell growth.

<table>
<thead>
<tr>
<th>Glue</th>
<th>Cell growth</th>
</tr>
</thead>
<tbody>
<tr>
<td>Super epoxy</td>
<td>OK</td>
</tr>
<tr>
<td>LocTite 401</td>
<td>OK</td>
</tr>
<tr>
<td>Araldit Rapid</td>
<td>OK</td>
</tr>
</tbody>
</table>

Table 3 Results of cell growth experiment.

The glue finally chosen was LocTite 401, since it was just as good as the other two who passed both test, but it is much simpler to use since it is an one-component glue.

### 5.2.2 Cell growth on stimulated piezoelectric material

Cell growth was initiated a couple of times in the bending equipment, but every time some problem occurred. The first problem was that one of the culture wells detached during an experiment, so the cell culture medium leaked out. The warmth in the incubator might have caused it, but since it just happened with one sample it can be a single error in the silicon stripe. This was fixed by applying extra silicon on all wells for future experiments, and the problem has not occurred again after that. The second problem that occurred was that the cells didn’t grow and the medium dried. No cells grew on sample B and C, and on sample A the cell culture medium had dried, but there were some indication of cell growth on sample A anyway. Probably this is a result of the fact that the bending of the samples started immediately after the cells had been attached to the surface, and then they never have time to attach. Sample C was at this occasion placed on-top of the bending equipment, and the vibrations might have caused these cells to not attach here either. This problem can be solved by letting the cells incubate on the material over night and then start the bending. The cells will then get a chance to attach to the surface.

After this experiment there was a mal-alignment problem. The sample holders that hold the microscope slides were not in the same position, as they should be, and the motor had not stopped in the correct stop-position. Some adjustments were done and the start-position was adjusted briefly. After this a new experiment started, but this time one of the glass samples broke. This must have been due to some miss positioning in the start-position. After the repositioning the equipment was just run for approximately 10 minutes and then no glass broke. But if the start-position was not repositioned well enough, it could be that the glass could withstand bending for a while, but not for several days. After this the experiments were dropped for a while to give time to readjustment of the start-position and a long-time run without any cells growing in the equipment. By this time, the time for the diploma work was over, but more experiments will be done, see section 6.1.
Chapter 6

Discussion

An equipment for applying stress and electric potential to cells has been built. Culture dish samples has been produced and used for some basic cell growth experiments. The equipment has been used for experiments for characterisation of the bending, and some initial cell growth experiments.

The strain magnitude used was measured to be ~200 microstrain, and the frequency is 3 Hz. In other experiments a higher strain is often used, but since a microscope slide is used for bending, it is not possible to achieve such high strains without breaking the glass. There are two ways to make it possible to use higher strain magnitudes: (1) another glass that is softer can be used, but it still has to be stiffer than the piezoelectric material or (2) a softer piezoelectric material can be used and thereby a softer glass. Within the time range of this diploma work it was not possible to change the materials, so the cell growth will be studied for the strain magnitude possible, that is ~200 microstrain.

More cell growth experiments must be performed before it is possible to say whether a piezoelectric material can stimulate cells. If so, a piezoelectric coating can be used on implants to prevent implant loosening due to stress shielding.

6.1 Suggestions for further work

This is the first step of a project that will become huge if things work out as expect in this initial phase. There are many things left to do and some things have to be redone to be able to do statistics on it. Thanks to financing by the Materials in Medicine strategy field, the research in this field will continue as a scholarship project for nine months. Some suggestions for further work are:

- More strain measurements to get a statistical value of the strain applied to the area between the supports of the four-point bend equipment
- Improved sample preparation
- More cell growth studies with fibroblasts
- Cell growth studies with osteoblasts
- Study in which area of the implant stress shielding is present
• Investigate which potential magnitude that is best for cells, and adjust the piezoelectric material’s polarisation to give this potential
• Test other piezoelectric materials than PZT, since PZT contains lead
• Produce a thin-film of the piezoelectric material
• Coat an implant with the piezoelectric material
• *In vivo* animal studies
• *In vivo* human studies
References

Zellverhalten humaner Osteoblasten nach physiologischen dynamischen Dehnungen, Der Ortopäde 29, 85-90, 2000
[38] Bassett, 1957, Not published
[44] Discussion with Tore Dahlberg, Professor IKP, Linköping University
[46] www.tasitechnical.com
Appendices

