Journal of Thermal Analysis and Calorimetry, Vol. 81 (2005) 307–310

# THERMAL ANALYSIS OF THE CRUCIATE LIGAMENTS OF THE HUMAN KNEE

*P. Than*[1*], *V. Halmai*[1], *L. Kereskai*[2] *and I. Gazsó*[1]

[1]Department of Orthopedics, University of Pécs, Medical School, Szigeti út 12, 7643 Pécs, Hungary
[2]Institute of Pathology, University of Pécs, Medical School, Szigeti út 12, 7643 Pécs, Hungary

Cruciate ligaments of the knee joint are important structures very often affected by the degenerative process in case of osteoarthritis. One of the most controversial issues in knee arthroplasty practice nowadays is the role of the posterior cruciate ligament. With foregoing studies authors have demonstrated the feasibility of DSC in the investigation of the musculoskeletal system. With current study authors established the thermal behaviour of healthy cruciate ligaments and detected the alterations in case of osteoarthritis. By establishing the DSC scans of the normal ligaments authors demonstrated clear differences between the posterior and anterior ligament. In addition alterations between normal and arthritic samples could be detected both in terms of changes in total enthalpy and heat capacity. Calorimetric findings have been verified by histological examinations as well.

***Keywords:*** *cruciate ligaments, DSC, histology, knee joint, osteoarthritis*

## Introduction

Osteoarthritis of the knee is one of the most common musculoskeletal disorders with clear pathological abnormality in the tissue elements building up the joint. The basic histological and biochemical alterations in osteoarthritis affect the hyaline cartilage, but secondarily soft tissue elements are also involved. Degeneration of the anterior cruciate ligament (ACL) is well-known, but the involvement of the posterior cruciate ligament (PCL) is a matter of discussion in [1, 2].

Total condylar knee arthroplasty is a routine intervention in today's orthopedic practice with long-lasting, good results in case of osteoarthritic patients. Besides of correctly implanted modern prostheses, well-functioning knee ligaments are unavoidable for surgical success. There is no doubt about the ACL which is usually badly affected by the degenerative process and therefore removed during surgery. In contrary one of the most discussed questions in knee replacement practice nowadays is the preferable implant in terms of sparing or sacrificing the PCL ('posterior stabilized' or 'PCL retaining' design). Since degeneration of the cruciate ligaments is well-known in the osteoarthritic knee, many authors question the option of retaining the posterior ligament [3]. Even though there are many publications about this topic, supportive objective structural studies of the cruciate ligaments are rarely to be found in literature.

Based on the forehand mentioned facts, probably the most widely used knee prosthesis philosophy worldwide is the posterior cruciate ligament retaining implant. The other concept, invented by Insall *et al*. removes the posterior ligament in order to restore normal kinematics of the joint thus avoiding wear of the polyethylene [4]. Both types of prostheses are promoted widely in literature; the question of retaining or sacrificing the PCL is still a matter of discussion.

For the use of a posterior retaining implant a morphologically and functionally intact PCL is obviously needed. The question is, if the function of the ligament in the arthritic knee is preserved and what are the morphological features of it. Investigating the posterior cruciate ligaments of 24 arthritic and 36 healthy control knees microscopically, Kleinbart *et al*. found degeneration of the ligament in 83 of the arthritic and in 55% of the control joints. In the later group severe histological degeneration has not been observed. Difference between sexes was not present but the number of degenerated ligaments was significantly higher over the age of 70 [5].

Allain *et al*. examined both the posterior and the anterior ligaments histologically and macroscopically in 52 osteoarthritic knee joints. While most of the ACL (35/52) were macroscopically affected, signs of macroscopic degeneration have not been found in none of the PCL. Histologically these normal posterior ligaments were intact in only 22 cases, the remaining 30 showed signs of stage 1 or 2 grade lesions [6].

Foregoing examinations have demonstrated that DSC is a useful and well-applicable method for the investigation of the organs of the musculoskeletal

* Author for correspondence: ThanP@clinics.pote.hu

1388–6150/$20.00

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

system [7, 8]. Besides describing the characteristic DSC scans of the normal human hyaline cartilage, the intervertebral discs of the spine and the muscles of the lower extremity, different authors have demonstrated thermal effects of degenerative processes in various human tissue samples [9–12].

