

SHORT THESIS FOR THE DEGREE OF DOCTOR OF

PHILOSOPHY (PHD)

**CURRENT MECHANO-BIOLOGICAL ISSUES IN
MUSCULOSKELETAL SURGERY**

by Ágnes Éva Kovács (MSc)

Supervisor: Sándor Manó (PhD)



UNIVERSITY OF DEBRECEN

DOCTORAL SCHOOL OF CLINICAL MEDICINE

DEBRECEN, 2024

Current mechano-biological issues in musculoskeletal surgery

by Ágnes Éva Kovács, MSc

Supervisor: Sándor Manó, PhD

Doctoral School of Clinical Medicine, University of Debrecen

Head of the Defense Committee:

Prof. Árpád Illés, DSc

Reviewers:

Prof. Rita Mária Kiss, DSc

Prof. Zoltán Szekanecz, DSc

Members of the Defense Committee:

Dr. Ilona Veres-Balajti, PhD

Prof. Imre Bojtár, DSc

The PhD Defense takes place at the Lecture Hall of Building A,
Department of Internal Medicine, Faculty of Medicine, University of
Debrecen

02nd of September 2024., 14:00

1. INTRODUCTION

Medical practice requires the replacement of tissues and organs in many diseases. Consequently, the exploration of the effects of metals in the living environment has inspired many researchers, including us. The evaluation of bone fusion solutions has raised mechano-biological issues that have attracted our interest.

In the first part of our research, I will report on biotribological studies of the spinal screws we are designing. The word *biotribology* was first used and defined by D. Dowson in 1970 as "*that aspect of tribology which relates to biological systems*". Within biotribology, the tribology of joints is considered in terms of the problems arising from the human musculoskeletal system, one of the most important functions of which is mobility. One of the most important components of the musculoskeletal system is the synovial joint; this includes the hip and knee. These joints are what can be described in engineering terms as a wristhing-like structure; they function throughout the human body over a lifetime, transmitting significant dynamic loads and capable of a wide range of movements. Severe wear and tear of the joint requires their replacement by artificially produced endoprotheses.

In friction, lubrication and wear testing of artificial joints, it is important to optimise performance to improve clinical outcomes and functionality. Currently, the main clinical problem associated with artificial joints is the loosening of prostheses, mainly due to adverse biological reactions caused by wear. Friction occurs between the moving surfaces of the prosthesis. Friction causes wear products, called particles, to be deposited from the surface. Due to the body's immune response to the detached particles, the polyethylene fragments induce bone resorption, which

can eventually lead to loosening of the prosthesis. Therefore, in addition to the traditional polyethylene-metal combination, ceramic-ceramic, ceramic-polyethylene combinations with less wear products are becoming more common.

Skeletal implants inserted into a living organism can be divided into four main groups based on mechanical features:

1. moving structures (joint prostheses) with appropriately designed sliding surfaces
2. stand-alone implants (screws, cerclages, etc.)
3. combined structures, montages, which predominantly exert shear forces to each other (plate-screw osteosynthesis, locked medullary nails)
4. implants capable of undesirable sliding along each other, where there are various clamping or twisting mechanisms to prevent displacement (certain spinal implants).

In the first group, proper lubrication is desirable, in the second and third groups it is practically indifferent, and in the fourth group it is a distinct disadvantage.

The American Society for Testing and Materials (ASTM) does not provide definitive guidelines for *in vitro* testing of mounted spinal implants with screws and hooks attached to longitudinal rods:

'12.5 In developing this test method, significant debate revolved around the question of whether to test in saline, in a simulated body fluid, or in air. Because it was impossible to define a fluid that exactly simulates the in vivo environment and because implants must be compared to one another until

performance standards are defined, the test environment is left to the individual investigator.'

In vitro studies were designed for spinal implants inserted from posterior approach to find the industrial lubricant that most closely resembles the tribological behaviour of biological materials in the surgical field. The use of such a material could standardise and bring the methods of *in vitro* mechanical testing closer to reality. The following questions were examined in the measurements:

- How does the presence of biological substances in real-life environments affect screw-rod connections tested under otherwise dry conditions?
- Which industrial lubricants can replace friction-reducing substances found in living organisms?

In the context of the other major theme of our research, the average age of people around the world has risen rapidly in recent decades. With the ageing of the population, the number of indications for total joint replacement is consistently increasing. In 2017, the number of orthopaedic procedures worldwide was approximately 22,3 million, making it one of the fastest growing surgical procedure categories.

Several materials such as ceramics, metals and polymers have been introduced to meet industrial demand, with the biomedical sector promoting the use of metals and their alloys for medical implant applications. The number of different types of metal implants implanted in the

human body is therefore increasing, and the quality of these implants is also improving significantly.

