MECHANICAL TESTS OF BIOLOGICAL TISSUES

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Abstract: The contribution deals with the mechanical tests of different biological tissues, their realization, the construction of the testing mechanisms for tensile tests of soft tissues and application of the results of these experiments.

Biomechanical compression strength experiments of pig's hip joint osteosynthesis were realized by means of commercial testing device.

Obtained results are applicable in department of tissue bank, accidental surgery, plastic surgery etc., or in technical sciences, especially in the area of biomedical engineering.

Introduction

There are three main ways how to analyse properties of the biological tissues: biomechanical (failure analysis), structure and geometry analysis (imaging techniques) and biocompatibility and cell response analysis.

The study of biomechanical properties of living materials is a fascinating and important task of continuum mechanics. Biological tissues are inhomogeneous, anisotropic and are often subjected to large deformations.

Important mechanical properties of interest in biomaterials applications are: stiffness, strength, toughness, hardness, fatigue (especially cyclic), fracture strength and wear resistance.

The solution of problems in the area of biomechanical tests of tissue calls for interdisciplinary cooperation of technicians and doctors. Interdisciplinary cooperation in realization of an experiment reposes on the need to use specific methodology and evaluation of the measured data.

Tens of biomechanical experiments of human and animal tissues in tension and pressure (tensile tests of human skin, human amniotic membrane, rat skin, compressive strength tests of ostheosynthesis of femur head) have been realized.

Scientific knowledge from the obtained output is operational in many clinical disciplines, mainly in surgery, implantology, and plastic and traumatic surgery. The results are also used in technical solutions of the methodics of experiments, in designing the devices for biomechanical tests of tissues, and also in modelling and simulation of biological structures.

One of the tests that are carried out on tissues taken away from the donor and designated for implantation is the biomechanical test. It determines the tensile strength of the biological material in tension and pressure, torsion, bend and cyclical ballast. Biological tissues submitted to biomechanical tests can be generally divided into soft (for example, skin, muscle and neural fibres, tendon) and hard (bone, cartilage).

The outputs of biomechanical tests are mostly diagrams of stress-time dependence, or more precisely, stress-strain dependence.

Experiments are carried out on mechanisms for biomechanical tests, specifically adjusted to individual tissues, or on multifunctional mechanisms that are able to test most of the tissues and cover more types of biomechanical experiments.

Tensile tests

For human amnion and rat skin tensile tests, a special tension mechanism was constructed that fulfilled conditions required for realization of that kind of experiments (dimensions, sensitivity, load range, etc).

Human amniotic membrane tensile strength test

Amniotic membrane (AM), as the natural cover of various traumatically or pathologically damaged body surfaces (e.g. bedsores, diabetic ulcers, etc.) was during the long centuries naturally used without the knowledge of origin of its biological effect in therapeutic or later on medical practice.

Foetal membranes for experimental purposes were at the Department of gynaecology and obstetrics removed from physiologic accouchements. Slovak valid standards for tissue banking were observed, with attention to ethic and moral standards.

The elaboration was realized at the temperature of 24°C. During the mechanical elaboration, AM was precisely separated from chorionic membrane. The AM was fractionised to adequate size, inserted into the labelled bottles with chemical solution and left for incubation in vacuum at the laboratory temperature until next day in sterilised solution. A part of the tissues was expanded and dried on the paper base. Finally, the tissue was cut to required size, precisely labelled and packed into the plastic case. Second part of tissues was moved into the plastic bottles with 80 % glycerine and preserved at the temperature of 4°C.

Any real body is not homogenous; therefore it is not possible to consider all miscellaneousness properties of AM at the calculations.

Calculations were realized using following theoretical basis:

- Material is continuously filling volume of the given body,
- Material is ideal elastic, for real materials only for certain load value,
- Stress-strain dependence is linear (Hook's Law),
- Virtual plane cross sections, normal to the axis of body remain planar and normal to the deformation axis after deformation.

Testing specimen were incised by means of special cut form, designed for uniform cutting by manual compressive force, sparing to the tissue.