The aim of current study was to set up the thermal characteristics of healthy cruciate ligaments of the human knee, and to investigate ligaments in case of degenerative joint disorders with DSC. The calorimetric examination of this kind, we hoped could give an answer to the following questions:

- Is it possible to detect differences between thermal features of the intact anterior and posterior ligaments?
- Is it possible to detect thermal effects of degenerative processes of the ligaments in case of osteoarthritis?
- Does the arthritic process result in the same thermal consequences in both ligaments or does their originally different anatomical structure influence the DSC scans significantly?

## Experimental

*Materials and methods*

Sample preparation

The healthy cruciate ligament samples were of cadaver origin. These samples remain as waste materials when several preparates are dispensed for the bone bank of our orthopedic clinic. We removed both the anterior and the posterior ligaments from four cadaver knees. The donors taken into our study were all under the age of 35 at their death, we considered these persons to be free of degenerative changes in their joints. We took samples only from knees where degeneration of the joint could not be verified macroscopically in any terms. All the medical interventions were made according to the ethic regulations of the University of Pécs.

The pathologic ligaments were derived during knee prosthesis implantations. We measured samples of 8 arthritic knees of seven female and one male patient being in average 65 years (49–72) of age. Three posterior and six anterior cruciate ligaments have been examined with macroscopically evident degeneration.

Histological examination

We removed the ligaments as one piece and longitudinally cut them into two parts. One part has been sent to histological examination the other underwent DSC investigation. The later samples were put into physiological saline solution and were stored separately at 4°C, no longer than 24 h. The samples subject for histological examination were fixed in 4% formaldehyde, longitudinal slides have been made and stained with hematoxylin and eosin. Light microscopic control has been performed.

DSC investigation

The calorimetric experiments were done as described earlier [13–19]. The thermal denaturation was monitored by a Setaram Micro DSC-II calorimeter. All the experiments were performed between 0 and 100°C. The heating rate was 0.3 K $min^{-1}$. Conventional Hastelloy batch vessels were used during the denaturation experiments with 850 μL sample volume (300–400 mg wet mass of ligament samples), on average. The sample and reference vessels were equilibrated with a precision of ±0.1 mg and there was no need to do any correction from the point of view of heat capacity between the sample and reference vessels. Origin 6.0 did the data treatment after ASCII conversion.

## Results and discussion

To our best knowledge thermal analysis of cruciate ligaments of the knee has not been performed and published in literature before.

With our own histological studies we could demonstrate that cadaveric ligaments showed no signs of degeneration. Regular collagenous structure could be seen in case of the posterior and the anterior ligaments either (Fig. 1). The arthritic samples showed marked signs of degeneration microscopically (Fig. 2). In four cases stage II type degeneration, in the remaining five, stage III type degeneration could be seen using the classification of Allain *et al*. [6]. According to this, degeneration and fibrillation of the normal collagenous structure is visible in 1/3–2/3 of the full

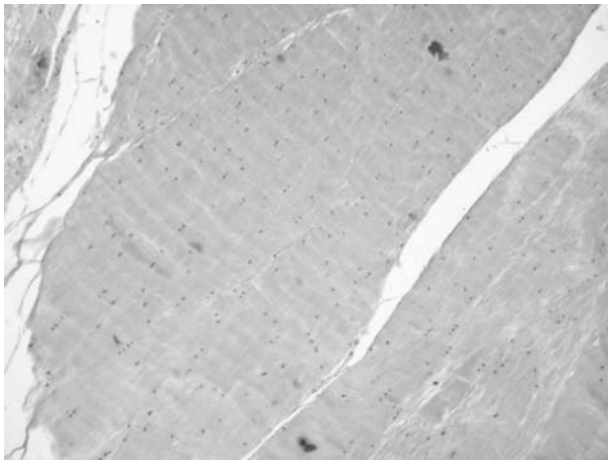

**Fig. 1** Histological examination of an intact posterior cruciate ligament demonstrating regular collagenous structure (hematoxylin and eosin, 100×)

