Thermoanalytical examination of different tissue samples collected from patients underwent shoulder replacement

PhD dissertation

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Introduction:

In the recent decades, the number of arthroplasties has been significantly increased, due to the continuous development of prostheses and surgical techniques. Among arthroplasties, knee and hip prosthesis implants are performed most often.

The third most frequent prosthesis implantation is the shoulder replacement. Because of the increasing average age, shoulder arthroplasties are performed with orthopedic indications due to degenerative changes and diseases of the shoulder joint more frequently. From a traumatological point of view, an increasing number of shoulder arthroplasty is done worldwide, for the treatment comminuted, intraarticular 4-part proximal humerus fractures. The first generation of shoulder prostheses was developed by Professor Neer in the early 1950s. Since then, almost 70 years passed and the shoulder implants have undergone significant development. Nowadays total, reverse total, hemi and partial resurfacing prostheses are available for the treatment of shoulder joint arthritis, advanced rotator cuff (ROC) damage and intraarticular 4-part fractures associated with significant displacement.

At our department (Traumatology – Orthopaedics, 'Balassa János' Hospital of Tolna County), we have been performing shoulder arthroplasties with orthopedic and trauma indications since 2019.

Scientific background:

There are several protocols available to support an orthopedic indication for shoulder replacement. In case of trauma indications (4-part fractures), the selection of the appropriate implant type could be challenging. The MRI examination, known as the 'gold standard' for assessing the condition of the shoulder joint, is not available in all cases. The use of MRI is limited by older metal implants, the presence of a pacemaker, or claustrophobia. Histological examinations can provide an accurate picture of the damage to a specific structure, but their preparation is time-consuming. In addition, most classifications (Hamada, Walch) based on non-MRI examinations (CT, US) indirectly assess the state of ROC and were basically developed for non-traumatic cases.

There is a need for novel examination methods that could be successfully used in the future as a supplement to medical imaging or histological examinations and could help determine the stage of shoulder joint degenerative diseases. Also, these methods could provide further details about the biochemical background of ROC, hyaline cartilage and subchondral bone damage. It would be important especially for trauma patients, since in their cases, the preoperative functional tests cannot be performed to assess the condition of the ROC and the range of motion (ROM) of the shoulder joint.

Aims and hypotheses:

Differential scanning calorimetry (DSC) has previously been used successfully in the research of degenerative and inflammatory diseases of the skeletal muscle system, to detect structural changes in collagen. Furthermore, TG / DTA (thermogravimetry / differential thermal analysis) also provides reliable information about the thermal stability of the bone stock and its composition based on mass loss.

<u>Hypotheses:</u>

- *We hypothesized that differential scanning calorimetry (DSC) and thermogravimetry / differential thermal analysis (TG / DTA) are effective tools for examining samples collected during shoulder arthroplasties with different (orthopedic, traumatological) indication, to assess the condition of ROC, hyaline cartilage and subchondral bone.*
- *We hypothesized that different thermoanalytical examination methods (DSC, TG, DTA) could help to determine the condition of ROC, hyaline cartilage and subchondral bone. Therefore, more data could be obtained about the structural, degenerative changes of human tissue samples.*
- *We further hypothesized thermoanalytical evaluations of structural changes of the examined tissues could provide clinically relevant information, beside the standard examination methods (imaging diagnostics, histology). These results could help in the selection of proper prosthesis type and thus could significantly the patients' further life quality.*

To confirm our hypotheses, we are proposing the experiments:

Planned examinations:

1) **Assessment of rotator cuff (ROC) degeneration:** samples collected from patients undergone shoulder joint prosthesis surgery will be subjected to macroscopic,

histological and calorimetric tests (DSC). The radiological stage of the ROC damage will be confirmed with radiological imaging performed prior to the operation.

With our investigations, we are planning to evaluate the following hypotheses:

A) Does differential scanning calorimetry reliably indicate the degenerative changes in the collagen content of the ROC tendon in human samples collected during shoulder arthroplasty?
B) Does the degree of damage measured by DSC correlate with the radiological stage determined by medical imaging?
C) Could the DSC test help in the more accurate selection of the type of prosthesis analyzing human tissue samples taken during shoulder arthroplasty?

2) **Assessment of hyaline cartilage and subchondral bone damage:** various cartilage and bone samples collected during shoulder replacement will be subjected to macroscopic, histological, differential calorimetry (DSC) and thermogravimetry (TG/DTA) tests. The radiological stage of joint degeneration will be determined with the help of imaging performed before the operation.

With our investigations, we are planning to evaluate the following hypotheses:

A) Does differential scanning calorimetry / thermogravimetry reliably indicate cartilage wear and aseptic bone necrosis in human samples taken during shoulder arthroplasty?
B) Does the degree of damage indicated by DSC / TG/DTA correlate with the stage determined by the radiological examination?
C) Could DSC / TG/DTA test help in the more precise selection of the type of prosthesis with analyzing human tissue samples taken during shoulder arthroplasty?

