*Journal of Thermal Analysis and Calorimetry, Vol. 95 (2009) 3, 805–808*

# CALORIMETRIC PROPERTIES OF DEGENERATIVE HUMAN SHOULDER JOINT HYALINE CARTILAGE

*J. Csotye*[1], *Z. Aigner*[2], *G. Sohár*[1], *Piroska Szabó-Révész*[2] *and K. Tóth*[1]*

[1]Department of Orthopaedics, University of Szeged, Faculty of Medicine, Semmelweis u. 6, 6725 Szeged, Hungary
[2]Department of Pharmaceutical Technology, University of Szeged, Faculty of Pharmacy, Eötvös u. 6, 6720 Szeged, Hungary

The glenohumeral joint is not a classical mass bearing joint, the treatment of primary osteoarthritis is conservative. In all other cases, when the arthritis is associated with unbalance of the soft tissues, the treatment solution of this pathology is arthroplasty. The purpose of this study was to examine the altered metabolism in human degenerated cartilage of the shoulder joint. With the rise of temperature an endothermic reaction was observed in all cases. The use differential scanning calorimetry as part of thermal analysis was a reliable method for differentiating normal hyaline cartilage from degenerated samples.

***Keywords:*** *DSC, human hyaline cartilage, osteoarthritis, shoulder*

## Introduction

The shoulder is the most mobile joint of the human body, it is able to place the arm and hand on all positions in space. A number of muscles, tendons, ligaments and bones, that move the shoulder joint have a harmonic coordination, and this very complex guidance makes the shoulder joint so mobile. For the stability of the joints, in all cases, there is the need for its components harmonic and well coordinated movement. In all other, deviant cases the movement of the joint becomes disharmonic, leading to instability [1–3].

The instability of the shoulder joint can cause osteoarthritis because of the changed movement, the changed placement of the humeral head. The stable points in the joint, which provides the safe motion, are changed. The tension of the inferior glenohumeral ligament is lower, therefore the proprioception suffers. In this status, the proper nutrition of the cartilage is damaged, and the smooth surface becomes rough, causing pain. By the time, osteophytes appear, and the range of motion decreases [4–6].

Because the glenohumeral joint is not a classical mass bearing joint, the treatment of the primary osteoarthritis is a conservative one, and with this the symptoms can be maintained in a good balance for a long time. In all other cases, when the arthritis is associated with unbalance of the soft tissues, the treatment is difficult, and the solution of this pathology is arthroplasty.

Osteoarthritis (OA) is a disease characterized by degeneration of cartilage and its underlying bone within a joint. The breakdown of these tissues eventually leads to pain and joint stiffness [7]. Arthritis is one of the most prevalent chronic health problems. OA is second only to ischemic heart disease as a cause of work disability in men over age 50 years [8]. The prior notion of osteoarthritis (OA) as a 'wear and tear' of the joint has given way to views of the new paradigm of OA, considering it as a heterogeneous disease with numerous factors (mechanical and molecular) leading to its pathologic hallmark of cartilage loss [9, 10].

DSC measures the temperatures and heat flow associated with transitions in materials as a function of time and temperature. There are many possible applications of thermoanalytical techniques: characterizations of active and inactive ingredients, routine analysis, and qualitative control. Calorimetry can be used for qualitative and quantitative analyses [11, 12]. $\Delta H$ can often be determined for an unknown reaction in a complex system, and the value of $\Delta H$ can then be used to assist in identifying the reaction of the system. $\Delta H$ is the enthalpy change of the process initiated by the temperature change. The change of energy in thermal processes can be measured. Analysis of thermodynamic and kinetic data from calorimetry always involves a model for the system, e.g. a set of chemical reactions, kinetic equations, or a theoretical model for the property as a function of temperature, pressure, or composition. Calorimetric data will be fitted to the model to obtain model parameters, and thus provide a description of the system as a function of the experimental variables [13].

