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THERMAL ANALYSIS OF THE OSTEOARTHRITIC HUMAN HYALINE CARTILAGE

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Arthritis of major joints, especially osteoarthritis of the knee is a very frequent disease of human beings mainly in the developed countries. The pathology of osteoarthritis has been subject of many publications before, using a wide spectrum of different methods to evaluate degenerative changes of hyaline cartilage.

The authors examined osteoarthritic human knee joint hyaline cartilage with differential scanning calorimetry. The different stages of cartilage degeneration have been verified by histological examinations.

The research group demonstrated thermal differences between various stages of osteoarthritis. Besides explaining possible causes for experienced thermodynamic effects, the authors reflect upon future research ways and the possibilities of applying the method in practice.

Keywords: *hyaline cartilage and calorimetry, knee joint, osteoarthritis*

Introduction

Osteoarthritis is one of the most frequent orthopedic diseases usually affecting joints of the lower extremities. From all tissues of the musculoskeletal system, in case of osteoarthritis the most visible degenerative deformities can be observed in the hyaline cartilage. The step-by-step damage of the healthy tissue leads to complete desintegration of the joint, resulting in severe complaints and disability. Pathomorphologic changes in osteoarthritis have been subject to research for decades, basic histological and biochemical features of the arthritic cartilage are well known [1–7].

There are several approaches to the research of degenerative abnormalities of the hyaline cartilage, a large number of histological, histochemical, biochemical methods can be applied to study the issue. Differential scanning calorimetry (DSC), described below, has widespreadly been established in the research of biological systems. In an earlier publication we introduced the method for the investigation of hyaline cartilage and established calorimetric standards of the healthy human cartilage [8].

The aim of current investigation was to prove the capacity of calorimetry to study the degenerated human hyaline cartilage.

Experimental

Material and method

Preparation of human samples

The investigated pathological human samples were derived from patients who underwent knee surgery because of osteoarthritis. In all patient uni- or total condylar knee replacement was carried out. Examinations were performed exclusively on cartilage taken from the medial femoral condyle. 17 (6 male, 11 female) patients suffering from different stages of osteoarthritis underwent sample harvesting during operation, in 9 cases from the left, in 8 cases from the right side. Average age of patients was 64 years (46–79). The stage of osteoarthritis was classified during operation according to Outerbridge [9]. Stage I was diagnosed in 2, stage II in 9, stage III in 6 patients. All ‘stage III’ samples showed signs of severe arthritis, in all ‘stage I’ and in 4 ‘stage II’ cases the arthritis of the medial femoral condyle was only moderate.

The samples were cut to final form under sterile circumstances after the surgery and put into storage liquids. Although the form of the sample does not influence the examination, our target was to standardize its size. Samples were prepared in a cylindrical form, measuring 3 mm in diameter and 15 mm in length.

Chemicals

The derived cartilage was given three rinses in PBS (sterile ‘phosphate-buffered saline’, pH 7.4) in order to

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eliminate other tissue remnants. Samples were placed in factory-made solutions (RPMI-1640, Sigma) containing 10% bovine-serum (Hyclone), antibiotic and antimycotic solution (1 U mL^{-1} penicillin, streptomycin, gentamycin and fungison, Gibco Lab.), non-essential amino acids (Gibco) and natrium-chloride. All samples were separately stored at a temperature of 4°C for not longer than 48 h and later measured in this form.

DSC experiments

The measurements have been done by a Setaram Micro DSC-II calorimeter as published earlier [10–22]. 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 process with 850 μL sample volume, on average. The pure RPMI-1640 solution served as 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 the sample and reference vessels. Origin 6.0 did the data treatment after ASCII conversion.

Results and discussion

Basic assumption for the calorimetric research of biological systems is that macromolecules are in complex interactions with their environment, the change in external chemical-physical variables (e.g. temperature) results in characteristic changes of the system, which can be detected by calorimetry. Obviously, if a biological structure undergoes a change for any reason, its thermodynamic characteristics will change; therefore its calorimetric graph will deviate from the original. In case of an adequate model simulating the research problem, processes important from a medical point of view can be monitored this way [23].