3) **Comparative analysis: comparison of orthopedics and traumatology samples:**

With our investigations, we are planning to evaluate the following hypotheses:

A) Is there a difference in the degree of ROC, cartilage and bone damage comparing orthopaedic and trauma patients, based on radiological analysis?
B) Is there a difference in the thermoanalytical parameters of the samples taken from orthopaedic and trauma patients?
C) Based on our data, could thermoanalytical measurements be utilized as an additional clinical test in the further biochemical analysis of samples taken intraoperatively from orthopaedic and trauma patients?

Patients:

Adult patients with acute shoulder joint injury (fracture) or chronic, degenerative joint changes (pain, loss of function, signs of inflammation) were included to the study. These patients, based on clinical evaluation, required shoulder replacement with trauma or orthopaedic indications.

Demographic data:

Since 2019, a total of 51 patients have received hemi or reverse shoulder replacement in our department. By gender, the male:female ratio was 13:38. At the time of surgery, the average age (mean ± SEM, min. – max., years) was 69 ± 1.1 (51 – 86) years.

We performed shoulder arthroplasty in a total of 36 cases with a traumatological indication. In this group, all patients had comminuted proximal humerus with significant dislocation, the fracture line extending to the collum anatomicum as well.

Regarding the mechanism of injury, low-energy injuries (n = 31) were predominantly found. Therefore, the existing osteoporosis may be a significant risk factor for the comminuted. 4-part proximal humerus fractures. High-energy injuries (n = 5) - such as "falling from a height" or "being run over by a vehicle" - occurred only in a small number of cases. Epilepsy (n = 1), drunkenness (n = 1) and general deterioration caused by COVID-19 infection (n = 1) were occasionally included as other factors in the cause of injuries.

In a total of 15 cases, the prosthesis implantation was performed with orthopedic indications. In these cases, reverse shoulder arthroplasty was done with cemented (n = 4) or cementless (n = 11) stem. In all patients with orthopedic indications, advanced rotator cuff tear arthropathy (RCA) was the indication for surgery, often accompanied by a history of degenerative and/or autoimmune disease.

Medical imaging studies:

The degenerative changes of the glenohumeral joint were analyzed using the different type of classifications: for ROC degeneration the Hamada classification, for degree of glenohumeral arthrosis the Walch classification, for degree of cartilage damage: Outerbridge classification was applied. Optimally, it would be important to perform both CT and MR examinations, since the different classifications are based on different imaging modalities. For the preoperative planning of surgeries with orthopedic indications, we also have the option for implant planning software, using native X-ray images or CT-scans.

CT scans were performed with a Siemens Somatom Perspective Dual 64/128, and MR examinations were done with a Siemens Magnetom Essenza 1.5 T type device. The radiological evaluation is part of the routine surgical planning and does not mean any extra burden for the patients.

Surgical technique and sample collection

During the operations, DePuy Synthes DeltaXtend reverse shoulder prosthesis or Global FX hemiprosthesis were implanted. We collected only tissues for our experiments that were supposed to be removed during the interventions, as part of the surgical procedure.

The operations were performed under general anesthesia (ITN), and scalenus blockade was used as part of postoperative pain relief. The shoulder joint was exposed through deltopectoral approach. After opening the joint capsule, we identified the tuberculum maius humeri, on which three of the four members of the ROC insert (m. supra- et infraspinatus, m. teres minor). During the implantation of a hemiprosthesis, the integrity and proper fixation of the tubercles determines the subsequent function of the shoulder joint.

We cut through the m. biceps brachii long tendon at its point of origin. The humeral head was removed with an oscillating saw. After opening the medullary cavity, we cemented the stem of the appropriate size into the medullary cavity. In case of hemiprosthesis implantation, the glenoid fossa was left intact, the remaining tuberculum maius was attached to the stem with suture material. If there was an advanced degeneration of the shoulder joint and the function of the ROC was already missing, a reverse total shoulder prosthesis was implanted, during which a cup was also implanted. In this case, we also took cartilage tissue (5x5x2.5 mm size) and subchondral bone sample (5x15 mm bone cylinder) from the glenoid fossa – of the so-called glenohumeral contact area (GCA).

For the control group, samples were taken from young patients who suffered proximal humerus fracture (4-part fracture with significant dislocation) and the restoration of the joint surface was no longer possible. The criterion was that patients in the control group cannot have degenerative or inflammatory changes in the shoulder joint.