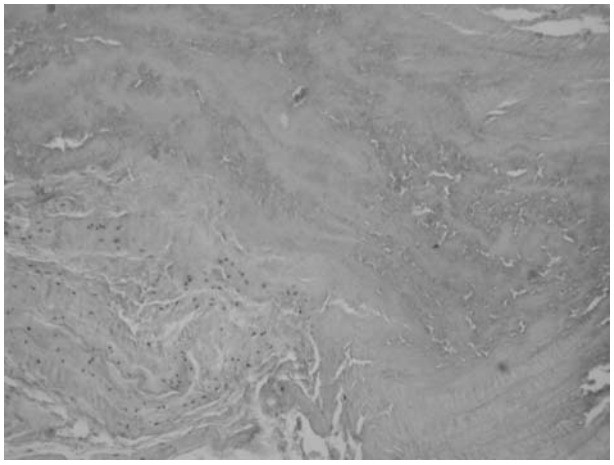

**Fig. 2** Histological examination of a posterior cruciate ligament in osteoarthritis. Marked degeneration is visible (hematoxylin and eosin, 100×)

ligament thickness (stage II) or in more than 2/3 of the full thickness (stage III).

The thermal denaturation results coincide with histological findings. As it can be seen on Fig. 3 the intact posterior ligament is more stable ($\Delta H$=5.64±0.4 J $g^{-1}$ [mean±SD]) than the anterior one ($\Delta H$=4.48±0.3 J $g^{-1}$) and the smaller half width of its DSC scan verifies a more cooperative structure. The melting temperatures do not differ significantly. The most striking effect in arthritic samples is, that these anterior cruciate ligaments have a significantly smaller enthalpy change ($\Delta H$=0.83±0.05 J $g^{-1}$) than the intact ones. The increase in half width of their curves refers to the loosening of internal structure. At the same time posterior cruciate ligaments of arthritic knees have a definitely greater thermal stability ($\Delta H$=2.18±0.15 J $g^{-1}$) than the anterior ones (Fig. 4).