Today, substances intended for implantation in the human body must meet the following requirements:

- *Mechanical requirements*: the implant material should ideally have a modulus of elasticity equivalent to that of bone.
- *Biocompatibility*: the compatibility of the implanted material with the tissues is critical, the implant must not have any adverse effects on the tissues, cause inflammation or allergic reactions.
- *High wear resistance and high corrosion resistance*: the lifetime of musculoskeletal implants is mainly determined by friction and wear resistance. Low wear resistance can lead to implant loosening and wear fragments can cause a number of adverse tissue reactions.
- *Osteointegration*: the direct linkage of the implant to the bone. The component material of the implant, the surface roughness and surface topography, and where appropriate the lattice structure, all have a role to play in the development of the appropriate degree of osteointegration.
- *Longevity*: when an implant is placed in the body, the implant is exposed to a range of cyclic stresses and therefore needs to have high fatigue strength to withstand the repeated stresses in the body.

With currently available metallurgical methods, it is possible to create a porous metal with properties very similar to those of cancellous and cortical bone. The latest Additive Manufacturing (AM) technologies can

produce a metal with a complex architecture and almost unlimited porosity, according to the anatomical and functional requirements of the bone to be replaced.

The other topic of our research is the evaluation of the use of 3D printer manufactured local lattice structures of different shapes for the manufacturing of Ti6Al4V custom implants, which could be the basis for the production of implants with a higher degree of biofunctionality for the human body, faster healing and significantly longer lifetime, using AM technology, which is substantially more time and cost efficient than the current methods.

In this research, I studied the behaviour of implants with lattice structure made of Ti6Al4V material in a biological, bony environment. The following main question was investigated:

- How does the geometry of the lattice structure of metal implants in a bony environment influence the tendency to bone ingrowth?

The ability to 3D print lattice metals has also made it possible to create a biological, bone ingrowth-based fixation between bone and implant without the need for other fixation materials.

2. OBJECTIVES

I have worked to achieve the following goals:

2.1. Biotribological analysis of implants

- a) Finding easily available (industrial) lubricants that have similar tribological behaviour to human materials used in a surgical procedure.
- b) To examine how the presence of biological substances in real-life environments influences the implant-component interactions to be tested under dry or physiological saline conditions according to relevant standards.
 - Study design, statistical design
 - Perform the measurements
 - Summary of the results
 - Recommendation for the modification of the standard
 - Recommendation on the tightening torque of the spinal implant fixation screw

2.2. Examination of different types of lattice specimens

- a) Design and 3D printing of six different trabecular specimens from Ti6Al4V material.
- b) Determination of the rate of bone ingrowth by histological processing and development and application of an image processing algorithm for each type of grid implants.
 - Study design, statistical design
 - Designing an animal experiment, and obtaining the required permissions
 - Performing experimental animal surgery, monitoring, extermination, sample collection and storage
 - Performing histological and mechanical tests, analysis with image processing algorithm
 - Summary of the results

In some cases, methodological improvements were necessary to achieve the objectives I had planned to implement. I considered these developments as part of the related work processes.

3. MATERIALS AND METHODS

3.1. Biotribological analysis of implants

We performed two series of measurements. First, we measured the relative lubricity of different substances followed by stability measurements according to a spine implant related ASTM standard.

3.1.1. Measurement of relative lubricity

The experiments were carried out at the Faculty of Technology of the University of Debrecen. The measurements were carried out with a special instrument previously developed by the working group, which is suitable for measuring the relative lubricity of different materials. We used this instrument for testing tissues and fluids present in the operative field and compared our results to readily available industrial lubricants. Three milliliters of the tested lubricants were introduced put between these two surfaces and the device measured the force required for the displacement of the two surfaces. Distilled water was used as the reference material. The conditions and materials used in the series of measurements were as follows:

1. Dry state without any lubricant.
2. Distilled water purified with a Millipore MilliQ I. (Merck KGaA, Darmstadt, Germany) water purification system.
3. Human blood (taken intra-operatively from patients undergoing elective surgery at our Department).
4. Human synovial fluid (taken intra-operatively from patients undergoing elective surgery at our Department).
5. Human subcutaneous fat tissue (taken intra-operatively from patients undergoing elective surgery at our Department).
6. Simulated body fluid (SBF).

7. Physiological saline solution (0.9%, B. Braun Melsungen AG, Melsungen, Germany).
8. Silicone (WOLF'S W340, Wolf Chemicals Ltd., Budapest, Hungary).
9. Vazelin (Vazelin Original, Vasenol, Unilever Plc., London, United Kingdom).
10. WD-40 (WD-40 Company, San Diego, CA, USA).
11. Turbine oil (Turbine-46 K, MOL Plc., Budapest, Hungary).
12. Engine oil (Castrol GTX3, Castrol Ltd., Pangbourne, United Kingdom).
13. Lubricant grease (Mobil Agri Grease Extra, Exxon Mobil Corporation, Irving, TX, USA).

