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CALORIMETRIC EXAMINATION OF THE HUMAN MENISCUS

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Meniscus degeneration is a very frequent disease of human beings mainly in the developed countries. The ability of the meniscus to participate in load bearing, shock absorption, joint lubrication, and joint stability depends on the maintenance of its structural integrity. Therefore the pathology of the degeneration has been subject of many publications before. These studies all agreed that the grade of the degeneration correlated with the patient's age, weight, profession, and athletic activity [1]. These reviews also described the biochemical changes in the structure, too [2, 3].

In the current study authors examined degenerated human meniscus with differential scanning calorimetry and demonstrated thermal differences between healthy and intraoperatively removed pathological samples.

Keywords: *DSC, meniscus degeneration, thermal denaturation*

Introduction

The meniscus injury is one of the most common lesions of the knee joint. These injuries affect both the adolescents, and the adult population. Even though the mechanism of the injury is different in these two groups, they both lead to early osteoarthritis of the joint, which in turn is a great problem for the healthcare systems, and a major source of work absenteeism in the affected groups. The step-by-step damage of the healthy tissue leads to complete desintegration of the cartilage, resulting in severe complaints and disability.

The pathology of meniscus degeneration process is well described in the literature [2, 3], therefore our aim was to find new a examination method for the description of the structural changes of the menisci. 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 tissue. Differential scanning calorimetry (DSC), described below, has widespreadly been established in the research of biological systems. Previous examinations have demonstrated that DSC is a useful for investigation of the human tissues [4].

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 [5–8].

The aim of the investigation was to find a new examination method for the description of the structural changes of the menisci.

Experimental

Materials and methods

Preparation of human samples

3 healthy and 21 degenerated menisci were included in the study. The healthy menisci were removed from young cadavers, they had all intact structure without signs of degeneration. The pathological human samples were obtained from patients who underwent uni- or total condylar knee replacement due to advanced osteoarthritis at the department of Orthopaedics, University of Pécs. These samples all showed signs of macroscopic degeneration. Examinations were performed using the posterior horn of the medial meniscus in all cases. 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.

Chemicals

The derived cartilage was rinsed 3 times in PBS (sterile 'phosphate-buffered saline', pH 7.4) in order to eliminate other tissue remnants. Samples were placed in factory-made solutions (RPMI-1640,

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Table 1 The most important themal parameters of denaturation

Age of patient	T_m/°C±s.d.		$T_{1/2}$/°C±s.d.		ΔH/J g^{-1}±s.d.	
	Woman	Man	Woman	Man	Woman	Man
Young		62.4±0.1		2.6±0.1		3.46±0.2
Middle age	63.4±0.1	63.3±0.1	3.1±0.1	3.1±0.1	6.03±0.25	4.93±0.2
Old	62.6±0.1	62.5±0.1	4.1±0.1	6.1±0.1	6.34±0.25	3.63±0.2

T_m=melting temperature, $T_{1/2}$=transition temperature, ΔH=calormetric enthalpy exchange

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.

DSC experiments

The measurements were done using 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}. 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. If a biological structure undergoes a change for any reason, its thermodynamic characteristics will change, and its calorimetric graph will deviate from the original. The changes in the molecular structure of the degenerated meniscus therefore can also be monitored this way.

The results of the thermal analysis can be seen in Table 1 and Figs 1–4.

Our diagrams show increasing difference in heat capacity with age. The underlying cause is due to the fact that thermal heat capacity of any biological system depends on the amount of water tied. These changes show the increase in the level of water content in the degenerated meniscus. A greater heat capacity change in case of older patients caused by severe degeneration can also be noticed. During denaturation the meniscus gradually loses its water