Appendix A  Pages  55-58
Appendix B  Page  59-60
Appendix C  Pages  61-64
Appendix D  Page  65-66
Appendix E  Page  67
Appendix A

Derivation of the piezoelectric effect [37]

Consider the stress tensor $T$ ($T_{11}, T_{12}, \ldots, T_{33}$), the electric field vector $E$ ($E_1, E_2, E_3$), the temperature $\theta$ and the magnetic field intensity vector $H$ ($H_1, H_2, H_3$) as independent variables. Then the Gibbs free energy of a piezoelectric crystal system can be written in the form

$$ G = U - S_y T_{kk} - D_m E_n - B_m H_n - \sigma \theta $$

$i, j, k, l = 1, 2, \ldots, 6$
$m, n = 1, 2, 3$

$U$ is the internal energy of the system, $S$ the strain tensor, $D$ the electric displacement vector, $B$ the magnetic induction vector (if it exists) and $\sigma$ is the entropy. In general, magnetic effects are not considered in piezoelectric phenomena, so the $B$ term is cancelled. $T, E$ and $\theta$ are independent variables and from now on a single subscript, instead of two is used to label the components of $S$ and $T$. Using Gibbs free energy $G=G(T, E, \theta)$, the dielectric, elastic and thermal equations of state can be written in the form

$$ S_i = - \left( \frac{\partial G}{\partial T_j} \right)_{E, \theta}, \quad D_m = - \left( \frac{\partial G}{\partial E_n} \right)_{T, \theta}, \quad \sigma = - \left( \frac{\partial G}{\partial \theta} \right)_{T, E} $$

These can be rewritten in differential form

$$ dS_i = \sum_j \left( \frac{\partial S_j}{\partial T_j} \right)_{E, \theta} dT_j + \sum_n \left( \frac{\partial S_j}{\partial E_n} \right)_{T, \theta} dE_n + \left( \frac{\partial S_i}{\partial \theta} \right)_{T, E} d\theta $$

$$ dD_m = \sum_j \left( \frac{\partial D_m}{\partial T_j} \right)_{E, \theta} dT_j + \sum_n \left( \frac{\partial D_m}{\partial E_n} \right)_{T, \theta} dE_n + \left( \frac{\partial D_m}{\partial \theta} \right)_{T, E} d\theta $$

$$ d\sigma = \sum_j \left( \frac{\partial \sigma}{\partial T_j} \right)_{E, \theta} dT_j + \sum_n \left( \frac{\partial \sigma}{\partial E_n} \right)_{T, \theta} dE_n + \left( \frac{\partial \sigma}{\partial \theta} \right)_{T, E} d\theta $$

Defining the following coefficients:

elastic compliance coefficients $s_{ij} = \frac{\partial S_j}{\partial T_i}$,

---

34 Gibbs free energy is defined as $G = H - TS$ and is derived from the second law of thermodynamics as a special case where the temperature and pressure are constant. $H$ is enthalpy, $T$ temperature and $S$ entropy. Gibbs free energy represents the maximum energy that can be extracted for mechanical work in a reaction where the temperature and pressure does not change when measured at the beginning and at the end of the reaction. An other interpretation of $G$ is as a sign of whether a reaction will occur or not.

\[ dG > 0 \] reaction will not occur

\[ dG = 0 \] reaction is at equilibrium

\[ dG < 0 \] reaction is allowed, "spontaneous"
piezoelectric constants
\[ d_{mj} = \frac{\partial S_j}{\partial E_m} = \frac{\partial D_m}{\partial T_j} , \]

thermal expansion coefficient
\[ \alpha_i = \frac{\partial S_i}{\partial \theta} = \frac{\partial \sigma}{\partial T_i} , \]

permittivities
\[ \varepsilon_{mn} = \frac{\partial D_m}{\partial E_n} , \]

pyroelectric coefficients
\[ p_m = \frac{\partial D_m}{\partial \theta} = \frac{\partial \sigma}{\partial E_m} , \]

the specific heat
\[ c = \frac{\theta}{\rho} \left( \frac{\partial \sigma}{\partial \theta} \right), \text{ where } \rho \text{ is the density.} \]