During the operations, samples were collected from the following tissues: humeral head cartilage, humeral head subchondral bone, rotator cuff (supraspinatus tendon attached to the tuberculum maius), biceps tendon intra-articular section, glenoidal cavity cartilage, subchondral bone of glenoidal cavity. To examine the ROC tendons, an approx. 5x5x10 mm tissue sample was usually taken from the supraspinatus tendon (an approx. 1.5 cm length of intraarticular part, corresponding to its critical zone). A 5x5x2.5 mm hyaline cartilage sample was taken from the surface of the humeral head (corresponding to the humerus - glenoid fossa contact surface). Another sample was collected from the subchondral bone of the humeral head and from the subchondral bone of the glenoid fossa with a 5 mm inner diameter cylinder drill.

The samples required for histological sections were placed in 4% formalin, and the tissue samples required for DSC and TG/DTA examinations were placed in cold physiological saline solution, then transported and stored deep-refrigerated until further processing.

Differential scanning calorimetry (DSC):

The stored samples were washed three times e and remained in a sterile buffer on 4°C before starting the calorimetric examinations (max. half an hour). The measurements were made by a SETARAM Micro DSC-II calorimeter between 0 and 100 °C with a heating rate of 0.3 K/min. Conventional closed Hastelloy batch vessels (V=1 mL) were used for the experiment to perform the thermal denaturation. Samples' masses were between in mgs: 100-150. The sample buffer was used as a reference. The sample and reference vessels were equilibrated with a precision of ±0.1 mg. There was no need to do any correction from the point of view of heat capacity between sample and reference vessels. With the help of a two-point setting SETARAM peak integration calorimetric enthalpy was calculated from the area under the heat absorption curve then the other thermal parameters (denaturation or melting temperature (T_m), range of denaturation (ΔT), half width of transition ($T_{1/2}$) and calorimetry enthalpy (ΔH_{cal}) data of samples) were compared. After ASCII conversion, the data was processed using the Origin 6.0 program.

If the thermal properties of the sample and the reference are the same, there will be no phase transition. Therefore, equal amount of energy is needed to the same temperature changes and thus the output signal will be zero. If an endothermic (heat-absorbing) process initiates in the sample as a result of structural transformation, its temperature can only follow the temperature change of the reference cell by supplying extra energy to maintain the difference between the two cells on zero. This extra amount of energy, which causes a change in heat capacity during a process at constant pressure (hermetically sealed cell), appears as an output signal as a function of temperature.

Thermogravimetry (TG) and Differential thermal analysis (DTA):

The thermoanalytical investigation of bone samples was performed by an SSC/5200 TG/DTA equipment made by SII Seiko Instruments (Japan). The sample holders were open aluminum crucibles with a diameter of 5.2 mm and a depth of 5 mm. The investigated temperature range was from ambient up to 550 °C (aluminum sample holders). The applied heating rate was between 10-40 K/min (in 10 K steps). Measurements were performed under an inert nitrogen gas with a flow rate of 100 mL /min. The detected signals were DTA („heat flow"), TG (mass loss in %) and DTG („speed" of mass loss) curves.

During the thermogravimetric analyses, the percentage change in mass of the tested material were registered as a function of the temperature increase. The obtained thermogram provides information about sample's thermal stability. DTG (derivative thermogravimetry) is registered at the same time as the TG curve and represents the derivative TG curve, calculated from the data points of the TG curve.

In case of differential thermal analysis (DTA), the tested sample (with an inert substance) is heated at a standard rate, while the temperature difference between the two substances is measured and registered as a function of heating. These data reflect to the enthalpy change in the examined sample.

Statistical analyzes:

The statistical analyses of DSC data were performed using GraphPad Prism 6 (GraphPad, San Diego, CA) and SPSS 2 6.0 (SPSS, Chicago, IL) software, where differences of $p < 0.05$ were considered statistically significant. For the evaluation of thermogravimetric tests, the correlation analysis and the creation of graphs were done using the MS Excel software.

DSC examination of rotator cuff samples

The results of both of the orthopaedic and trauma samples were compared to the control sample. The heat flow curves were calculated from the average of three runs, normalized to the weight of the wet sample. The consequence of the advanced tendon damage (B1) as well as specifically advanced tendon damage (B2) compared to the control is well demonstrated by the shape and the running of the curves.

The significant decrease of the calorimetric enthalpy well demonstrates the structural consequence of the medical abnormality. The change in the baseline after the denaturation can be a sign of a heat capacity increase, demonstrating a more compact (more densely packed) structure as a consequence of less elasticity of the tendon (Fig. 1 – Fig. 2).

In case of trauma samples, the moderately degenerated tendons (A2) exhibited significant difference from the control in the shape of the scans and in the thermal parameters, too. The heat denaturation curve of the A2 sample compared to the other trauma samples confirms the degeneration of the ROC existing prior to injury. According to the X-ray examination performed before the operation, it was classified as "stage 4b", based on the Hamada classification. At the same time, the other samples of trauma origin showed much smaller thermodynamic differences compared to the control.