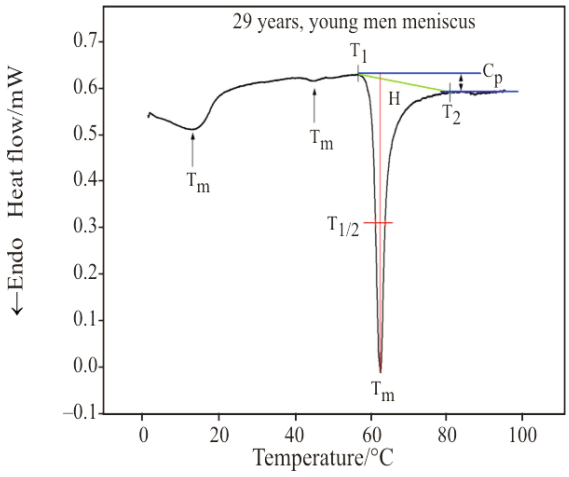

Fig. 1 Calorimetric curve of healthy meniscus from young patients

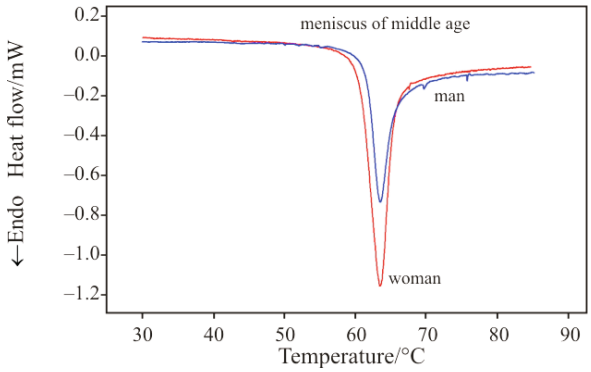

Fig. 2 DSC investigation in case of middle aged patients

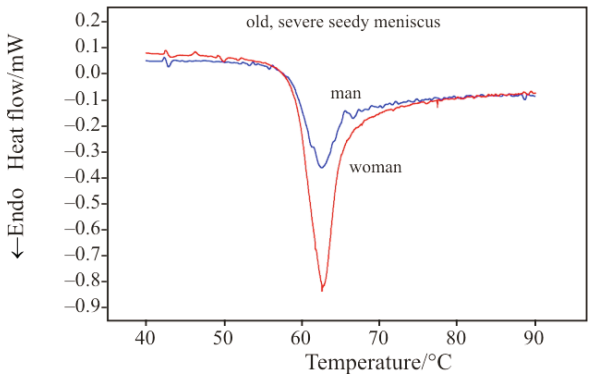

Fig. 3 DSC investigation in case of old patients

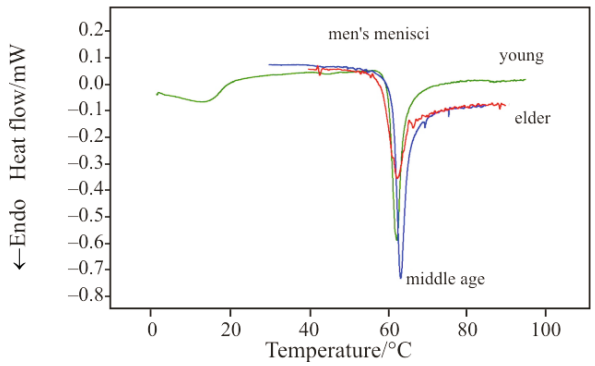

Fig. 4 Summary of results of DSC investigation

content, and the graph stabilizes at a lower value at the end of the heating process.

After thermal excitation a main transition zone can be seen at 62°C and two lower temperature transition zones at 15 and 45 degrees. The cause of the low temperature transition zone is due to the presence of the elastic tissue.

The greatest change is in the enthalpy which shows, that the structure of the meniscus becomes more compact after the degeneration, and functions as one compact system.

A summary of the calorimetric curves in figure 4 demonstrates the significant difference in the heat capacity between healthy and degenerated menisci.

To conclude our results we have found significant differences in the heat capacity and in the enthalpy of healthy and degenerated menisci. The structure became more compact and the amount of water tied increased.

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