Using these definitions, the differential equations of state can be rewritten into (let \(dS_i=S_i\) and \(dD_m=D_m\), because the choice of origins is arbitrary)

\[ S_i = s_i^{E,T} T_i + s_{i2}^{E,T} T_2 + \ldots + s_{i6}^{E,T} T_6 + \]
\[ + d_i^{E} E_1 + d_{i1}^{E} E_2 + d_{i2}^{E} E_3 + \alpha_i^{E} d \theta \quad (i = 1,2,\ldots,6) \]

\[ D_m = d_m^{E} T_1 + d_{m2}^{E} T_2 + \ldots + d_{m6}^{E} T_6 + \]
\[ + \varepsilon_m^{T,E} E_1 + \varepsilon_{m1}^{T,E} E_2 + \varepsilon_{m2}^{T,E} E_3 + p_m^{E} d \theta \quad (m = 1,2,3) \]

\[ dQ = \partial d \sigma = \partial \left( \alpha_i^{E} T_i + \alpha_2^{E} T_2 + \ldots + \alpha_6^{E} T_6 \right) + \]
\[ + \partial \left( p_1^{E} E_1 + p_2^{E} E_2 + p_3^{E} E_3 \right) + \rho c_p^{E} d \theta \]

Superscripts in these coefficients indicate that the electric field, the stress, or the temperature is kept constant when the others are measured.

If \(dQ=0\), the isothermal piezoelectric equations are obtained. By letting \(dQ=0\) and dropping the terms with \(d \theta\) in the two first equations the adiabatic piezoelectric equations are obtained. These equations can be simplifier stated by using Einstein’s summation convention

\[ S_i = s_i^{E,T} T_i + d_{i1}^{E} E_m \]
\[ D_m = d_m^{E} T_1 + \varepsilon_{mn}^{T,E} E_n \]

This is one set of piezoelectric equations that can be derived from Gibbs free energy. By letting \(dQ\neq0\) and considering the internal energy \(U\) as a state function of the system another set is obtained, looking like:

\[ T_j = c_j^{B} S_j - h_j D_n \]
\[ E_n = -h_{ny} S_j + \beta_{mn}^{B} D_m \]
where \( c_{ij} \) are elastic stiffness constants, \( \beta_{mn} \) are dielectric impermeabilities, and \( h_{nj} \) are piezoelectric constants.

Another set of piezoelectric equations can be obtained by the same procedure as above, by considering elastic enthalpy \( H_1 = U - S_iT_j \) as a function of state of the system with \( T_j, D_m, \) and \( \sigma \) as independent variables, or electric enthalpy \( H_2 = U - E_nD_m \) as a function of state of the system with \( S_i, E_n, \) and \( \sigma \) as independent variables.

Table A.1, column one, lists the various forms of the piezoelectric equations. In second column some boundary conditions are presented and in the third column the results of boundary conditions on the piezoelectric equations are given.

<table>
<thead>
<tr>
<th>Piezoelectric equations</th>
<th>Boundary conditions</th>
<th>Piezoelectric equations under boundary conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td>( S_i = s^e_i T_j + d_{im} E_m ) ( D_m = e^T_m E_n + d_{im} T_i )</td>
<td>T=0 (unclamped(^{35} )) Apply E, Measure S and D</td>
<td>( S = dE ) ( D = \varepsilon^T E )</td>
</tr>
<tr>
<td>( S_i = s^d_j T_j + d_{im} E_m ) ( D_m = e^T_m E_n + d_{im} T_i )</td>
<td>E=0 (short circuit) Apply T, Measure S and D</td>
<td>( S = s^e T ) ( D = dT )</td>
</tr>
<tr>
<td>( S_i = s^o_j T_j + g_{im} D_m ) ( E_m = \beta^T_m D_n - g_{im} T_i )</td>
<td>T=0 (unclamped) Apply D, Measure S and E</td>
<td>( S = s^o T ) ( E = \beta^T D )</td>
</tr>
<tr>
<td>( S_i = s^c_j S_j - e_{im} E_m ) ( D_m = e^c_m E_n + e_{im} S_i )</td>
<td>D=0 (open circuit) Apply T, Measure S and E</td>
<td>( S = s^c T ) ( E = -gT )</td>
</tr>
<tr>
<td>( T_i = c^c_i S_j - e_{im} E_m ) ( D_m = e^c_m E_n + e_{im} S_i )</td>
<td>S=0 (clamped) Apply E, Measure T and D</td>
<td>( T = -eE ) ( D = \varepsilon^c E )</td>
</tr>
<tr>
<td>( T_i = c^e_i S_j - e_{im} E_m ) ( D_m = e^c_m E_n + e_{im} S_i )</td>
<td>E=0 (short circuit) Apply S, Measure T and D</td>
<td>( T = c^e S ) ( D = eS )</td>
</tr>
<tr>
<td>( T_i = c^o_i S_j - h_{im} E_m ) ( D_m = \beta^c_m D_n - h_{im} S_i )</td>
<td>D=0 (open circuit) Apply S, Measure T and E</td>
<td>( T = c^o S ) ( E = -hS )</td>
</tr>
<tr>
<td>( T_i = c^s_i S_j - h_{im} E_m ) ( D_m = \beta^c_m D_n - h_{im} S_i )</td>
<td>S=0 (clamped) Apply D, Measure T and E</td>
<td>( T = -hD ) ( E = \beta^s D )</td>
</tr>
</tbody>
</table>