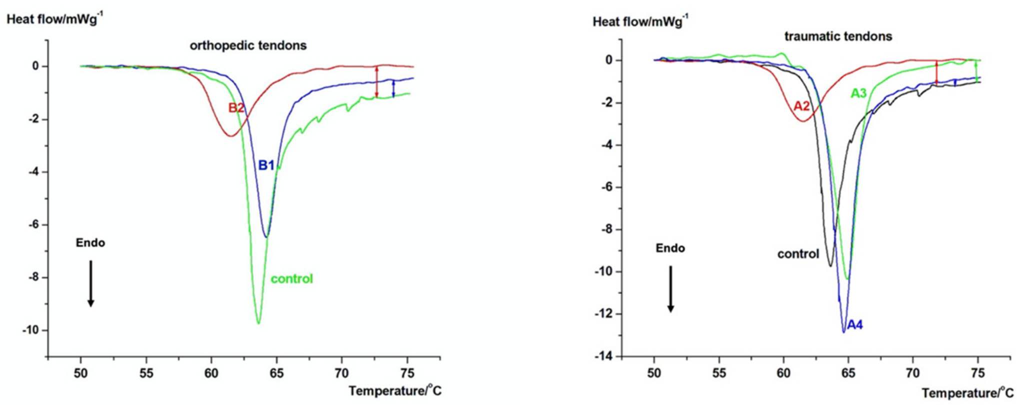


Fig. 1. – 2.: Denaturation curves of the ROC tendons (orthopedic indication: B1 and B2; traumatological indication: A2, A3 and A4, and the healthy (control) tendon)

The DSC curves demonstrated a clear difference in the thermal parameters of healthy and pathologic rotator cuff samples of patients underwent reversed shoulder replacement with orthopaedic and trauma indications.

DSC examination of hyaline cartilage samples

The hyaline cartilage contains type II. collagen. The different types of collagen proteins can form a rigid (bone), soft (tendon) or transitional (cartilage) structure. Differential scanning calorimetry can be used to determine the enthalpy (heat quantity) changes associated with physical-chemical transitions and the temperature at which structural changes occur.

The denaturation is the process during which the macromolecules lose their quaternary and then tertiary structure, i.e. the polypeptide chains unfold.

ΔT (ºC) indicates the difference between the beginning and end of denaturation. T_m is the denaturation temperature at which almost 50% of the sample has been unfolded. The more thermally stable the sample is, the higher the temperature the collagen will "disintegrate". ΔH is the mass-normalized amount of energy required for the given structural change to occur.

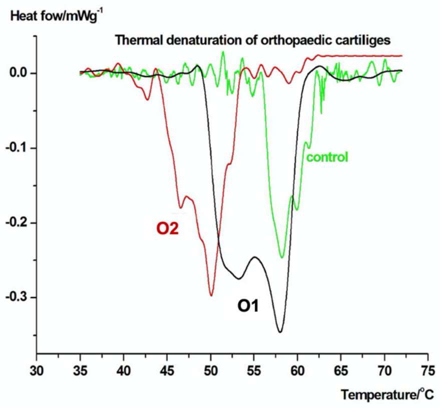
The changes of the thermal parameters can reflect to the structural differences of the cartilage, especially to type II. collagen's structural changes.

Our main observation regarding the origin of the samples and the consequence of the disease is that the denaturation temperature range and the half-width of the heat flow curves are significantly wider in the orthopedic samples compared to the control and traumatic samples (Fig. 3-4). These facts show the thermal consequence of the less cooperativity among the different structural domains of the orthopaedic cartilage.

The calorimetric enthalpy exhibited also significant differences in both samples referring to the control. Because we have no biochemical background to separate the different compounds of cartilage, it would be difficult to interpret the 'big jumps' in O1 and O2, compared to the control. The melting temperatures (T_m) show that structural change caused by disease (O2) is greater than in case of a traumatic impact.

Compared to orthopedic samples, in case of trauma samples, T_m shifts towards higher temperatures. The broadening of the heat denaturation curve appears less marked, thereby indicating that the cooperativity is greater, so the collagen content of the samples is more thermally stable, i.e. they have better preserved their tertiary and quaternary structure compared to the orthopedic samples.

Based on the shape of the DSC curves and the thermal characteristics, we found that the structural changes caused by degenerative diseases are greater than the changes observed in patients operated with a trauma indication. Structural damage seen in trauma patients can be either a pre-existing condition or the effect of the trauma itself.