Clinical experiences at the application of amniotic membrane show, that next factor, which can also affect success of its application is its thickness, narrowly relating to the strength of the tissue.

It is known, that AM is transparent, stiff, tough, and elastic and its thickness varies in the range from 0.02 to 0.5 mm. AM consists of five layers.

Because of mentioned mechanical properties of amniotic membrane (especially elasticity), it is not possible to measure its thickness by mechanical principles.

We had following technical possibilities for thickness measurements:

- Measurements by direct comparison,
- Differential method,
- Optical method.

After measurements by all mentioned methods, we selected the optimal one: optical measurements of the AM specimen thickness by Kerato Analyzer, Conan Company.

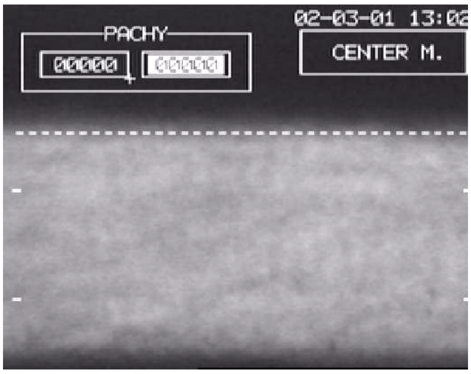


Figure 1: Zero position adjustment on upper AM layer.

It is device, normally used in clinical practice for cornea transplants analysis. The principle uses light reflection from the mirror and the light ray's transition through the examined specimen.

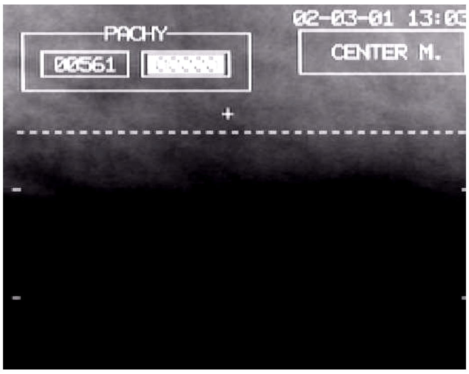


Figure 2: Subtracted thickness of AM by setting its lower layer.

Tensile strength of AM was for comparison purposes of the tensile strength of single specimen specified.

We consider a following outline at the calculations: "Normal stress at the bare tension is constant all over the cross section and it is equal to fraction of internal normal force and the cross section area". Internal forces are uniformly distributed over the cross section, and resultant force affect in the centre of gravity – in the axis of the load.

Maximal tensile strength is equal to fraction of maximal load force to cross section area of the given specimen (1):

$$\sigma_{tmax} = F_{max}/A \text{ [Pa]} \qquad (1)$$

where F_{max} is maximal break load of the specimen [N], A is the cross section area [m^2]; A= bxh, where b is constant width of the specimen and h is measured thickness of the specimen.

Comparison of the measured values shows, that technique of chemical treatment and preservation of amniotic membrane affects its tensile strength properties.

AM chemically treated by propylglycol with the highest concentration and preserved by drying, has the highest tensile strength.

Obtained results confirm that strengthening of the cross collagen ligatures by chemical membrane treatment increases its resistance against the mechanical load, and consequently improves expected membrane properties after in vivo application.

Rat skin tensile strength test

Measurement of the tensile strength of skin can be realized by biomechanical tensile strength test by means of tensile strength tester adapted to that specific purpose. In rats the tensile strength of the wound achieves only 3% of the tensile strength of the healthy skin after 7 days of healing. The low skin tensile strength of the wound during the first 7 days promoted us to application of a specific high-sensitive tensile strength tester.

Female Spraque-Dawley rats of the age of 6 months were used for experiments (n=5).