Table A.1 The various forms of the piezoelectric equations and standard boundary conditions used to isolate linear relationships. [46]

---

\(^{35}\) Clamped refers to either a condition where the material is physically clamped, or is driven at a frequency high enough above the mechanical resonance so that the device cannot respond to the changing electric field.
Appendix B

Derivation to show that momentum is proportional to strain.

\[ M = Fa \text{ when } a < x < L - a \]

\[ S = \frac{T}{Y} = /(*)/ = \frac{Mz}{YI} = \left\{ \text{when } a < x < L - a \right\} = \frac{Faz}{YI} \quad \text{where } z = \frac{t}{2} \]

\[ F = \frac{2yi}{at} \quad S \]

\[ \Rightarrow M = \frac{2yi}{t} \quad S = CS \]

(*) It is this relation that is not valid for plates. For a plate the theory of so called Stimle plates should be used, which would introduce a term \( \frac{1}{1-\nu^2} \). This would for the dimensions used here give a change in stress of approximately 0.1 percent [44].
Appendix C

Derivation of how the strain depends on the deflection of the plate’s edges

Calculations are made to find out how much the edges of the microscope slide glass must be deflected to give a certain strain in the area between the supports. These calculations are in fact not valid for plates but for beams, but the error due to applying beam theory to a plate is only in the size of 0.1 percent [44], so it is not considered further here.

The curvature of a beam’s centre line can be written as [47]

\[ K = \frac{1}{R} = \frac{M}{YI} \quad (a) \]

where \( R \) is the radius of curvature, \( M \) is the bending momentum, \( Y \) is the modulus of elasticity or Young’s modulus, and \( I \) is the second moment of area. The product \( YI \) is the flexural rigidity.

Figure C.1 Coordinate system xz. w(x) describes the curve and \( w'(x) \) is the derivative.

Figure C.1 illustrates an arbitrary curve. As illustrated \( w(x) \) is the deflection of the curve. The curvature, \( K \), can be mathematically deducted to be:

\[ K = \frac{-w''}{(1 + (w')^2)^{3/2}} \quad (b) \]

where

\[ w' = \frac{d w(x)}{dx} \quad \text{and} \quad w'' = \frac{dw'(x)}{dx} \quad (c) \]

For small deflections, \( w'(x) \) is small (in the order of beam height divided by beam length) and therefore \((w')^2 << 1\). Equation (b) can then be expressed as

\[ K = -w'' \quad (d) \]
Combining equations (a) and (d) gives the relation
\[ w''(x) = \frac{-M}{YI} \tag{e} \]

The solution to this differential equation is
\[ w(x) = -\frac{M}{YI} \frac{1}{2} x^2 + Ax + B \tag{f} \]

where A and B are constants that are determined by the boundary conditions. In the four-point bend case, the boundary conditions are
\[ w(a) = 0 \]
\[ w(L - a) = 0 \tag{g} \]

Combining equation (f) with these boundary conditions gives the expressions for the constants A and B in the case of four-point bend. The results are
\[ A = \frac{ML}{2YI} \quad \text{and} \quad B = \frac{Ma}{2YI} (a - L) \tag{h} \]