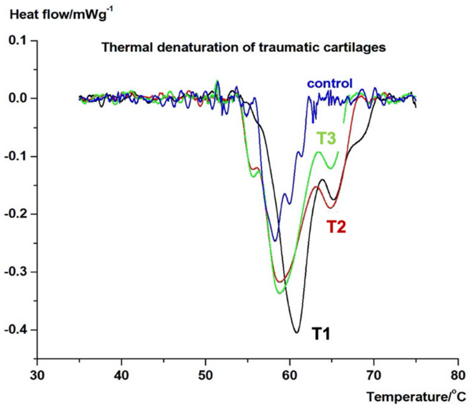


Fig. 3. – 4.: Heat denaturation curves of hyaline cartilage samples obtained from orthopaedic (O1, O2) and trauma patients (T1, T2 and T3), compared to healthy hyaline cartilage samples.

Thermogravimetric analysis of cancellous bone samples of humerus head

Prior to operation, quality of cancellous bone stock of humerus head cortical-like bone of the glenoid should be evaluated. The existence of severe osteoarthritis and AVN (avascular necrosis) of the head require different planning and may exclude the use of certain type of prosthesis, such as stemless implants. Therefore, a bone cylinder with a diameter of 5 mm and a length of 15 mm was collected from the removed humerus head during surgery.

Thermogravimetric examinations have already been proven useful *in vitro* and *post mortem* analyses of bone tissue. Therefore, we hypothesized that thermogravimetric analysis of human humerus head samples would indicate difference in the bone quality of orthopaedic and trauma patients. We expected poorer bone stock and less mineralization in orthopaedic samples with existing osteoarthritis or cuff tear arthropathy (CTA), compared to trauma samples. We also hypothesized that the bone loss in orthopaedic patients would correlate with the grade of degenerative changes.

The control sample was collected from a young patient with dislocated 4-part proximal humerus fracture and was considered macroscopically as a healthy cancellous bone. This finding was confirmed by histological examination. Samples from orthopedic surgery ranged in mass from 14 to 110 mg, while traumatic test samples ranged in mass from 12 to 49 mg.

The thermoanalytical investigation of bone samples was performed by an SSC/5200 TG/DTA equipment made by SII Seiko Instruments (Japan). The sample holders were open aluminum crucibles with a diameter of 5.2 mm and a depth of 5 mm. The investigated temperature range was from ambient up to 550 °C (aluminum sample holders!). The applied heating rate was between 10-40 K/min (in 10 K steps). Measurements were performed under an inert nitrogen gas with a flow rate of 100 mL/min. The detected signals were DTA („heat flow"), TG (mass loss in %) and DTG („speed" of mass loss) curves. Data are averages of three measurements.

The DTA result in case of control sample exhibited four well separable thermal transitions (75-103-335 and 407 °C) and about a total 40 % mass loss in the scanned temperature range. The DTG curves (they are not plotted) followed the DTA curves in case of the first two peak temperatures with 1.5 mg/min and 2.1 mg/min, but in case of the higher thermal transitions we could detect only one DTG peak around 402 °C with 2.7 mg/min value.

The sample A1 showed transitions around 50 (inflection) and 120 °C with twice a bigger calorimetric enthalpy as the control. The denaturation in higher temperature range shifted to 348 and 423 °C with practically same enthalpies. The mass loss was smaller, about 30%. The DTG exhibited at 100 °C higher, 4 mg/min value, and at 414 °C 2.7 mg/min, also higher than for control.

A2 sample showed the biggest difference compared with either control or any orthopaedic and traumatic case. A single transition at ~ 50 °C, a well separable double denaturation in the vicinity of 150 °C, as well as the others at 340; 420 and 500 °C. It is very surprising that the total calorimetric enthalpy in 20-220 °C much lower than in case of control. The enthalpy contribution in each three denaturation phases higher compared with the control. The final mass loss is ~ 60%, the DTG values ~ 48 °C 0.23 mg/min, ~ 137 °C 0.25 mg/min and ~ 150 °C 0.18 mg/min. In the higher range these values are at 338 °C 0.31 mg/min, at 410 °C 1.2 mg/min.

In case of A3 sample we can distinguish thermal transitions at 90, 110 and 150 °C. The enthalpy contribution of these three peaks is in the range of the control in 20-210 °C interval. The enthalpies at higher temperature range are also similar to the control. The full mass loss 35 %, the DTG values are at 85 °C 1.12 mg/min and at 408 °C 3.3 mg/min.

At first glance a very definite different denaturation process can be seen, compared with the orthopedic samples. The graphs clearly show the differences between the trauma and orthopedic samples (Fig. 5. – 6.).

The O1 orthopedic sample showed twice a big thermal effect than the control one. The lower temperature peaks shifted to 50 and 107 °C and the endotherm effect was four times bigger than in case of control. The higher transitions remained roughly at the same temperatures

(333 and 404 °C). The twice a greater final mass loss jumped out from the three graphs (DTG was at 107 °C ~ 4.6 mg/min, and 1.3 mg/min at 390 °C).