3.1.2. Measuring the effect of lubricants on implant stability

The stability measurements were performed according to ASTM 1798–97 (2008) standard arrangements, with an Instron 8874 material testing machine (Instron Ltd., High Wycombe, United Kingdom) using 6 mm diameter Ti6Al4V rods and 45 mm length transpedicular set screws (Sanatmetal Ltd., Eger, Hungary.) The measurement was also performed with the tightening of the set screw at 2 Nm and 4,5 Nm as recommended by the spinal implant manufacturers.

3.1.3. Statistical analysis

The normality of the population was determined using the Shapiro–Wilk test. Statistical differences between the different lubricants were analysed by two-way ANOVA supplemented with Tukey post hoc test. A $p < 0,05$ was considered statistically significant. Data are presented as

mean $\pm$ SD. To determine whether the difference in loosening torque between dry and lubricated screws was statistically significant, the two-sample t-test was used.

Statistical calculations were performed using GraphPad Prism 7 program (GraphPad Software Inc., San Diego, CA, USA).

3.2. Examination of different types of lattice specimens

3.2.1. Ethical permission

The animal experiment was carried out in the operating room of the Experimental Surgical Institute of the Semmelweis University in Herceghalom, with an ethics reference number of PE/EA/573-8/2019. For ethical reasons and in accordance with the current directive 2010/63/EU, we worked with the smallest possible number of experimental animals that still allowed for statistical evaluation.

3.2.2. Design and manufacture of implants

We aimed to create the most comprehensive experimental model possible, by including in the experiments not only the most commonly studied lattice shapes in the literature, but also those for which no data were available in the literature. In view of this, the lattice shapes studied were: gyroid, cube, cylinder, tetrahedron, double pyramid, Voronoi. The implants were fabricated on an EOS M290 (EOS GmbH, Krailling, Germany) 3D printer with Direct Metal Laser Sintering (DMLS) technology using Ti6Al4V (Grade 23) material.

3.2.3. Selection of the model animal, animal experiment

Thanks to the work of the Swiss Arbeitsgemeinschaft für Osteosynthesefragen (AO) working group, sheep have become the gold standard for bone surgery experiments worldwide. AO experiments have shown that the results obtained using sheep can be applied to human bone surgery in many respects. The sheep, as an experimental animal, therefore appeared to us to be an ideal candidate.

Because the spongy bone of the distal femur condyle of sheep most resembles that of humans at a young age, i.e., 3–4 years old, we used sheep of this age in our animal experiment. Our study included 12 female Merino sheep each weighing 45–50 kg. We implanted three implants into the two femoral condyles of each sheep. One of the three implants was always a control, which was solid and without a trabecular structure. The other two were lattice-structured implants of different lattice types. Bone ingrowth was studied by exterminating the animals in two terminals. Exterminations were performed at weeks 8 and 12 after surgery.

3.2.4. Mechanical evaluation

We conducted push-out tests to determine the force required to displace the implants with different lattice structures from their positions, and to measure the resistance created by bone growth. The greater the force required for displacement, the greater the assumed degree of bone growth. The tests were performed in our Biomechanical Materials Testing Laboratory using an Instron 8874 (Instron, Norwood, Massachusetts, USA) materials testing machine.

3.2.5. Histological processing

The implants were removed from the femur condyles with approximately 5 mm of their bony surroundings. These were used to make the sections for further processing. Sections were ground and polished on an Ecomet II Grinder Polisher (Buehler, Lake Bluff, USA) used for geological sample preparation, using different grit sizes of abrasive papers. The sections were then cleaned in Sonorex RK100H (Bandelin, Berlin, Germany) ultrasonic cleaner in isopropanol for 1 min and then blown with high pressure air. Optical microscopy images of the sections were taken with a Keyence VHX-6000 (Keyence International, Mechelen, Belgium) digital microscope. A VH-Z20R/Z20T objective was used to take the images.

3.2.6. Manual image processing

Microscopic images were manually drawn to determine the extent of bone ingrowth using GIMP (The GIMP Development Team, GIMP 2.0., <http://www.gimp.org/>) and Image J (National Institutes of Health, Bethesda, MD, USA) software. Images were processed independently by two people.

3.2.7. Histomorphological examination

After the removal of the implants, we also examined the histomorphology of the implants, and histological processing was performed on tissue samples from both the implant surface and the internal cavities. The specimens were embedded in paraffin after decalcification in ethylenediaminetetraacetic acid (EDTA) according to routine histological protocol.

3.2.8. Statistical analysis

We evaluated two hypotheses:

- There is no significant difference in the push-out force between the solid and the other lattice types.
- There is no significant difference in ingrowth between the examined lattice types at week 8 and week 12, and the ingrowth over time within each lattice type does not change significantly.

We examined the normality of the population using the Shapiro–Wilk test. We analyzed statistical differences between different grids and the solid grid using one-way ANOVA supplemented with Dunnett’s post hoc test. We used a linear regression model to compare the algorithm method and the manual method. We used two-way ANOVA complemented by Sidak’s multiple comparison test to assess the statistical differences between the investigated grids at different time points. We considered $p < 0,05$ to be statistically significant. We present the data as the mean $\pm$ 95% confidence intervals. We performed statistical calculations using GraphPad Prism 7 program (GraphPad Software Inc., San Diego, CA, USA).

