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NOVEL CALORIMETRIC INVESTIGATION OF DIFFERENT DEGENERATIVE DISORDERS OF THE HUMAN HYALINE CARTILAGE

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The purpose of this investigation was to further elucidate calorimetric properties of cartilage samples from femoral head necrosis and osteoarthritis from live surgeries. The natural course of this disease is one of steady progression with eventual collapse of the femoral head, followed by secondary osteoarthritis in the hip joint. All samples showed a clear denaturation peak on the calorimetric curve. Cartilage obtained from necrotic femoral head required the lowest amount of energy for decomposition. The use differential scanning calorimetry as part of thermal analysis was a reliable method for differentiating.

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

Introduction

An increasing number of publications have been published with the use of calorimetric techniques in the examination of human hyaline cartilage. Previously, thermoanalytical studies were used for the investigation of normal and degenerative human hyaline cartilage [1, 2]. The first paper from this field was the study of Than *et al*. [3]. 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 twofold, while in an earlier study enthalpy changes in the intact hyaline cartilage was in some cases higher and in some cases lower [4–6]. 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.

The goal of this series of calorimetric investigation was to study the thermal effects cartilage samples derived from live surgeries because of osteoarthritis, avascular necrosis of the femoral head (AVN), spondylolisthesis, rheumatoid arthritis and shoulder instability. The purpose of this investigation was to further elucidate calorimetric properties cartilage samples from femoral head AVN.

Avascular necrosis of the femoral head is an increasingly common cause of musculoskeletal disability as well as a major diagnostic and therapeutic challenge. AVN of the femoral head is a pathologic process that results from interruption of blood supply to the bone. AVN of the hip is poorly understood, but this process is the final common pathway of traumatic or nontraumatic factors that compromise the already precarious circulation of the femoral head. Femoral head ischemia results in the death of marrow and osteocytes and usually results in the collapse of the necrotic segment. AVN is extremely rare in healthy individuals. Although initially patients are asymptomatic, AVN usually progresses to joint destruction, requiring total hip replacement in individuals, usually before the fifth decade. No universally satisfactory therapy has been developed, even for early disease. Since joint preservation measures have a much better prognosis when the diagnosis of AVN is made early in the course of the disease and since the results of joint replacement therapy are poorer in younger age groups, diagnosing AVN as early as possible is critical. AVN is characterized by areas of dead trabecular bone and marrow extending to involve the subchondral plate. Elderly persons are at decreased risk for developing AVN. Incidence of AVN is increasing. The causes include greater use of exogenous steroids and an increase in trauma [7–9].

Osteoarthritis (OA) on the other hand is a disease characterized by degeneration of cartilage and its un-

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derlying bone within a joint as well as bony overgrowth. The breakdown of these tissues eventually leads to pain and joint stiffness [10]. Arthritis is one of the most prevalent chronic health problems. One in five (21%) adults in the United States report having doctor diagnosed arthritis and an estimated 21 million adults have osteoarthritis [11, 12]. The specific causes of osteoarthritis are unknown, but are believed to be a result of both mechanical and molecular events in the affected joint. 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 [13].

Thermoanalytical techniques measure the change in physical or chemical properties of the sample as a function of temperature. Calorimetric data can be used to gain fundamental insight into a process or property of a material. Current uses of calorimetry are primarily on complex, often poorly defined, systems, and the most common use is probably kinetics. Calorimetry can be used for qualitative and quantitative analyses. Differential scanning calorimetry (DSC) involves the heating or cooling of a sample and reference and the measurement of the differential heat flow. ΔH is the enthalpy change of the process initiated by the temperature change. ΔH can often be determined for an unknown reaction in a complex system, and the value of ΔH can then be used to assist in identifying the reaction of the system. The change of energy in thermal processes can be measured. Analysis of data from calorimetry always involves a 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 [14].

Experimental

Materials

During arthroplasty procedures performed at the Orthopedic Department, University of Szeged, degenerative human hyaline cartilage was obtained from 38 hips and normal cartilages from 11 knees. As part of these procedures, pathological femoral head is cut and removed in order to implant the prosthesis. Normal samples were derived when total knee arthroplasty was performed and the unaffected femoral condyle had to be sacrificed for the procedure. Usually, in arthritis of both medial and lateral knee compartments total knee replacement is performed, when only one compartment is affected and ligamental stability is intact unicondylar prosthesis is implanted. Normal cartilage was obtained from those cases where one compartment was degenerated and the other was normal but ligamental instability was the indication for total knee arthroplasty. 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. The sample was taken under sterile conditions, and excess 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, max: 26 h).