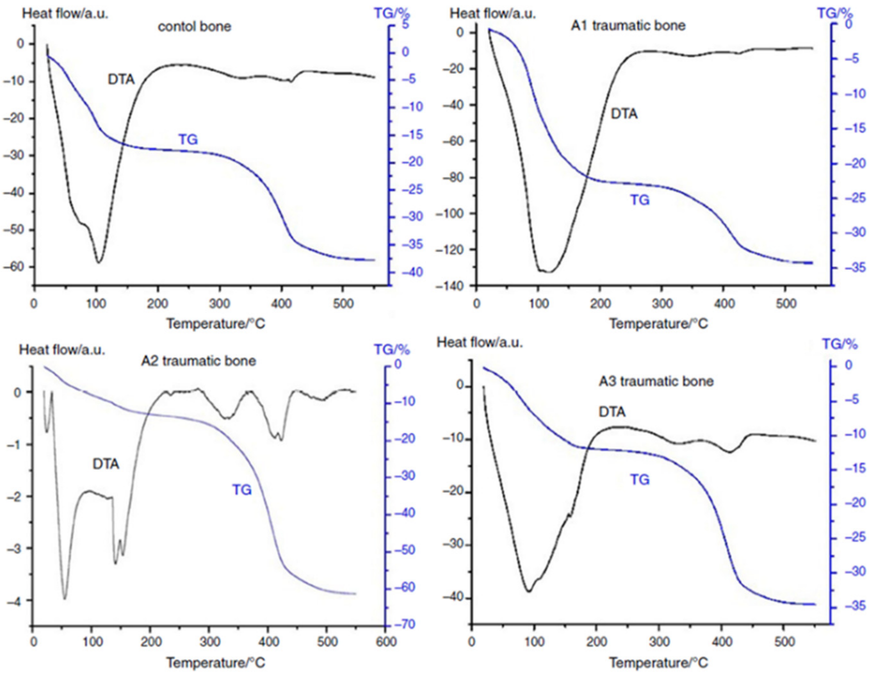


Fig. 5.: TG/DTA curves of the control and trauma samples (the data are averages of three measurements)

The O2 sample exhibited in the characteristic and denaturation heat similar tendency as it was in case of the control. The two lower denaturation peaks separated more clearly (38 and 118 °C) resulting same calorimetric enthalpy. In the higher temperature range instead of two (see control) three denaturation peaks appeared (333, 388 and 485 °C), where the enthalpy contribution of denaturation at 333 °C was greater than that of control at 335 °C and much greater than the second one at 388 °C (this last one was smaller than that of the similar control at 407 °C). The total mass loss was about 40%, and DTG values for the biggest lower transition were 1.43 mg/min and at 380 °C 2.3 mg/min.

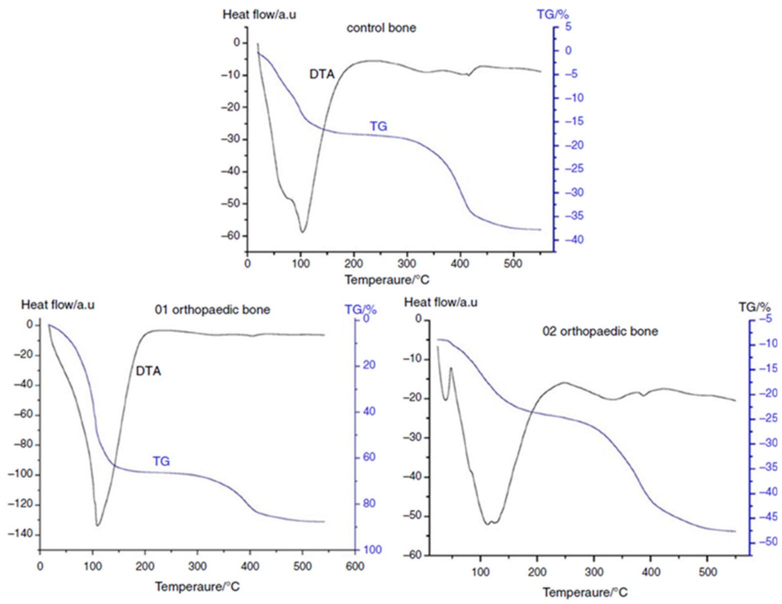


Fig. 6.: TG/DTA curves of the control and orthopedic samples (the data are averages of three measurements)

Relationship between the level of tissue damage, degeneration and thermal parameters

Regression analyzes have been performed to find a relationship between the thermal parameters and other indicators of degenerative processes, such as macroscopic scores or radiological evaluations.

Of the cartilage samples, ‘moderately strong’ relationship was observed between osteoarthritis score and denaturation temperature range ($R^2 = 0.74$, $p = 0.06$, trend to statistical significance). Furthermore, plotting the osteoarthritis score as predictor and half width at the highest heat flow as a dependent variable also demonstrated similar pattern ($R^2 = 0.76$, $p = 0.05$, trend to statistical significance).