4. RESULTS

4.1. Biotribological analysis of implants

4.1.1. Determination of relative lubricity

The average lubricity values of the tissues in the surgical field and the industrial lubricants were compared with the results obtained using distilled water as a reference (lubricity of distilled water=100%) under a load of 300 N. The average lubricity of blood and subcutaneous fat tissue was similar to that of industrial lubricants. The lubricity of the tested human tissues was between the motor oil and lubricating grease, but closer to the motor oil, therefore stability measurements were performed with Castrol GTX3 motor oil.

The relative lubricity of the simulated body fluid recommended by the ASTM standards was lower than the physiological saline solution, and only slightly higher than the results obtained from measurements in the dry state without lubricant.

Based on the statistical results, there was no significant difference between the engine oil and the kinetic forces of the three human materials (engine oil vs. human blood: $p=0,111$; engine oil vs. human synovial fluid: $p=0,998$; engine oil vs. human adipose tissue: $p=0,999$) at 300 N compression force.

The main result of the experiment was that the lubricity of Castrol GTX3 engine oil was similar to the lubricity of human tissue in the surgical field. In the context of this result, future researchers will find it easier, simpler and cheaper to study the stability of spinal and other implants in a truly

realistic test environment using industrial oils, without any ethical problems.

4.1.2. Stability measurements

For the stability tests, Castrol GTX3 engine oil was used as a lubricant. As a control, a dry state was also tested at two tightening torques. At 2 Nm of torque, an average of 330 N was required to push the rod out, while this increased to 607 N at 4.5 Nm. When engine oil was used, the average extension force decreased to 293 N and 595 N, respectively. At 4.5 Nm of torque, 1.84 times more force was needed to push the rod out when dry, and 2.02 times more force when lubricated with oil than at 2 Nm.

Our results showed that the use of the lubricant reduced the force required to destabilise the montages by 10 % at 2 Nm torque and by 2 % at 4.5 Nm.

It was found that the lubricated screws could be loosened with lower torque (1.49 Nm vs. 1.24 Nm on average). A result of $p=0.038$ was obtained between the loosening torque of dry and lubricated screws, significance was obtained.

4.2. Examination of different types of lattice specimens

4.2.1. Definition of the surgical site

In contrast to many authors, the femoral condyle was approached from the inside rather than the outside. There were several practical reasons for this:

- In decubitus sheep, both sides could be operated on simultaneously with one isolation.
- This positioning provided a better surgical approach.
- Surgical wounds on the medial side were involuntarily spared more by the experimental animals.

4.2.2. Surgical technique

Surgery was performed in the usual human practice, with full respect for sterility, following regular surgical scrubbing and dressing. An oblique incision was made on the inner surface of the knee, partly along the medial fibres of the vastus and partly through them. The medial femoral condyle was then exposed and the periosteum mobilised. After exploration, one shaft of the targeting needle was inserted into the retracted part of the medial femoral condyle at the anterior-inferior edge of the condyle after a mini-arthrotomy, and the other needle was inserted through the arthrotomy opening to the accessible edge of the medial femoral condyle. With the target laid on the freed bone surface, the entire width of the femoral condyle was drilled through with a 6 mm diameter drill, including the overlying femoral condyle. In the three holes prepared in this way,

implants 0.25 mm thick were press-fitted in a well-identifiable manner according to a predefined pattern.

4.2.3. Specific targeting device for surgery

We have developed a targeting device for preparing the site of the implants, which has been developed through several prototypes. An important factor in the final shape was the presence of a small distinct indented groove on the antero-medial margin of the distal femoral condyle of each sheep bone, which, being located in a nearly identical position, provided a good orientation point for the design of the targeting. Our laboratory, the Biomechanical Laboratory of the University of Debrecen, is also well equipped for printing sterilizable plastic devices, and the targets used during the surgeries were produced from MED610 translucent biocompatible photopolymer material using an Objet260 Connex (Stratasys, Revohot, Israel) printer. A 6 mm diameter sterilizable drill stem was used adapted for the grafting specimens. To prevent chipping of the plastic target, the final targets were supported by 3 metal bushings with flanges on top.

4.2.4. Histological analysis

The implants were removed from the femoral condyles with their bony circumferences of approximately 5 mm. From these, longitudinal sections for further processing were formed using a waterjet I510-G2 (TECHNI Waterjet, Campbellfield, Australia) instead of the standard microtome solution.

4.2.5. Image processing algorithm

In collaboration with the Faculty of Informatics of the University of Debrecen, we have developed a computer image processing method that is suitable for the quantitative evaluation of newly grown bone tissue on microscopic images of implants. This automated method efficiently measures the quantitative assessment of bone ingrowth in implants with different grid structures. The method consists of two basic steps. The first step is to segment the implants by accurately separating them from the background. The second step is to detect three different textures on the implant. Identify the surface of the alloy implant, the areas of osseointegration and the gaps in the lattice structure where no osseointegration was observed.