Degenerated cartilage from the shoulder joint has not been studied previously. Only one research

* Author for correspondence: tothk@orto.szote.u-szeged.hu

*1388–6150/$20.00*

*Akadémiai Kiadó, Budapest, Hungary*
*Springer, Dordrecht, The Netherlands*

group has reported their findings on differential scanning calorimetric and histological examinations of the long head of the biceps in cadavers and the thermal effects of shoulder electrothermal arthroscopic capsulorrhaphy [14, 15]. A limited number of papers have been published before on the subject of thermal analysis of normal and degenerative human hyaline cartilage of other joints. They have concluded that structural manifestation of osteoarthritis appears as a remarkable change of thermal stability of hyaline cartilage samples. The healthy cartilage samples used in these studies were of cadaver origin as waste material, pathological cartilage was derived as intraoperative tissue fragments. The measurements were conducted in 48 h of sample deriving. The reported data on the calorimetric enthalpy changes proved to be inconsistent. In severely affected osteoarthritis the $\Delta H$ has increased almost twofold, while in an earlier study enthalpy changes in the intact hyaline cartilage was in some cases higher and in some cases lower [16–18].

The main purpose of this study was to examine the altered metabolism in human degenerated cartilage of the shoulder joint. Previous thermoanalytical studies used cadaver samples for the investigation as normal human hyaline cartilage. All samples that were extracted for this study were obtained during live surgeries. A new protocol had to be established before the detailed investigation could be performed. Most of the known changes in the extracellular matrix in OA come from animal models since human samples for investigation are not widely available for experiment.

## Experimental

### *Materials*

The material (cartilage) for this study was collected from seven patients during arthroscopic procedures performed at the Orthopedic Department, University of Szeged. The patients had different pathologies of the shoulder joint: impingement syndrome, rotator cuff tear, rotator cuff degeneration, partial rotator cuff tears. The prelevation of the material was performed in general anesthesia as a first step of the arthroscopic intervention, with a special instrument. The sample was a cylindrical cartilage about 5 mm prelevated from the space between the tuberculum maius and capitulum humeri. After the prelevation, the surgical interventions were performed by the regular steps. Degenerative human hyaline cartilage was obtained from 16 hips and normal cartilages from 11 knees during arthroplasty. Pathological femoral head is cut and removed as part of these procedures. Normal cartilage was obtained from those cases where one knee compartment was degenerated and the other was normal but ligamental instability was the indication for total knee arthroplasty and the unaffected femoral condyle had to be sacrificed for the procedure. Usually, when only one compartment is affected and ligamental stability is intact unicondylar prosthesis is implanted. All tissues were yielded in accordance to legal regulation, international ethical concerns, and patients' consent.

After the operation, a disc (5 mm in diameter) was removed from the unhealthy and healthy cartilage surface. While smaller sample sizes were also acceptable measurements with this diameter were more reproducible. The sample was taken under sterile conditions, and subchondral bone was removed. The disc was first washed in sterile saline, and then stored in 20 mL saline for transportation at room temperature.

Preoperatively the diagnosis of the patient was established on basis of the patient history, clinical signs and radiological findings. The state of the hyaline cartilage was determined intraoperatively. In order to conduct the thermoanalytical study, 34 samples were collected. Based on the patient diagnosis, 11 samples were analyzed as normal hyaline cartilage, seven were obtained from patients with shoulder joint instability, and 16 were collected from Grade 4 osteoarthritic cartilage.

### *Methods*

The thermal properties of samples were determined by differential scanning calorimetry (Mettler-Toledo DSC 821e apparatus, Mettler-Toledo GmbH, Switzerland). Samples were heated from 0 to 80°C. The heating rate was 0.3°C $min^{-1}$. Conventional Hastelloy batch vessels were used with 40 μL sample volume. All the DSC measurements were preceded in Ar atmosphere and the flow rate was 100 mL $min^{-1}$. From the DSC curves the decomposition temperature, the transition temperature range and the total calorimetric enthalpy change were calculated. Fisher LSD method by the Statistica for Windows statistical program was used to compare enthalpy change in the different groups.