The used anesthetic mixture consisted of a combination of sedative Xylazine (Rometar a.u.v, Spofa, CR) (10mg/kg), anesthetic Ketamin (Calypsol, richter Gedeon, Hungaria) (30mg/kg) and analgesic Tramadol (Kramadol-K, krka a.s., Slovenia) (5mg/kg) was used. The anesthetic mixture was applied i.m.. Atropine (Atropin, Hoechst – Biotika s.r.o., SR) was applied to animals 20 minutes before anesthesia in the dose of 0,05 mg/kg.

Two 4cm long parallel skin incisions were performed under aseptic conditions on the left and right of the experimental rat's spine. The incisions were then closed in a conventional manner by 4 simple sutures with an atraumatic monofil material Chiraflon 3/0 (Chirmax CR).

Tensile strength measurement of the wound: The animals were killed by ether inhalation 7 days after the operation. The samples were taken and the tensile strength of the skin was measured immediately after killing the animals to prevent postmortem transformation of the skin wound. Strips of 5x5cm form the wounded area were prepared. The adequate size of the sample prevented mechanical damage of the sample. The sample was dampened by physiological solution during the measurement procedure to prevent drying up and changes of the mechanical properties. Sutures were removed and the skin strip was adjusted to optimal 2cm width. The skin strip was then placed into the fixation device. Parts overhanging the fixation clamps were removed. Subcutaneous tissue was scarified near both clamps. This approach was applied, as the large tensile strength of subcutaneous tissue does not allow precise defining of the breaking point of the sample, which is the result of the continual pulling force increase caused by pulling of the subcutaneous tissue (Fig.3).

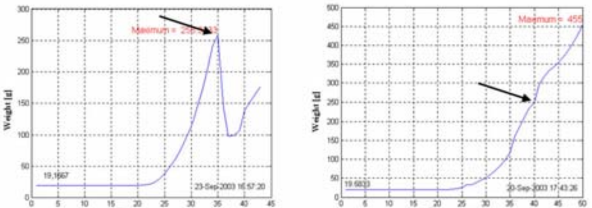


Figure 3: Tensile strength of the wound measurement with incised subcutis (left picture) and without incised subcutis (right picture).

Apparatus and parts of the pulling system: The main construction unit of the tensile strength tester is a stand with a moving arm, which transfers force form the sample to connected Honeywell's piezoelectronic sensor FSG15N1A, working with frequency of 10 Hz. A PC was used for final processing of the data. An intelligent module ADAM 4011by Advantech Company was used as a sensor computer interface. Designing and creating the fixation mechanism for fixing the skin was little problematic as the fixation mechanism had to comply with the following parameters:

- Simplicity of manipulation preventing unintentional damage of the sample during manipulation and fixation
- Elimination of shear and slide forces in the place of fixation
- Minimal mass (Fig. 4)

Data processing and analyzing: Data transmission to the computer was realized through the serial port. Software solution of data processing, recording and analyzing was realized by means of Matlab software. Data were processed and recorded into beforehand-defined files with the possibility of graphical visualization (Fig. 5).

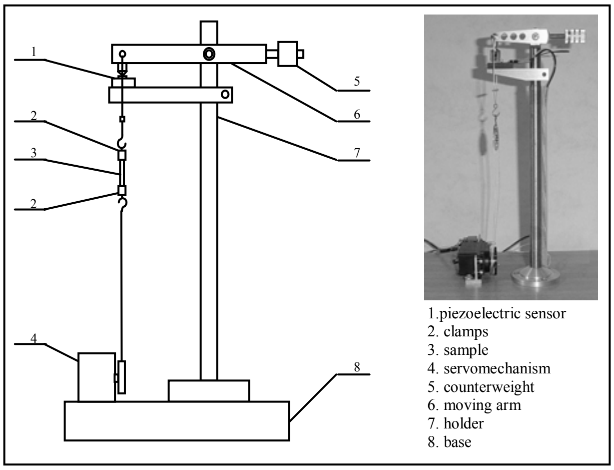


Figure 4: Mechanical parts of pulling system.

