Available online at www.sciencedirect.com
ScienceDirect
Thermochimica Acta 464 (2007) 75–77
thermochimica acta
www.elsevier.com/locate/tca

Short communication

Further characterization of degenerated human cartilage with differential scanning calorimetry☆

K. Tóth [a], G. Sohár [a,*], E. Pallagi [b], P. Szabó-Révész [b]

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

Received 16 April 2007; received in revised form 19 July 2007; accepted 20 July 2007
Available online 25 July 2007

Abstract

A limited number of papers have been published before on the subject of thermal analysis of normal and degenerative human hyaline cartilage. They have concluded that structural manifestation of osteoarthritis appears as a remarkable change of thermal stability of hyaline cartilage samples. The reported data on the calorimetric enthalpy changes proved to be inconsistent. 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. With the rise of temperature, an endothermic reaction was observed in all cases. The enthalpy change of the process initiated by the temperature change showed marked difference between the normal and pathological groups. The use of 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; Enthalpy

1. Introduction

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 [1]. 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 [2]. 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 [3–5]. Pathologic changes in cartilage composition and molecular organization, as well as elevated water content, alter the exquisite balance of biomechanical properties, thus causing excessive joint loading. Loss of cartilage stiffness decreases with increasing stages of OA [6,7]. The first alteration seen within days after joint destabilization is an increase in cartilage water content. The increase in water content in OA cartilage is due to loss of the collagen network's elastic restraint, enabling the hydrophilic polyanionic proteoglycans to swell more than normal. A study of laser-induced structural alterations in the cartilage matrix carried out by scanning force microscopy proposed that the transition of bound to free water at 70 °C can cause spatial re-organization within the cartilage matrix leading to an irreversible alteration [8–12].

A major obstacle to understanding osteoarthritis' (OA) natural history and its modifications by therapy has been the lack of consensus. Pathologic features of the cartilage characteristic for OA biologic activity and progression were not well defined. Further, common histopathologic assessment methods (Grade) under both clinical and experimental conditions are very nonlinear over the range from mild to advanced phases of the disease [13–14].

There are many possible applications of thermoanalytical techniques: characterizations of active and inactive ingredients, routine analysis and qualitative control. Differential scanning calorimetry (DSC) involves the heating or cooling of a sample and reference and the measurement of the differential heat flow. Calorimetry can be used for qualitative and quantitative analyses. ΔH can often be determined for an unknown reaction in a

☆ Presented at the 17. Ulm-Freiberg Kalorimetrietage in Freiberg.
* Corresponding author. Tel.: +36 62 545 423; fax: +36 62 545 418.
E-mail address: gsohar@yahoo.com (G. Sohár).

0040-6031/$ – see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.tca.2007.07.013

complex system, and the value of ΔH can then be used to assist in identifying the reaction of the system. ΔH 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 [15].

A limited number of papers have been published before on the subject of thermal analysis of normal and degenerative human hyaline cartilage. Only one research group has reported their findings on differential scanning calorimetry studies of osteoarthritic cartilage. 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 samples were washed in sterile phosphate-buffered saline and stored in complex solution containing fetal bovine serum, antibiotic, antimycotic solution and amino acids. 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 ΔH has increased almost two-fold, while in an earlier study, enthalpy changes in the intact hyaline cartilage was in some cases higher and in some cases lower [16–20].

The main purpose of this study was to further characterize the altered metabolism in degenerated cartilage that promotes disease progression. 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.

2. Experimental

2.1. Materials

Degenerative human hyaline cartilage was obtained from 28 hip and normal cartilages from 7 knees during arthroplasty procedures performed at the Orthopedic Department, University of Szeged. 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. Mean storage time was 6 h (min: 1 h and max: 26 h), 29 samples out of 35 were studied within 4 h of preparation. Six samples were stored overnight at 5 °C.

Pre-operatively 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, 35 samples were collected. Based on the patient diagnosis, 7 samples were analyzed as normal hyaline cartilage, 12 were obtained from patients with femoral head necrosis (taken from above the necrotic area) and 16 were collected from Grade 4 osteoarthritic cartilage.

2.2. 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. 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. 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.

3. Results

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).

Greatest change in the enthalpy was observed in normal cartilage: −811.496 J/g (S.D. = 46.82). In the necrotic sample

Table 1
Thermal parameters of denaturation (mean ± S.D.) of normal and degenerated samples

Sample group	Sample number	ΔH (J/g)	DSC peak (°C)	Beginning (°C)	Ending (°C)
Normal	7	−788.346 (83.181)	50.18 (3.31)	≈32.5 (3.45)	57.09 (5.35)
Necrotic	12	−567.083 (120.17)	48.93 (5.93)	≈34.16 (4.37)	53.38 (4.28)
Arthritis	16	−543.838 (88.572)	50.340 (2.937)	≈33.8 (4.3)	54.9 (3.1)

The values in the parenthesis indicate S.D.