## Results and discussion

With the rise of temperature an endothermic reaction was observed in all of the cases. The enthalpy change of the process initiated by the temperature change showed marked difference between the normal and pathological groups (Table 1).

**Table 1** Thermal parameters of denaturation (mean±SD) of normal and degenerated samples

| Sample group | Sample number | $\Delta H$/J g$^{-1}$ | DSC peak/°C | Beginning/°C | Ending/°C |
|---|---|---|---|---|---|
| Normal | 11 | –1493.31<br>SD: 193.04 | 49.79<br>SD: 5.09 | 21.82<br>SD: 3.64 | 55.20<br>SD: 5.43 |
| Shoulder | 7 | –1250.96<br>SD: 199.49 | 46.31<br>SD: 9.22 | 17.99<br>SD: 6.55 | 52.66<br>SD: 9.14 |
| Arthritis | 16 | –1414.78<br>SD: 135.81 | 48.47<br>SD: 3.16 | 19.99<br>SD: 6.08 | 52.48<br>SD: 7.00 |

Greatest change in the enthalpy was observed in normal cartilage: –1493.31 J g$^{-1}$ (SD: 193.03). In the shoulder joint samples 1250.96 J g$^{-1}$ (SD: 199.49), while in cases of end-stage osteoarthritis –1414.78 J g$^{-1}$ (SD: 135.81) was measured. Therefore, denaturation caused by heating was largest in the normal human hyaline cartilage. Consequently these samples required the largest amount of energy for decomposition. Denaturation peak in normal cartilage was at 49.79°C (SD: 5.09), in the shoulder samples it was lower at 46.31°C (SD: 9.22) however in osteoarthritis 48.47°C (SD: 3.16) was similar to the normal (Fig. 1).

All samples showed a clear denaturation peak on the calorimetric curve, therefore a volume of the curve was easily calculated giving the enthalpy change of the sample. The use differential scanning calorimetry as part of thermal analysis was a reliable method for differentiating normal hyaline cartilage from degenerated samples. The calorimeter that was available for use proved to be adequate for these measurements.

Characterization of the altered metabolism in cartilage that promote disease progression should lead to future treatment options that can prevent structural damage. Since damaged articular cartilage has a very limited potential for healing, prevention is fundamental in treatment. The future goal is to find a correlation between the status of the soft tissues and the status of the cartilage. In this way we can predict the status of the cartilage during a rutin surgical intervention performed for different pathologies of the shoulder joint.

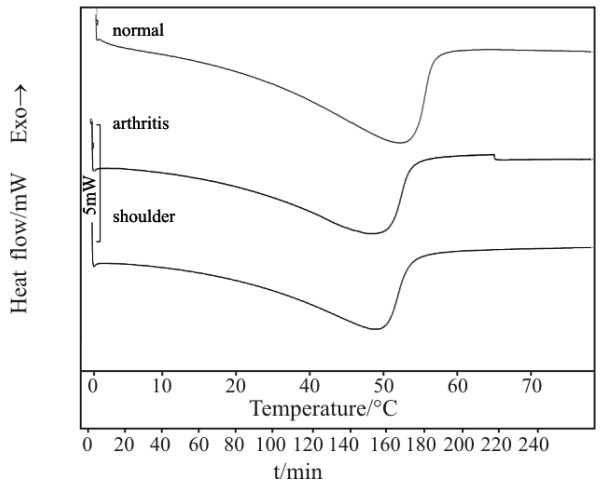


**Fig. 1** DSC curve of normal and degenerated human hyaline cartilage samples (the downwards deflection means endothermic effect)

Further understanding of the initiating events in cartilage destruction, the relationship between the different pathologic influences, and the role of the chondrocyte in maintaining extracellular matrix homeostasis will be necessary to reveal potential targets of therapy. Clinical trials are currently underway for a number of potential disease modifying agents that may significantly change the treatment approach for OA. With the possibility of disease-modifying OA drugs (DMOADs), the necessity for instruments sensitive to change in clinical trials has become very apparent. The current solutions of grade 4 osteoarthritis of the shoulder joint are the anatomical and reverse prosthesis. The development of cell implantation will create another way for the recreation of the cartilage. Thermal analysis of the experimental trials could be a reproducible and fast way of controlling healing of the hyaline cartilage layer.