To predict the effectiveness of different types of grid structures and to calculate how much they help the process of osseointegration, the ratio between the segmented area of the osseointegrated region (A_{Oss}) and the number of regions containing gaps (BI) was calculated using the following formula:

$$BI = \frac{A_{Oss}}{A_{Oss} + A_{Hole}},$$

where A_{Hole} refers to the detected gaps.

4.2.6. Results of the mechanical test

We compared the push-out force of implants with different grid types to that of the solid control implant.

The push-out force of the gyroid ($p=0,019$) and cube ($p=0,003$) grid types was significantly higher compared to that of the solid control implant. In the case of the cylindrical grid, a borderline significance was obtained compared with the control ($p=0,053$). The push-out force was also higher in the case of the double pyramid grid, although the difference was not significant ($p=0,215$). There were no significant differences in the push-out force between the tetrahedron ($p=0,884$) or Voronoi ($p=0,997$) grids and the solid grid. Overall, because there were several significant differences compared to the control, we rejected our first hypothesis (Section 3.2.9.).

4.2.7. Comparison of the results of the image processing algorithm and the manual drawing - determination of the rate of bone ingrowth

In our experience, the two different measurements showed a very strong correlation ($R^2=0,924$; $p<0,001$).

4.2.8. Results of the histomorphological examination

During histological examination, we invariably observed signs of normal bone formation, with slightly irregular but well-developed pseudo-lamellar bone tissue detectable in all cases. The locations of the titanium mesh

appear as empty holes in the sections, surrounded mostly by newly formed bone, with little adipose or connective tissue, and minimal bone marrow filling the cavities, without any signs of irritation or inflammation. All of these observations support the results obtained using the image processing algorithm, indicating that the digitally segmented and measured areas on the water-cut surface can be considered relatively homogeneous and properly formed mature bone based on tissue composition.

4.2.9. Efficiency of bone ingrowth rate - depending on grid type and time after implantation

We ranked the bone ingrowth efficiency of the six types of lattice implants according to the above results. In the case of the tetrahedron, ingrowth was significantly lower compared to all other grid types both at week 8 (tetrahedron vs. Voronoi, $p=0,005$; tetrahedron vs. cylindrical, gyroid, double pyramid, cubic, $p<0,001$) and at week 12 (tetrahedron vs. Voronoi, cylindrical, gyroid, double pyramid, cubic, $p<0,001$).

Although the Voronoi grid showed significantly greater ingrowth than the tetrahedron grid, compared to all other grid types, its performance was significantly lower in terms of ingrowth both at week 8 ($p<0,001$ in Voronoi vs. cylindrical, gyroid, double pyramid, cubic) and at week 12 ($p<0,001$ in Voronoi vs. cylindrical, gyroid, double pyramid, cubic).

There was no significant difference between the cylindrical and the gyroid grids either at week 8 ($p=0,742$) or at week 12 ($p=0,995$).

While there was no significant difference between the cylindrical and the double pyramid grids at week 8 ($p=0,113$), the double pyramid grid showed significantly greater growth at week 12 ($p=0,044$). At both week 8 ($p=0,037$) and week 12 ($p=0,003$), the cubic grid showed greater ingrowth compared to the cylindrical grid.

Furthermore, we did not find a significant difference between the gyroid and the double pyramid grids at any time (week 8, $p=0,857$; week 12, $p=0,138$).

There was no significant difference between the gyroid and the cubic grids at week 8 ($p=0,423$); however, in the case of week 12, we saw that the ingrowth was significantly greater in the case of the cubic grid ($p=0,01$).

Finally, there was no significant difference between the double pyramid and the cubic grids at any time point (week 8, $p=0,889$; week 12, $p=0,443$).

In terms of bone ingrowth, the most effective lattice types were ranked as follows: gyroid, double pyramid, and cubic. The order of these three lattice types was the same for both weeks, i.e., the ranking order remained the same at both week 8 and week 12 after euthanization. For this reason, we also rejected our second hypothesis (Section 3.2.9.).

During the time comparison, only in the case of the Voronoi grid was a significant difference between week 8 and week 12 ($p<0,001$); for the other grid types, there were no significant differences (tetrahedron, $p=0,999$; cylindrical, $p=0,648$; gyroid, $p=0,999$; double pyramid, $p=0,088$; cubic, $p=0,078$).

5. DISCUSSION

5.1. Biotribological analysis of implants

A number of rheological, mechanical and tribological properties of human tissues have been identified, but the literature on their lubricity is very limited. Lubricity is one of the most important aspects of tribology. The lower the lubricity, the higher the wear of the material under test. The lubricity of a material cannot be measured directly and is significantly influenced by other factors such as the shape, microgeometry and extent of surfaces, the viscosity and density of the lubricant, temperature and pressure.