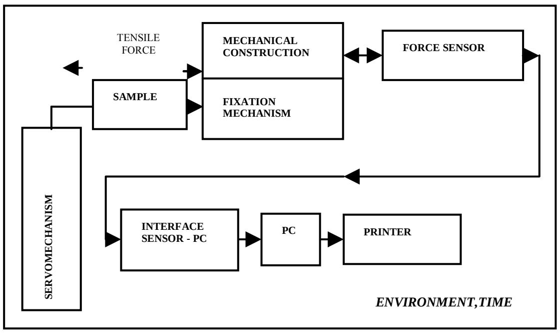


Figure 5: Block diagram of the measurement chain.

Measurement of the area of the wound and determining the tensile strength: After each tensile strength measurement the length and thickness of each ruptured wound was measured. By multiplying these values the wound area was calculated, which was used for dividing of the recorded strength – thus break point in grams. This way the tension for each wound was obtained.

The tensile strength of skin wound in a rat reached the value of 19,09 ± 1,47 $g.mm^{-2}$ (19,09 ± $1,47.10^{-9}$ $kg.m^{-2}$) after 7 days of healing.

Results are comparable to other published studies in rats after 7 days of healing. This proves quality of our testing apparatus and credibility and accuracy of our measurement. In future we would like to use the designed and examined technology of the tensile strength of wound for further experimental studies related in particular to physical factors effecting wound healing (magnetic field, low-frequency laser radiation).

On the skin in rats we compared the resistance of prepared skin wounds in fixed intervals after the realization of suture with healthy and undisturbed skin.

Mechanical properties of skin

Skin, cutis (derma in Greek) is a complicated organ, which serves as a protection cover for the body and is therefore the biggest and most damaged body part.

Elasticity and ability of the skin to move against lower layers protects body against mechanical influences [5]. Corrugated flow of collagenous fibres supports skin tensibility and elastic fibrils have a tension compensating function when skin tensiblity loosens. Consequently, it follows that the function of collagen from a mechanical point of view is to prevent tissue damage at extreme stress. The function of elastic fibrils is to restore the shape of skin at the beginning of strain [6]. This is explained in the figure 6.

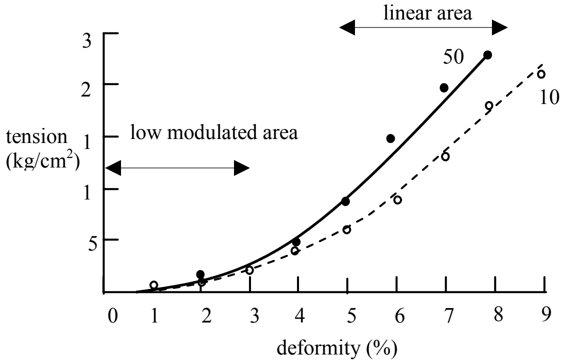


Figure 6: Dependence graph σ-ε for skin

For the experiment we used an electric shredder ZD 100 from the Department of Materials Science, with an applied range (interface) 400N, decuple enlargement of diagram deformation axis and with a load velocity circa 10^{-3} s^{-1}. Measurement accuracy was –2,29%.

Skin was taken after lower extremity amputation at a 72 year-old female patient. The patient suffered from a metabolic disease (diabetes) and ischemia of a right lower extremity due to arterio-sclerotic artery narrowing. Skin samples were taken in a way that Langer lines were longitudinally, crosswise and tangentially oriented. Consequently, subcutaneous fat was removed and the samples were wrapped into gauze wetted by a physiological solution.

The skin samples were cut into the shape shown in figure 7 at the place of experiment.

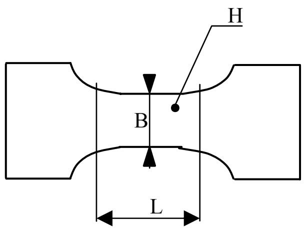


Figure 7: Schematic outline of the sample with dimensioned measures.

After setting the tensile strength test device, the adjusted samples were fixed into fixation mechanisms and mechanical stress was applied until the sample was broken. During the experiment a problem with sample fixing occurred due to the fact the sample had a tendency to slip from the fixation clamps of the device. The problem was cleared by an application of an abrasive paper between the sample and the clamps. The fine paper caused no damage to the skin structure and therefore had no influence on the overall measurement.