The purpose of this study was not to duplicate what was previously reported in the literature, but to clarify the thermoanalytical results with acquiring normal cartilage from live surgery was important to provide similar sample environment, and to perform the investigation in a relatively short period of time compared to the earlier reports. This way extracorpal degeneration was minimized.

Finally, common histopathologic assessment methods (Grade) under both clinical and experimental conditions reflect poorly mild phases of the disease, and are very non-linear over the range from mild to advanced disease. Therefore, a detailed thermal examination is needed on the same joint surface with samples taken from different grades of degeneration within the same joint.

## Acknowledgements

Study was supported by OTKA T-047166.

## References

1 H. L. McLaughlin and W. U. Cavallaro, Am. J. Surg., 80 (1950) 615.
2 H. L. McLaughlin and D. I. MacLellan, J. Trauma, 7 (1967) 191.
3 L. Hovelius, J. Bone Joint Surg., 69-A (1987) 393.
4 C. R. Rowe and H. T. Sakellarides, Clin. Orthop., 20 (1961) 40.
5 F. Buscayret, T. B. Edwards, I. Szabo, P. Adeleine, H. Coudane and G. Walch, Am. J. Sports Medicine, 32 (2004) 1165.
6 H. M. Van der Zwaag, R. Brand, W. R. Obermann and P. M. Rozing, J. Shoulder and Elbow Surgery/American Shoulder and Elbow Surgeons, 8 (1999) 252.
7 J. Buckwalter, C. Saltzman and T. Brown, Clin. Orthop., 427S (2004) 6.
8 R. C. Lawrence, C. G. Helmick, F. C. Arnett, R. A. Deyo, D. T. Felson, E. H. Ciannini, S. P. Heyre, R. Hirsch, M. C. Hochberg, G. G. Hunder, M. H. Liang, S. R. Pillemer, V. D. Steen and F. Wolfe, Arthritis Rheum., 41 (1998) 778.
9 C. W. Wu and K. C. Kalunian, Clin. Geriatr. Med., 21 (2005) 589.
10 G. S. Firestein, R. C. Budd, E. D. Harris, I. B. McInnes, S. Ruddy and J. S. Sergent, Kelley's Textbook of Rheumatology, 7th Ed., Elsevier Saunders, Philadelphia 2005, p. 1496.
11 D. Lőrinczy and J. Belágyi, J. Therm. Anal. Cal., 90 (2007) 611.
12 T. Dergez, D. Lőrinczy, F. Könczöl, N. Farkas and J. Belágyi, BMC Struct. Biol., 7 (2007) 41.
13 L. D. Hansen and D. J. Russell, Thermochim. Acta, 450 (2006) 71.
14 G. Bognár, I. Szabó, L. Bálint, B. Hepp, L. Kereskai and D. Lőrinczy, Thermochim. Acta, 464 (2007) 78.
15 I. Szabó, G. Bognár, L. Kereskai, K. Szász and D. Lőrinczy, J. Therm. Anal. Cal., 88 (2007) 343.
16 P. Than and D. Lőrinczy, Thermochim. Acta, 404 (2003) 149.
17 P. Than, I. Domán and D. Lőrinczy, Thermochim. Acta, 415 (2004) 83.
18 P. Than, I. Domán and D. LÞrinczy, The nature of biological systems as revealed by thermal methods, D. Lőrinczy Ed., Kluwer Academic Publishers 2004, p. 285.

DOI: 10.1007/s10973-008-9414-3