In orthopaedic surgical practice, lubricity plays both a positive and negative role. In some cases, increased lubricity is a useful property, especially in joint replacement surgery. However, in the case of spinal implants with numerous connections, lubricity can be especially harmful, as lubrication from surrounding tissues and blood can cause these implants to loosen due to their high viscosity. Spinal implants inserted from the posterior approach consist of various pedicle screws and hooks that are connected to two longitudinal rods. Spinal implants that are fixed to rods are subjected to high loads after deformities have been corrected, which are compensated by the clamping force of the set screws that hold the implant parts together. In the surgical field, both the loosening of the fixation screws and the sliding of the implants on the rods are facilitated by the undesirable lubricity of the environment.

The implant manufacturers usually specify the implant placement torque values of set screws. The primary stability measurements are performed

in standardized laboratories working mostly according to ASTM directives. However, most of the tribology tests are conducted with water or saline, and do not take into consideration the lubricity of surrounding human tissues. These test methods are used to quantify the static and dynamic mechanical characteristics of different designs of spinal montages. ASTM mentions that the tests performed with simulated body fluid or physiological saline solution may cause fretting, corrosion, or lubricate the interconnections, and thereby affect the relative performance of tested devices. The results obtained cannot be used directly to predict the *in vivo* performance, as they could significantly differ from the real values obtained in the intraoperative setting. A considerable amount of information is available on the lubrication of endoprostheses, but there is a relatively modest amount of literature on the stability of spinal implants tested under physiological conditions.

Our investigations were motivated by the idea of finding a synthetic material with tribological properties that are closest to those of substances with lubricity in living organisms. We would also like to suggest a more consensus proposal to perform more realistic mechanical tests and to make it mandatory for manufacturers to provide the required torque values for screw-rod spinal implants.

Although the performed experiments were technically easy, the results showed that tissues found in the surgical field have a larger lubricant property than expected. In cases where the manufacturer does not provide the value of the torque and the screw is tightened without torque meter, the torque could be low. At low torque values (2 Nm), these lubricants showed a significant decrease in their stability (10 %).

On this based, it is therefore recommended to perform stability tests not only under dry conditions but also under physiological conditions. These tests, in the light of the results obtained from our tests, can be carried out with Castrol GTX3 oil, as its lubricity is similar to that of human tissues in the field of surgical interventions. Thus, stability tests can be easily performed without the need to analyse human tissues, avoiding ethical and related public health concerns such as access to and storage of human samples and problems in obtaining consent for their use.

5.2. Examination of different types of lattice specimens

The requirements for bone joint implants may vary depending on the body region and the purpose of the application. For long-term endoprostheses, fusion of the bone implant by bone ingrowth is desirable. In these cases, there is a role for bone-growth-promoting surface. In recent studies, the use of various lattice structures, especially for custom-made implants that can be produced by 3D printing, has become increasing. The determination of the ideal lattice size for bone growth can be rather clearly defined based on relevant studies in the literature. In view of this, we chose the 600 μm size, which proved to be the right choice in the experiments. However, the effect of the lattice shape on bone growth still raises many unanswered questions. This fact motivated our research.

In our study, we have used an experimental approach and methods that are partly different from those found in the literature. Six different grid shapes (gyroid, cube, cylinder, tetrahedron, double pyramid, Voronoi) were investigated simultaneously with a follow-up period of 12 weeks,

and the implants were placed in the unloaded area of the femur. Our results are broadly in line with those reported in similar studies in the literature but provide several new insights. Based on our experiments and results, we can state that there are major differences in bone ingrowth between the lattice shapes. We found that there are factors involved in the processes of bone growth *in vivo* in a metallic lattice environment that cause more significant bone ingrowth in round and approximately round cavities such as gyroid, double pyramidal and cubic lattice types. The results of our experiments lead us to the following conclusions:

- The shape of the lattice structure of 3D-printed Ti6Al4V implants has a great influence on bone ingrowth. Lattice structures containing circular and approximately circular cavities, namely the gyroid, the double pyramid and the cube, promote larger, more efficient bone formation.
- The results of statistical and classical histological processing confirm that our newly developed image processing algorithm is capable of accurately and precisely determining the extent of bone growth.
- The best performing lattice forms show a significantly higher bone ingrowth rate (60-80%) after 12 weeks.

6. SUMMARY

The common element linking the two parts of my research is the analysis of the impact of metals in the living environment. In the first half of the research, I presented my investigations on the stability of spinal implants. Stability testing of spinal implants with numerous connections depends on a wide range of factors from design to material selection, and there are also important biotribological testing parameters (e.g. kinematics and loading) that can significantly influence the outcome of a given stability test. The implant testing standards and guideline specifications have many open questions, which make it essential to compare the *in vitro* testing method with the *in vivo* method, as the correct evaluation of the test results can lead to avoiding loosening of the montages. We aimed to find an industrial lubricant whose use could be standardised, and it could bring *in vitro* mechanical testing methods closer to the reality. In consideration of our results, we proposed the use of a commercially available engine oil for mechanical testing.