Recorded values were processed into tables and also graphically (Fig. 8) for all mentioned types of samples.

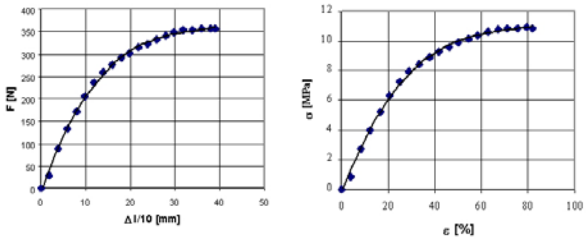


Figure 8: Obtained graphs of stress-strain dependence.

The experiment apparently shows that mechanical properties of skin at tensile strength test depend on the orientation of the Langer lines. Strength needed to break samples is biggest at the longitudinally oriented fibres. The weakest strength is needed at crosswise orientation of fibres in the skin.

Although the age and diagnosis of the patient, inaccuracy of the device and other external factors influenced the test results, the recorded values correspond to those of other published studies or to the Internet published studies. Therefore, it is possible to consider their use for handling specific problems.

Compressive strength tests

During compressive strength tests we ballasted pork head of femur where the artificially created defect was connected with different types of osteosynthesis. The aim was to find out the optimal form of osteosynthesis by means of comparing maximum pressure by the joint impairment. For the pressure tests we used the Shredder Heckert FP 100/1 mechanism, for tensile strength tests of steel and other technical materials, adjusted to our experiment.

Bone fractures are connected by a conservative method (plaster cast) or by an invasive surgery method called osteosynthesis. The experiment focused on connecting bones by means of screws, wires and so on., more specifically on the osteosynthesis of neck of femur. With this method of connecting bones a sample of bone tissue is taken, which weakens the structure of bones. Therefore, it is very important to select an appropriate type of osteosynthesis. Hip joint is one of the most frequently stressed joints in the human organism from the point of view of static stress (standing) as well as dynamic stress (walking, running). To detect influence of osteosynthesis on maximal static stress, an artificial osteotomy was performed on a pig's neck of femur. The osteotomy was performed under a 65 degree angle to horizontal plain /type of fracture Pauwels III/ to reduce the stabilization compressive strength affecting a break line to minimum and to let shear strength work dislocationally. (Fig. 9)

Figure 9: Osteotomy of a pig's neck of femur.

Later on the osteosynthesis of the bone was performed by means of the 4 most frequently used methods (Fig. 10). Compressive load test was preformed to the point of integrity damage of the tissue on the Shredder Heckert FP 100/1 with the following parameters: strain range decuple, force range 20kN and strain velocity 5mm/min.

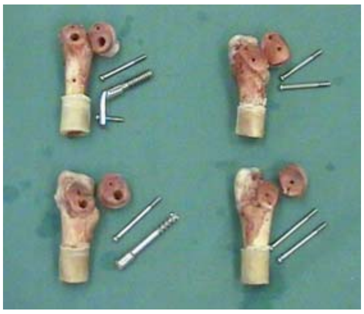
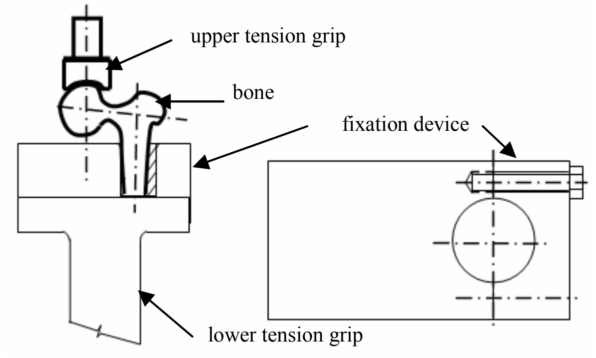


Figure 10: Methods of osteosynthetic technique.