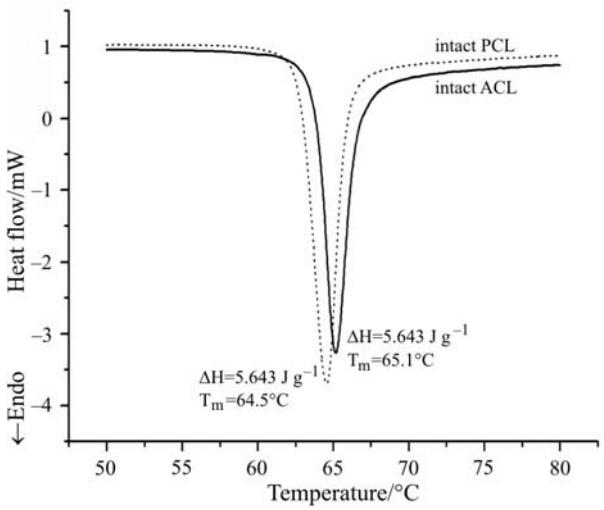


**Fig. 3** Endothermic meltings of intact anterior and posterior cruciate ligaments

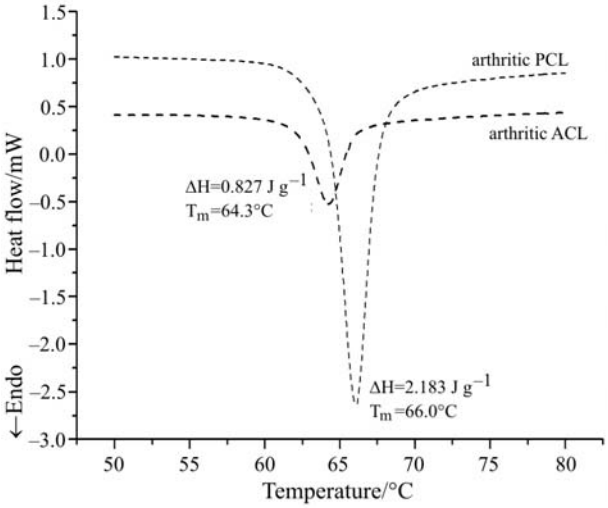


**Fig. 4** Thermal denaturation scans of arthritic anterior and posterior cruciate ligaments

To sum up we can say that we have found significant difference between intact and arthritic ligaments including anterior and posterior ones too. In both cases the posterior ligament has a greater thermal stability and a more compact structure than the anterior one. With our calorimetric investigations – in accordance with the surgical experience – we have observed less damage in posterior cruciate ligaments than in anterior ones in osteoarthritis. One of the reasons of this could be the well-known anatomical fact, that the PCL is an extrasynovial structure, thus less exposed to the inflammatory process provoked by the cartilage degeneration. Even though the thermal features of the arthritic PCL are significantly better than those of the ACL, our DSC scans demonstrated that the posterior cruciate is compromised in osteoarthritis, having a thermal characteristics inferior to the intact ligament. Thermal analysis has been proved to be a useful tool in following the changes in ligaments affected by different pathologic conditions.

## Acknowledgements

This work was supported by research grants from OTKA T-043281, the Setaram Micro DSC-II was purchased from CO-272 fund.

## References

1 M. Alexiades, G. Scuderi, V. Vigorita and W. N. Scott, Am. J. Knee Surg., 2 (1989) 153.
2 D. L. Gardner, J. Anat., 184 (1994) 465.
3 C. A. Carpenter and T. S. Thornhill, In: J. J. Callaghan, A. G. Rosenberg, H. E. Rubash, P. T. Simonian and T. L. Wiczkiewicz (Eds) The Adult Knee, Lippincott W&W, Philadelphia 2003, p. 1135.

4 J. N. Insall, P. F. Lachiewicz and A. H. Burstein, J. Bone Joint Surg., 64A (1982) 1317.
5 F. A. Kleinbart, E. Bryk, J. Evangelista, N. Scott and V. J. Vigorita, J. Arthroplasty, 11 (1996) 726.
6 J. Allain, D. Gouttalier and M. C. Voisin, Acta Orthop. Scand., 72 (2001) 266.
7 Z. Szántó, L. Benkő, B. Gasz, G. Jancsó, E. Rőth and D. Lőrinczy, Thermochim. Acta, 417 (2004) 171.
8 P. Than, I. Domán and D. Lőrinczy, Thermochim. Acta, 415 (2004) 83.
9 I. Domán, G. Tóth, T. Illés and D. Lőrinczy, Thermochim. Acta, 376 (2001) 117.
10 I. Domán, T. Illés and D. Lőrinczy, Thermochim. Acta, 405 (2003) 293.
11 I. Gazsó, J. Kránicz, Á. Bellyei and D. Lőrinczy, Thermochim. Acta, 402 (2003) 117.
12 P. Than, Cs. Vermes, B. Schäffer and D. Lőrinczy, Thermochim. Acta, 346 (2000) 147.
13 D. Lőrinczy and J. Belágyi, Thermochim. Acta, 259 (1995) 153.
14 D. Lőrinczy and J. Belágyi, Biochem. Biophys. Res. Commun., 217 (1995) 592.
15 D. Lőrinczy and J. Belágyi, Thermochim. Acta, 296 (1997) 161.
16 D. Lőrinczy, F. Könczöl, B. Gaszner and J. Belágyi, Thermochim. Acta, 322 (1998) 95.
17 D. Lőrinczy and J. Belágyi, Eur. J. Biochem., 268 (2001) 5970.
18 M. Kiss, J. Belágyi and D. Lőrinczy, J. Therm. Anal. Cal., 72 (2003) 565.
19 D. Lőrinczy, M. Kiss and J. Belágyi, J. Therm. Anal. Cal., 72 (2003) 573.

Received: March 3, 2005

DOI: 10.1007/s10973-005-6966-3