Comparing the outcome of the radiological evaluation and the calorimetric measurements, causal relationship between the degree of arthritis (osteoarthritis score) and the calorimetric enthalpy was found at the lower temperature peaks ($R^2 = 0.61$, $p = 0.067$, trend toward significance). It must be noted that by removing one outliner, the correlation would be much stronger ($R^2 = 0.92$). Authors suggest that with a larger sample size, the data could show a statistically significant, strong correlation between radiological and thermal data.

We have demonstrated moderately strong relationship between thermal parameters and osteoarthritis scores, using regression analysis in case of hyaline cartilage samples and subchondral cancellous bone samples. Interestingly, in case of the rotator cuff samples, there were no statistically supported correlation found between the radiological scores and outcome of thermal measurements. Possible explanation is that osteoarthritis score is based on radiological morphology of glenohumeral joint, describing the bone consistency, joint space, etc. Neither conventional radiographs, nor CT-scans can provide details about the degree of rotator cuff tendon damage. The likely degenerative changes of rotator cuff tendon could only indirectly be concluded from radiological morphology of the joint.

The differences between samples of orthopaedic or trauma origins are not always clear. Trauma patients may have symptoms of pre-existing osteoarthritis, and orthopedic patients may develop signs of AVN as a result of previous trauma. We probably observed a combination of the increased effect of degenerative disease and acute trauma in the "outlining" trauma pattern.

Therefore, we believe that data showing differences in the state of ROC, hyaline cartilage, and subchondral bone between trauma patients and orthopedic patients can help in surgical planning and improve therapeutic protocols.

Discussion:

Based on our results, DSC has been proved to be an effective method for examining hyaline cartilage and ROC samples of traumatological and orthopedic origin. DSC curves showed a clear difference between the thermal parameters of the healthy and pathological rotator cuff samples of patients undergoing reverse shoulder joint prosthesis implantation. Similarly, the degree of hyaline cartilage damage can also be evaluated based on on the DSC curves.

Therefore, DSC could be a useful additional method in evaluating the degree of GH joint arthrosis and rotator cuff tear arthropathies in future studies.

DTA/TG analysis of human humeral head samples showed significant differences in the bone quality of orthopedic and trauma patients, compared to controls.

Despite recent advances in protocols and guidelines, there is still a need for studies investigating the biochemical basis of ROC degeneration, hyaline cartilage, and subchondral bone damage. These tests could give the surgeon a more detailed picture of the expected extent of arthritis, especially in circumstances where MRI scan is not available.

Novel findings:

1. The thermal characteristics of rotator cuff (ROC) samples collected from patients undergoing reverse shoulder arthroplasty were found to reliably reflect changes in collagen structure and the amount of degradation using differential scanning calorimetry.

2. With the help of DSC studies, we could demonstrate significant differences comparing the thermoanalytical parameters of ROC samples collected during operations with orthopedic and traumatological indications, compared to the healthy control.

3. DSC thermal characteristics of hyaline cartilage samples obtained during reverse shoulder surgeries with orthopaedic and trauma indications followed the degenerative changes of the hyaline cartilage structure.

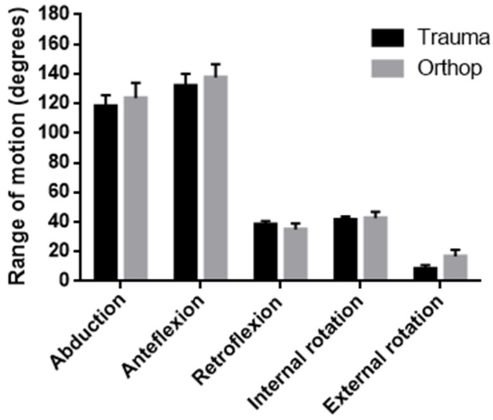
4. The thermal parameters of hyaline cartilage samples of orthopaedic and trauma origin showed a clear difference, based on differential scanning calorimetric measurements.

5. The TG / DTA (thermogravimetry / differential thermal analysis) examination of the subchondral bone collected during surgeries indicated significant differences in the thermal parameters of the orthopedic and trauma samples, compared to the control. In case of orthopedic samples, thermogravimetry (TG) confirmed a lower quality of bone stock with less mineral contents.

6. Analyzing the outcome of radiological evaluations and thermal measurements, in case of hyaline cartilage, the denaturation temperature (T_m) and half-width of maximum heat flow ($T_{1/2}$) showed a moderately close relationship to the osteoarthritis score (OA). Similar correlation was found between the OA score and the calorimetric enthalpy ($\Delta Hcal$) of subchondral bone samples.

7. The thermal analyzes provide a deeper insight into the changes in the damaged structures of the operated shoulder joint (collagen structural change, bone structure changes, degree of mineralization) and some denaturation parameters also have predictive value. Based on tour findings, thermal analysis can be useful in the further investigation of the complex processes underlying orthopedic and post-traumatic shoulder diseases and in the selection of the proper type of prosthesis.