In the second part of the research, I presented an analysis of the behaviour of our designed and 3D printed lattice implants in a biological, bony environment. Six different lattice structures (gyroid, cube, cylinder, tetrahedron, double pyramid, Voronoi) were tested in sheep in animal experiments. The implants were made of Ti6Al4V alloy using DMLS 3D printing technology. Mechanical, histological and image processing analyses were performed on the samples. The samples were cut using a novel waterjet method. By statistically evaluating the results of our image processing algorithm, and by supporting it with the results of classical histological processing, we have concluded, that the digitally segmented areas can be used to accurately quantify the extent of bone ingrowth. The bone ingrowth performance of the six types of grids was ranked as described above: implants with gyroid, double pyramid and cubic grids had the highest bone ingrowth. The order of these three types of grids was also the same in terms of elapsed time, i.e. they were true at both the 8th and 12th week after extermination. In addition to the cubic lattices, which have been reported in the literature to have good bone ingrowth values, the gyroid and double pyramid lattice types also showed similarly good results.

7. NEW RESULTS

7.1. Biotribological analysis of implants

- a) We have developed experimental methods for measuring relative lubricity and implant stability. The results obtained after the experiments were performed were subjected to statistical analysis.
- b) A proposal has been made to complete the ASTM F1798-97(2008) standard; to use motor oil for testing, which would standardise and bring the results of *in vitro* mechanical tests closer to reality.

7.2. Examination of different types of lattice specimens

- a) In addition to the lattice types already studied in the literature, we also included our own lattice types in the research, based on our own ideas.
- b) We have created a single-use, sterilizable, universal targeting device for animal testing.
- c) We used a novel approach to the surgical technique: implants were placed in the unloaded area of the femur of the sheep.
- d) In a novel way, the waterjet cutting method was used to create longitudinal sections of the bony femoral condyle pieces.
- e) We have developed an automated computer image processing algorithm in cooperation with the Faculty of Computer Science of the University of Debrecen. The algorithm is suitable for the quantitative characterisation of newly grown bone tissue on microscopic images of implants.

ACKNOWLEDGEMENTS

I would like to thank my supervisor Dr. Sándor Manó, a research associate of the Biomechanical Laboratory of the Department of Orthopaedics and Traumatology, University of Debrecen, for the many professional, practical, and human guidance and support provided during my work.

I express my gratitude to Prof. Dr. Zoltán Csernátó, who recently passed away. He was the Director of the Department and Clinic of Orthopaedics and Traumatology at the University of Debrecen and Head of the Biomechanics Laboratory. Thank you for allowing me to be involved in the work of the Biomechanics Laboratory and for the opportunity to write my thesis. He supported my progress with his valuable insights and advice.

I am grateful to Prof. Dr. Gábor Méhes, Director and Professor of the Institute of Pathology, Faculty of General Medicine, University of Debrecen, for his help in the histomorphological examination and analysis of bone implant samples.

I would like to thank Prof. Dr. Norbert Németh, Head of the Department of Surgical Surgery, Faculty of General Medicine, University of Debrecen and Dr. Ádám Deák, Adjunct Professor, Faculty of General Medicine, Department of Surgical Surgery, University of Debrecen for their advice and help in the design and implementation of the animal experiment.

Thanks to Dr. Dániel Szabó, a medical specialist at the Department of Orthopaedics and Traumatology, for his help in performing animal experiments.

Thanks to Dr. János Szabó, Associate Professor at the Department and Clinic of Orthopaedics and Traumatology, for his valuable and important professional advice and suggestions in the process of finalizing the thesis.

I would like to thank Dr. Mihály Braun of Isotoptech Zrt. for his support and professional and practical knowledge during the histological processing of bone implant samples, and Mihály Veres, the head of Isotoptech Zrt. for his support with the research equipment.

I would like to thank Dr. Balázs Harangi, Associate Professor of the Department of Data Science and Visualization at the University of Debrecen and Norbert Serbán PhD student for their help and active participation in the development and implementation of our image processing algorithm idea.

We would like to thank Prof. Dr. Géza Husi, Dean of the Department of Electrical Engineering and Mechatronics, Faculty of Technology, University of Debrecen, and Dr. Zsolt Tiba, lecturer of the Department of Automotive Engineering, Faculty of Technology, University of Debrecen, for their helpful advice and contribution during the biotribological investigation of lubricants.

Many thanks to Dr. Eszter Anna Janka, statistician, for her many useful tips and pragmatic insights.

Thanks to György Falk, director of Varinex Zrt., consortium leader of the GINOP 2.2.1-15.2017-00055 grant, for involving the Biomechanics Laboratory in the implementation of the research.

I would also like to thank the staff of the Biomechanics Laboratory for their help.

I would like to thank my family and friends for their support and understanding throughout the many years of my studies.