These expressions have been controlled by checking that the deflection of the two edges are equal, i.e. \( w(0) = w(L) \), which has to be valid in the four-point bend case. The deflection can now be written as
\[ w(x) = -\frac{M}{YI} \frac{1}{2} x^2 + \frac{ML}{2YI} x + \frac{Ma}{2YI} (a - L) = \]
\[ = -\frac{M}{2YI} \left( x^2 - Lx - a(a - L) \right) \]
\[ = -\frac{M}{2YI} \left( x(x - L) - a(a - L) \right) \tag{i} \]

In-between the supports the momentum is constant (derived in section 3.1), i.e.
\[ M = Fa \quad \text{when} \quad a < x < L - a \tag{j} \]

From Appendix B it is known that momentum is proportional to strain, i.e.
\[ M = \frac{2YI}{t} S \tag{k} \]

The deflection can now be written as
\[ w(x) = -\frac{2YI}{t} S \frac{1}{2YI} \left( x(x - L) - a(a - L) \right) = -\frac{x(x - L) - a(a - L)}{t} S \]
Now the relation between the strain and deflection is known, and the value of interest here is the deflection of the edges, i.e. $x=0$ and $x=L$.

\[ w(0) = w(L) = \frac{a(a-L)}{t} S \]

Finally the strain corresponding to a certain deviation of the edges of a beam can be calculated from

\[ S = \frac{tw(0)}{a(a-L)} \]
Appendix D

Code sequences to control the motor

Simple example of sequence for bending.

1SV400  Set the velocity to 400 steps/sec  
1DS1  Define sequence 1  
1MA60  Move to absolute position 60  
1MA0  Move to absolute position 0  
1XS1  Execute sequence 1  
1ES  End sequence  
Command: 1XS1  Execute sequence 1

Sequence for bending and a start/stop sequence that uses switch A. This is the sequence used in the experiments. Figure D.1 illustrates how switch A is implemented.

\[ Q = \{Q_1, Q_2, Q_3, Q_4\} \]

Figure D.1 Q has the value (1110) when switch A is ON, this will start the motor. When switch A is put to OFF it means that Q will have the value (1111)

1DS1  Define sequence 1  
1SN2221 Skip next row as long as Q = 1111  (Loop sequence 1 until switch A is OFF)  
1XS2  Execute sequence 2  
1MA60  Move to absolute position 60  
1MA0  Move to absolute position 0  
1XS1  Execute sequence 1  
1ES  End sequence  
1DS2  Define sequence 2  
1SV400  Set velocity to 400 steps/sec  
1MA0  Move to absolute position 0  
1SN2220 Skip next row as long as Q = 1110  (Loop sequence 2 until switch A is ON)  
1XS1  Execute sequence 1  
1XS2  Execute sequence 2  
1ES  End sequence  
Command: 1XS2  Execute sequence 2
Appendix E

Strain gauges

A foil strain gauge is a sensor for strain. Figure E.1 shows the schematic for a strain gauge. A very thin resistive foil extends back and forth.

![Figure E.1 The foil extends back and forth so the whole foil will lengthen quite much from a small deformation, which is why foil strain gauges can be very sensitive and accurate.](image)

The resistance of the foil changes as the foil is stretched. Tension causes a resistance increase and compression gives a resistance decrease. The resistance change is very small and cannot be measured directly. A Wheatstone bridge can be used to detect small resistance changes. The bridge is balanced when the strain gauges are unstrained and the potential difference measure by the voltmeter is then zero. As soon as the strain gauges are strained, the resistance changes, and the voltmeter gives a voltage. Temperature will affect the resistance of the foil, but there is a simple way to eliminate the temperature dependence; that is, to use two strain gauges, glued on opposite sides of the bending plate. By connecting the two strain gauges to the bridge as in Figure E.2, the maximum sensitivity is obtained.

![Figure E.2 A Wheatstone bridge with two strain gauges connected (half-bridge).](image)

There are equipments available where the strain gauges are just connected to the appropriate connector and then the equipment power supplies the bridge internally and performs all calculations and just displays the strain. One example is DMD20A from HBM\(^{36}\), which has been used here.

---

\(^{36}\) Hottinger Baldwin Messtechnik, www.hbm.com