8. The comparison of the range of motion (ROM) of the orthopedic and traumatological cases indicated that there is no significant difference between the two group in terms of the range of motion achieved after surgery (Graph. 7.).



Graph. 7.: Comparison of the postoperative ROM of orthopaedic and trauma patients, 12 months after surgery. Statistical analysis: two-way ANOVA, data are mean ± SEM (standard error of the mean). There is no significant difference in ROM between the two groups.

9. In our study, we evaluated the degree of pain by using the visual analogue scale (VAS). It is a one-dimensional pain measurement scale, which practically consists of a horizontal axis on which the patient must indicate the degree of pain. One endpoint of this is "no pain at all" (= 0), and the other is "unbearably painful" (= 10). The average of the values before and after orthopedic surgery (12 months) is shown in Graph. 8., which demonstrates that the surgery significantly reduced the patients' pain.

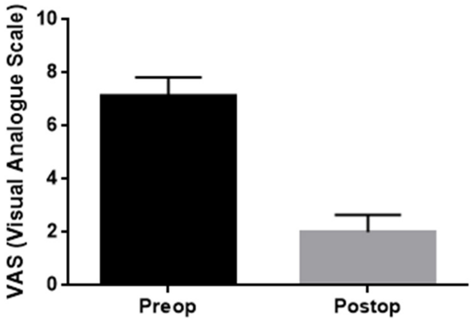


Fig. 8: Orthopaedic cases. VAS (visual analog scale) before and 12 months after operation. Statistical analysis: Student's t-test, data are mean ± SEM. The VAS showed a significant decrease 12 months after surgery, indicating a significant decrease in the shoulder pain.

7. Future plans:

- In the future, we plan to further investigate the structural differences between samples of different origins in a larger number of cases.
- We also plan further DTA/TG examination of the bone samples in order to distinguish the calorimetric parameters of different bone layers.
- The separation of the different layers of the cartilage is beyond the scope of our current study; however, further investigation could be conducted.
- With a larger number of cases, we propose further statistical analyzes to support the correlation between the radiological findings and thermal parameters.

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- To all **co-workers of the Thermoanalytical Laboratory** of the Department of Biophysics
- To **Dr. Hajnalka Szabó** – radiologist consultant ('Balassa János' Hospital of Tolna County)
- To **Dr. János Cifra** – pathologist consultant ('Balassa János' Hospital of Tolna County)
- To our **patients** who participated in the study and consented to the sampling required for the experiments

Publications related to the dissertation:

Nöt LG, **Bata A**, Szabó H, Cifra J, Lőrinczy D. DSC examination of rotator cuff damage in patients with total shoulder arthroplasty. *J Therm Anal Calorim.* 2021;146:165–70. https://doi.org/10.1007/s10973-020-10402-w ***IF: 4.755***

Bata A, Nöt LG, Szabó H, Cifra J, Lőrinczy D. DSC examination of cartilage damage of patients undergoing shoulder replacement. *J Therm Anal Calorim*. 2022;147:1275–80. https://doi.org/10.1007/s10973-020-10421-7 ***IF (2021): 4.755***

Bata A, Nöt LG, Szabó H, Cifra J, Lőrinczy D. Thermogravimetric analysis of cancellous bone of humerus head in patients undergoing total shoulder arthroplasty. *J Therm Anal Calorim.* 2022;147:3107–15. https://doi.org/10.1007/s10973-021-10702-9 ***IF (2021): 4.400***

Sum of impact factors of first authored publications: ***9.510***
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Conference abstracts related to the dissertation:

1. **Bata A,** Nőt LG, Lőrinczy D: Vállízületi protézis műtéten átesett betegek porckárosodásának vizsgálata termoanalítikai módszerrel. *Magyar Traumatológus Társaság 54. Kongresszusa.* Tapolca, 2021. október 8., *A-0004*

2. Lőrinczy D, **Bata A**, Lábas Z, Szabó H, Cifra J. Nöt LG: Thermal analysis of tendon, cartilage and bone samples of patients underwent shoulder replacement with differential scanning calorimetry (DSC) and thermogravimetry (DTA/TG). *Chemistry towards Biology (CTB10)*. Bratislava, Slovakia, 11-14. 09. 2022. Oral presentation.

3. Nöt LG, **Bata A**, Lábas Z, Szabó H, Cifra J, Lőrinczy D: Thermal analysis of biceps tendon of patients underwent reversed shoulder arthroplasty due to comminuted proximal humerus fractures. *3rd Journal of Thermal Analysis and Calorimetry Conference and 9th V4 (Joint Czech-Hungarian-Polish-Slovak).* June 20-23 2023, Poster presentation.