Our thermal denaturation results can be seen in Figs 1 and 2 and in Table 1.

In samples with slight osteoarthritis (Fig. 1) the endothermic reaction at around 60°C could be observed in all cases. The severe advanced cases (Fig. 2) also showed this thermodynamic effect between 60 and 70°C, but two important differences could be observed in all samples.

As Fig. 2 demonstrates, with severe arhritis there is smaller difference between the thermal heat capacities of the starting and ending condition than in mild one. The underlying reason could be that the thermal heat capacity of any biologic system is basically dependent on the amount of water tied. In a tissue showing little water content at native state (which is the case in advanced osteoarthritis) the heat capacity of starting and ending stage is closer to each other. Contrarily, as can be seen in Fig. 1, the less affected cartilage showing larger water content will have definite differences in heat capacities, since during denaturation it will gradually loose its water content, the graph stabilizes at a lower value at the end of the heating process.

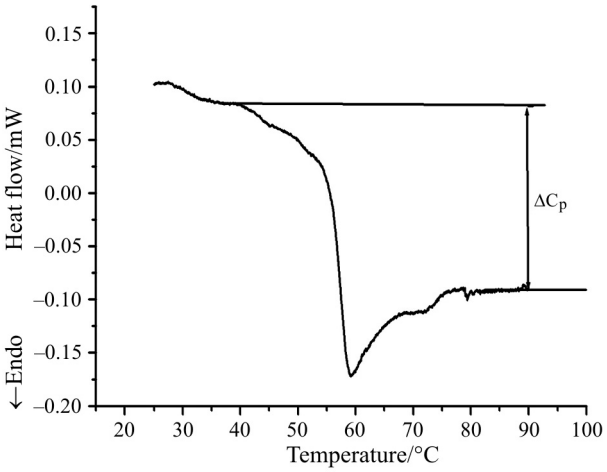

Fig. 1 Calorimetric scan of hyaline cartilage of a human femoral condyle with slight osteoarthritis

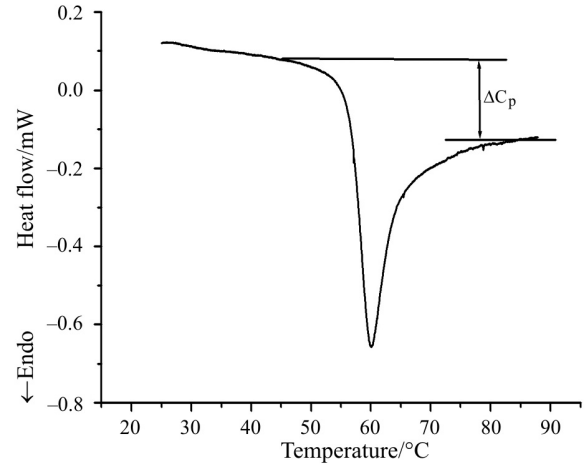

Fig. 2 Calorimetric curve of human femoral condyle hyaline cartilage with severe osteoarthritis

Table 1 The most important thermal parameters of denaturation (T_m=melting temperature, ΔH=calorimetric enthalpy change)

Stage of osteoarthritis	T_m/°C ±s.d.	Transition temperature range/°C	ΔH/J g^{-1} ±s.d.
slight	59.2±0.4	~48.5–87.8	0.53±0.08
severe	60.2±0.6	~48.2–87.7	1.305±0.1

The other evident difference between Figs 1 and 2 is the fact that the endothermic reaction at 60°C shows a narrower 'peak' in case of the severe affected cartilage. The reaction takes place as a single compact unit. This can theoretically be due to the reduced quantity of collagen, its abnormal structure or the deviations of the surrounding basic material. This way it appears as a more densely packed system. It is supported by the calorimetric enthalpy which is twice a higher than in mild osteoarthritis (Table 1). In less affected osteoarthritis the peak is wider, having at least two separable denaturated compounds. The thermal effect resulting presumably from collagen denaturation of higher amount is significantly slower with the same melting temperature and transition temperature range (Table 1).