During preparation and performance, problems related mainly to storing and fixing bones occurred. A bone is instable at room temperature just like any biological tissue.

Samples had to be taken from pigs of the same age and of approximately of the same weight in order to be accepted for the experiment. Mechanical hardness of a sample is affected not only by age and gender, but also by desiccation and conservation of a sample. During testing it is important to pay attention to the selected parameters of stress and to fixing sample in the tensile strength test device.

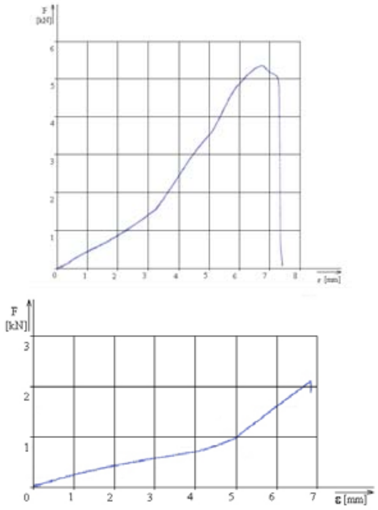
As a diaphysis does not have a constant cross-section or shape it was difficult to fix bones. Therefore, it was necessary to create a special device for fixing. Proximal part of diaphysis was sealed into a tube of a cylinder shape whose walls were coated with a layer preventing adhesion. Superakryl was used for sealing femur since the bone is covered by synovia and its excrements are also greasy. The device consisted of a 30 mm high divided metal plate into which the femur was fixed. Both parts were connected to each other by means of screws. Round opening was in its perimeter 6 tenths of mm smaller than sealed ends of the femur, which enabled perfect fixing of the distal part of diaphysis. Figure 11a schematically shows fixing the femur into the device and its placement in the tensile strength test device. Figure 11b shows the device from the top view.



a.) b.)

Figure 11: a) placement of the femur into the fixation device. b) superior view of the fixation device.

During the compressive load test, flow of strength depending on stress (Fig. 12) and variation in angle during stress influence was recorded (Fig. 13).



a.)

b.)

Figure 12: The course of strength depending on sample stress a) with osteotomy b) with osteotomy using the DHS screw.

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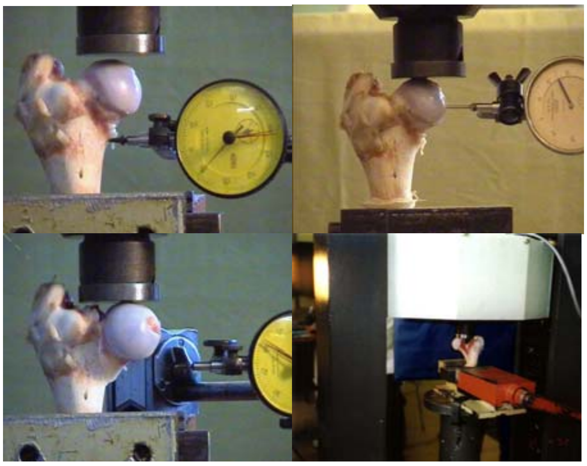

Figure 13: Measurement devices and technique.

Individual measurements provided valuable data about performance of each type of femur osteosynthesis at static stress. In order to accomplish better results it is necessary to adjust bone fixing and respect the position of the femur in the human organism.

Conclusions

Realization of biomechanical experiments pursues several aims. It is a case of determining the mechanical or resistance properties of tissue and its suitability for the consecutive clinical application, and determining and verification of suitability of certain therapeutical methods and procedures. These are connected mostly with invasive application of medical mechanisms and instruments.The realized biomechanical tests called attention, from the viewpoint of their accomplishment, on the importance of solving the clutching mechanisms or jaws (the contact of biological tissue with technical material under the load) and importance of methodology of experiment realization.

It is also important to achieve the conditions of the environment in which the experiment is realized as similar as possible to the physiological environment in human body, from which follows also the need for correct and suitable conservation and preservation of biological tissues.

Acknowledgement

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