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, 49 samples were collected. Based on the patient diagnosis, eleven samples were analyzed as normal hyaline cartilage, 22 were obtained from patients with femoral head necrosis, and 16 were collected from osteoarthritic cartilage. All patients in the AVN group were classified as Ficat stage 4 and articular surface deformation in the osteoarthritic group were considered to be grade 6.

Methods

The thermal properties of samples were determined by differential scanning calorimetry (Mettler-Toledo DSC 821^e 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

An endothermic reaction was observed in all of the cases with the rise of temperature. The enthalpy change of the process initiated by the temperature change showed marked difference between the normal and necrotic groups. The average ΔH value that was measured for osteoarthritic samples was in between the average ΔH value of the normal and necrotic samples.

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

Sample group	Sample number	ΔH/J g^{-1}	DSC peak/°C	Beginning/°C	Ending/°C
Normal	11	–1493.31 SD=193.04	49.79 SD=5.09	21.82 SD=3.64	55.20 SD=5.43
Necrotic	2	–1357.70 SD=212.72	49.07 SD=5.69	19.99 SD=4.71	55.14 SD=5.56
Arthritis	16	–1414.78 SD=135.81	48.47 SD=3.16	22.30 SD=3.66	54.54 SD=2.76

All samples showed a clear denaturation peak on the calorimetric curve. Cartilage obtained from necrotic femoral head required the lowest amount of energy for decomposition. Change in the enthalpy was observed in the AVN samples: –1357.70 J g^{-1} (SD=212.72). Denaturation caused by heating in the normal human hyaline cartilage needed –1493.31 J g^{-1} (SD=193.04) energy. The average enthalpy change during the calorimetric measurements in the osteoarthritic samples was –1414.78 J g^{-1} (SD=135.81) (Table 1). Statistical tests proved the difference between the normal and necrotic samples enthalpy change to be significantly different (p<0.05). The results of the calorimetric measurements of the normal and osteoarthritic samples did not prove to be significantly different. Denaturation peak was similar in all cases: in normal cartilage it was at 49.79°C (SD=5.09), in necrotic samples it was at 49.07°C (SD=5.69) and in osteoarthritis at 48.47°C (SD=3.16) (Fig. 1).

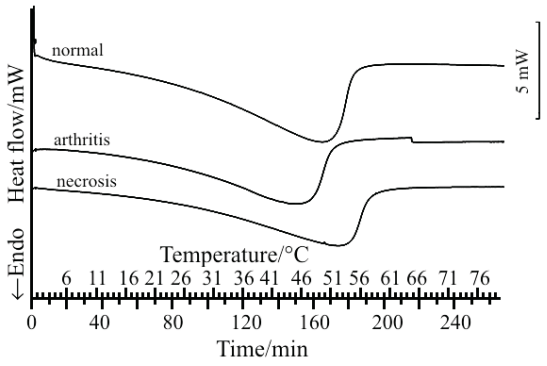

Fig. 1 DSC curve of normal, necrotic and osteoarthritic human hyaline cartilage samples

Avascular necrosis of the femoral head is a common cause of painful hip in young adults. The natural course of this disease is one of steady progression with eventual collapse of the femoral head, followed by secondary osteoarthritis in the hip joint. Molecular pathology is under intense investigation since biomechanical factors result in chemical alteration within the joint. In AVN of the femoral head interference with blood supply to femoral head results in infarction of a segment of the head leading to drastic early degeneration of the femoral head surface hyaline cartilage. Patient undergoing arthroplasty procedure showed clinically and radiological end-stage degradation of the cartilage, with very little resemblance to normal cartilage. Therefore the use differential scanning calorimetry as part of thermal analysis was a reliable method for differentiating normal hyaline cartilage from necrotic samples. The calorimeter that was available for use proved to be adequate for these measurements.

Conclusions

The purpose of this study was to clarify what was previously reported in the literature, 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. Due to the increased number of samples acquired for this study the results were much better reproducible, and the difference between the normal and necrotic samples was statistically measurable.

Characterization of the altered metabolism in cartilage that promotes 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. However, prevention is not possible without the knowledge of the basic pathomorphological mechanism leading to cartilage degeneration. Further investigation is needed to examine the effectiveness of currently used for resolving cartilage matrix degeneration.

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