The hyaline cartilage showing calorimetric graphs of Figs 1 and 2 underwent histological examination as well. Even by simple haematoxylin-eosin method, clear differences were evident. In slight osteoarthritis cases some irregularities could be observed in the ground substance. Cartilage cells were typical, no cell destruction could be observed (Fig. 3). In the arthritic sample massive destruction of the cartilage could be observed, more over, cartilage cells disappeared in large areas, in other places they formed groups as a sign of regeneration. There were also signs for the multiplication of anorganic material, the picture showed signs of advanced cartilage damage (Fig. 4).

Comparing the results of current investigation to our earlier studies [8] our basic observation was, that curves of slight arthritis were more similar to the graphs defined earlier as arthritic samples than to the graphs of intact cartilage.

Our studies repeatedly proved that the calorimetric scans of arthritic and healthy cartilage differ significantly; the thermal consequences of degeneration could be verified. Calorimetry itself is not suitable for structural studies, but together with histological examination the possible causes for the effects can be listed. The measurements carried out verified that morphologic differences between various stages of osteoarthritis could be demonstrated in an indirect way, even if not based on classic clinical categories.

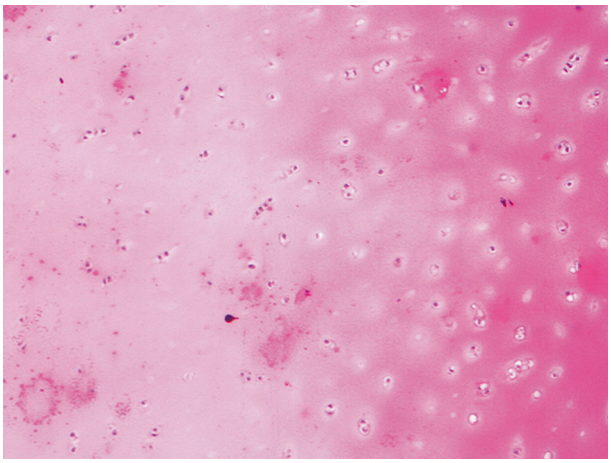

Fig. 3 Histological examination (hematoxylin-eosin, 200×) of the sample analyzed in Fig. 1

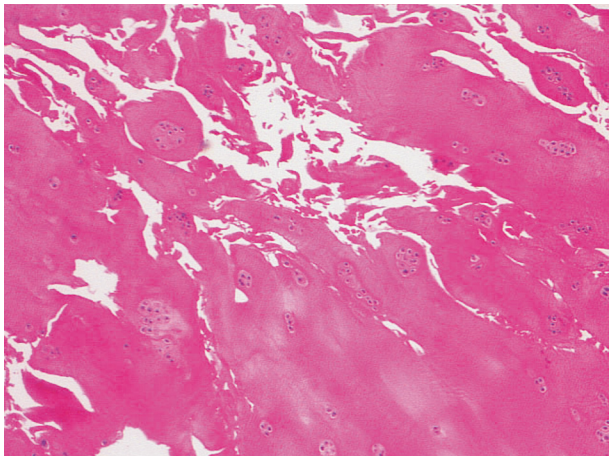

Fig. 4 Histological examination (hematoxylin-eosin, 200×) of the sample analyzed in Fig. 2

We are convinced that a number of questions have to be clarified which arise as a consequence of the studies carried out so far. The most recent problem is the question which component of the cartilage can be made responsible for the deviations measured with DSC, and if the method is sensitive enough to study that. To answer the question the main components of the cartilage have to be separated and separately examined by calorimetry. The values gained this way can be compared to the values of the complete structure.

Another interesting point is the question what practical benefits can be achieved by the studies since we know that calorimetric data serve as basic reference values in several other (e.g. industrial) fields. The most probable option could be the use of the method in controlling the effectivity of certain medical treatments. Among these the demonstration of effectiveness of the often used oral chondroitin sulfate therapy, or the controll of the effect of frequent intraarticular injections can be listed.

Acknowledgements

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