My doctoral thesis was supported by the GINOP grant 2.2.1-15.2017-00055.



Registry number: DEENK/522/2023.PL
Subject: PhD Publication List

Candidate: Ágnes Éva Kovács
Doctoral School: Doctoral School of Clinical Medicine
MTMT ID: 10076895

List of publications related to the dissertation

1. Kovács, Á. É., Csemátó, Z., Csámer, L., Méhes, G., Szabó, D., Veres, M., Braun, M., Harangi, B., Serbán, N., Zhang, L., Falk, G., Soós Horváth, H., Manó, S.: Comparative Analysis of Bone Ingrowth in 3D-Printed Titanium Lattice Structures with Different Patterns. *Materials*. 16 (10), 1-16, 2023.
DOI: <http://dx.doi.org/10.3390/ma16103861>
IF: 3.4 (2022)
2. Csemátó, Z., Manó, S., Tiba, Z., Husi, G., Jónás, Z., Váradi, T., Csámer, L., Kovács, Á. É.: Critical analysis of in vitro stability testing of spinal implants and proposal for standardization. *Expert Rev. Med. Devices*. 19 (3), 281-286, 2022.
DOI: <http://dx.doi.org/10.1080/17434440.2022.2054331>
IF: 3.1

List of other publications

3. Csemátó, Z., Manó, S., Szabó, D., Soós Horváth, H., Kovács, Á. É., Csámer, L.: Acetabular Revision with McMinn Cup: Development and Application of a Patient-Specific Targeting Device. *Bioengineering-Basel*. 10 (9), 1-13, 2023.
DOI: <http://dx.doi.org/10.3390/bioengineering10091095>
IF: 4.6 (2022)
4. Csámer, L., Csemátó, Z., Novák, L., Kövári, V. Z., Kovács, Á. É., Soós Horváth, H., Manó, S.: Custom-made 3D printing-based cranioplasty using a silicone mould and PMMA. *Sci. Rep.* 13 (1), 1-15, 2023.
DOI: <http://dx.doi.org/10.1038/s41598-023-38772-9>
IF: 4.6 (2022)





5. Kovács, Á. É., Csernátó, Z., Szabó, D., Csámer, L., Somoskeőy, S., Manó, S.: Csípőzületi vápadefektus-klasszifikáció megjelenítése 3D nyomtatással készült modellek segítségével. *Biomech. Hung.* 15 (2), 43-48, 2022.
DOI: <http://dx.doi.org/10.17489/biohun/2022/2/563>
6. Csernátó, Z., Deák, Á., Csámer, L., Kovács, Á. É., Soós Horváth, H., Csukás, D., Radovits, T., Manó, S.: Javaslat csontpótló anyagok juh teherbíró csonton végzett vizsgálatának standardizált módszerére. *Biomech. Hung.* 14 (2), 66-73, 2021.
DOI: <http://dx.doi.org/10.17489/2021/2/07>
7. Csernátó, Z., Zhang, L., Takács, K., Csámer, L., Kovács, Á. É., Manó, S.: Metal implants and MRI: a mythbuster study. *Glob. Imaging Insights.* 6, 1-4, 2021.
DOI: <http://dx.doi.org/10.15761/GII.1000215>
8. Manó, S., Kóvári, V. Z., Szabó, J., Csámer, L., Kovács, Á. É., Soós Horváth, H., Csernátó, Z.: 3D nyomtatás alapú cranioplasztika szilikon öntészeti módszerek és csontcement alkalmazásával. *Biomech Hung.* 13 (1), 49-56, 2020.
DOI: <http://dx.doi.org/10.17489/biohun/2020/1/05>
9. Manó, S., Kovács, K., Kovács, Á. É., Csámer, L., Csernátó, Z.: 3D nyomtatás és csontcement alapú cranioplastica mérése mechanikai szempontból. *Biomech Hung.* 13 (1), 29-39, 2020.
DOI: <http://dx.doi.org/10.17489/biohun/2020/1/03>
10. Csernátó, Z., Kovács, Á. É., Csámer, L., Zhang, L., Manó, S.: Állatkísérleti modell kidolgozása rágcsos mikroszerkezetű titán implantátumok értékelésére. *Biomech Hung.* 13 (1), 17-28, 2020.
DOI: <http://dx.doi.org/10.17489/biohun/2020/1/02>





11. Kovács, Á. É., Manó, S., Csámer, L., Somoskeőy, S., Csernátó, Z.: Scoliosis klasszifikáció szemléltetése 3D nyomtatással előállított modellgyűjteménnyel.
Biomech Hung. 13 (1), 7-15, 2020.
DOI: <http://dx.doi.org/10.17489/biohun/2020/1/01>

Total IF of journals (all publications): 15,7

Total IF of journals (publications related to the dissertation): 6,5

The Candidate's publication data submitted to the iDEa Tudóstér have been validated by DEENK on the basis of the Journal Citation Report (Impact Factor) database.

24 November, 2023