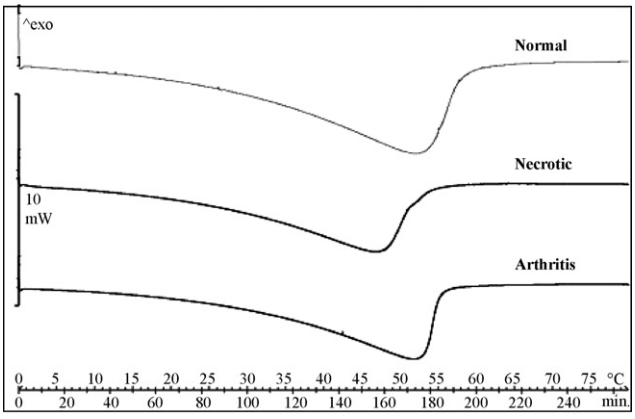

Fig. 1. DSC curve of normal and degenerated human hyaline cartilage samples (the downwards deflection means endothermic effect).

−510.832 J/g (S.D. = 157.66), while in cases of osteoarthritis −543.838 J/g (S.D. = 88.572) 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. Statistical tests proved these calculations to be significant (Fisher LSD method, $p < 0.05$). Denaturation peak in normal cartilage was at 50.88 °C (S.D. = 2.19); in necrotic samples, it was lower at 46.577 °C (S.D. = 7.94), however, in osteoarthritis 50.34 °C (S.D. = 2.937) was similar to the normal (Fig. 1).

4. Discussion

The pathogenesis of OA progression likely revolves around a complex interplay of numerous factors. The major contributors include chondrocyte regulation of the extracellular matrix, genetic influences, local mechanical factors and inflammation. 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.

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.

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 promise of biomarkers has yet to be fulfilled in OA. Type II collagen, cartilage oligomeric matrix protein, hyaluronan and aggrecan have been some of the many biomarkers investigated. Although numerous clinical studies have suggested that specific or combinations of biomarkers can have predictive value in terms of disease presence and severity, the wide variability in these values limits use for individual patients. The use of thermal analysis could be a simple and effective method for controlling the relationship between these markers and disease progression. The revised protocol for sample taking eliminates the presence of disturbing substances during the examination.

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.

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. In the OARSI (OsteoArthritis Research Society International) Scoring System grade is defined as OA depth progression into cartilage irrespective of its horizontal extent. Stage is defined as the horizontal extent of cartilage involvement within one side of a joint compartment irrespective of the underlying grade. Therefore, a detailed thermal examination is needed on the same joint surface with samples taken from different grades of degeneration within the same joint.

Acknowledgement

Study was supported by OTKA T-047166.

References

[1] J. Buckwalter, C. Saltzman, T. Brown, Clin. Orthop. 427S (2004) 6–15.
[2] R.C. Lawrence, C.G. Helmick, F.C. Arnett, et al., Arthritis Rheum. 41 (1998) 778–799.
[3] C.W. Wu, K.C. Kalunian, Clin. Geriatr. Med. 21 (2005) 589–601.
[4] M. Lethbridge-Çejku, J.S. Schiller, L. Bernadel, Natl Center Health Stat. 10 (2004) 222.
[5] E.D. Harris, et al., Kelley's Textbook of Rheumatology, 7th ed., Elsevier Saunders, Philadelphia, 2005, p. 1496.
[6] M.A. Soltz, G.A. Ateshian, J. Biomech. 31 (1998) 927–934.
[7] C.C.-B. Wang, J.-M. Deng, G.A. Ateshian, C.T. Hung, J. Biomech. Eng. 124 (2002) 557–567.
[8] H.J. Mankin, K.D. Brandt, Osteoarthritis: Diagnosis and Medical/Surgical Management, WB Saunders, Philadelphia, 1992, p. 109.
[9] D. Herbage, A. Huc, D. Chabrand, et al., Biochim. Biophys. Acta 271 (1972) 339.
[10] H. Muir, Ann. Rheum. Dis. 36 (1977) 199.
[11] H.J. Mankin, V.C. Mow, J.A. Buckwalter, et al., Orthopaedic Basic Science, AAOS, Chicago, 1994, p. 1.
[12] E. Sobol, A. Omelchenko, M. Mertig, W. Pompe, Laser Med. Sci. 15 (2000) 15–23.
[13] K. Ostergaard, C.B. Andersen, J. Petersen, K. Bendtzen, D.M. Salter, Ann. Rheum. Dis. 58 (1999) 208–213.
[14] K.P.H. Pritzker, et al., Osteoarthritis Cartilage 14 (2006) 13–29.
[15] L.D. Hansen, D.J. Russell, Thermochim. Acta 450 (2006) 71–72.
[16] P. Than, Cs. Vermes, B. Schäffer, D. Lőrinczy, Thermochim. Acta 346 (2000) 147–151.
[17] P. Than, I. Domán, D. Lőrinczy, Thermochim. Acta 415 (2004) 83–87.
[18] P. Than, L. Kereskai, J. Therm. Anal. Cal. 82 (2005) 213–216.
[19] D. Lőrinczy, J. Belagyi, Thermochim. Acta 296 (1997) 161.
[20] D. Lőrinczy, F. Könczöl, B. Gaszner, J. Belagyi, Thermochim. Acta 322 (1998) 95–100.