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Differential scanning calorimetric examination of the human intervertebral disc: a preliminary study

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Abstract

Intervertebral disc degeneration is a common orthopaedic disorder with significant social and economic impact. The major pathological changes occur in the structure of anulus fibrosus and nucleus pulposus. Differential scanning calorimetry (DSC) is a well-established method for the demonstration of thermal consequences of local and global conformational changes in biological systems. It has never been applied for the investigation of intervertebral disc. According to the present study, the thermograms may prove and follow the changes in the structure of degenerated intervertebral discs. Differences were clearly demonstrated between the two major parts of the intervertebral discs as well as healthy and degenerated samples with the changes in total enthalpy and heat capacity.

Keywords: DSC; Intervertebral disc; Degeneration; Anulus fibrosus; Nucleus pulposus

1. Introduction

Intervertebral disc (IVD) degeneration is a common orthopaedic disorder with significant social and economic impact. The degenerative processes begin early in adulthood and progress thereafter. It has been attributed to the accumulation of environmental effects, primarily mechanical insults and injuries, imposed on normal ageing changes. Degeneration alters the disc morphology and the mechanical properties and leads to its gradual destruction. Certain features of degenerated discs are believed to play a role in the pathomechanism of pain production, leading severe disability and decrease in the quality of life [1,2].

The IVD is a discoid fibrocartilage tissue, processing elastic properties allowing absorbance and dispersion of loads on the spinal column and providing for smooth movements of the spine. It is generally considered to consist of a gel-like nucleus pulposus (NP) surrounded by sheets of interlacing lamellae of collagen forming the anulus fibrosus (AF). The disc is limited above and below by a sheet of hyaline cartilage, which constitutes the cartilage end plate separating the NP from the adjacent vertebral body. The NP consists of a gelatinous fluid containing a loose meshwork of randomly distributed collagen fibres in a proteoglycan matrix and shows high affinity with water. It is highly hydrated, containing 80–90% of water. Approximately 65% of its dry weight is accounted for by proteoglycans, 20% by collagens and the remainder by elastin and other minor components. The AF consists of interlacing lamellae of coarse collagen fibers interconnecting the adjacent

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vertebral bodies, merging into the cartilaginous endplates and attaching to the vertebral bodies. The fibres interlace obliquely at a constant angle to form a three-dimensional collagen framework. Approximately 60% of its dry weight is collagen, and 20% is proteoglycans. Elastic fibres are also present as a minor component. The AF is less hydrated than the NP, with water content of 60–70%. Very few cells are present in the IVD, not more than 1–5% of the tissue volume [1].

The pathogenesis of IVD degeneration in humans has been the subject of ongoing research; the basic biomechanical and biochemical alterations had been already described. The structural changes occurring in the IVD during degeneration can be described as follows: the water content of NP decreases, whereas that of the AF changes relatively little. The aggregation and the amount of proteoglycans decrease particularly in the NP. The interstitial collagen types demonstrate quantitative and qualitative changes in the tissue pattern with a significant disturbance of the composition of the collagenous matrix. The repetitive mechanical insults results in an increased release of proteolytic enzymes (mainly matrix metaloproteinases) which accelerate the degradation of collagen and proteoglycan structure. As a result of these changes, the integrity of the IVD gradually weakens and morphological changes, as radial fissures, circumferential clefts and rim tears in the anulus, reduced disc heights, etc., appears [3–8].

Differential scanning calorimetry (DSC) technique was successfully applied for the demonstration of alterations between pathological abnormalities in the tissue elements building up the hyaline cartilage in osteoarthritis and in healthy case [9].

2. Hypothesis-objectives

Our hypothesis was that in IVD degeneration there is a clear pathological abnormality in the tissue elements building up both the NP and AF, which is responsible for the disease. Besides, examining healthy discs with DSC we planned to carry out investigations of degenerated IVDs. A calorimetric examination of this type has not yet been carried out in international level.

Our aim was to demonstrate that there is a definitive, reproducible difference in the structure of the healthy and pathological disc tissue.

Objectives of our research were

1. introducing the application of a new method in the research of IVD degeneration,
2. setting up of calorimetric standards of the normal NP and AF of IVD,
3. applying calorimetric methods for the investigation of different samples from clinically proven degenerated IVDs,
4. demonstrating differences in the samples of normal and degenerated conditions.

3. Materials and methods

3.1. Sample preparation

The IVD samples considered healthy were originated from scoliotic patients. The discs were removed during therapeutic surgical procedures and the samples were considered to be waste material. The healthy donors taken into our study were around their 15–16 years of age, and therefore, were considered to be free of degenerative changes. The applicability of the intervertebral discs taken from scoliotic patients for IVD research is widely accepted.

The IVD samples considered pathological were originated from cadavers. All samples were obtained during autposy within 24 h post mortem. All donors taken into this group were over the age of 70 years and free of clinical symptoms of connective tissue pathology.

The samples were obtained by devices specially designed for this task, with standard methods and from the same anatomic regions both in cadavers (L4–L5 and L5–S1) spinal segments) and scoliotic patients (L4–L5 spinal segments). The shape of the sample was prismatic with 5 mm of length and 10 mm of width. All samples were identical in size. Samples were washed three times in PBS (streile phosphate-buffer saline, pH 7.4) in order to eliminate tissue remnants, than the NP was separated from the anulus fibrosus in all specimens. Samples were than put into RPMI-1640 solutions (SIGMA) containing 10% fetal bovine serum (HYCLONE laboratories), antibiotic, antimycotic solution (1 U/ml penicilline, streptomycin, gentamycin and fungisone, GIBCO lab.), non–essential amino acids (GIBCO) and sodium carbonate. All the

individual samples were stored separately at 4°C, no longer than 24 h, then were subjected to calorimetric measurements. Our activities were done under the proper law paragraphs and valid permissions.

3.2. DSC measurements

The thermal unfolding of IVD was monitored by a SETARAM Micro DSC-II calorimeter (SETARAM, France). All the experiments were performed between 0 and 100°C with a scanning rate of 0.3 K/min. Conventional Hastelloy batch vessels were used during the denaturation experiments with 850 μl sample volume in average. RPMI-1640 buffer was used as reference sample. 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. The samples were irreversible denaturated during each cycle.

3.3. Evaluation of DSC scans

The repeated scan of denaturated sample was used as baseline reference, which was subtracted from the original DSC scan. Calorimetric enthalpy was calculated from the area under the heat absorption curves using two points setting SETARAM peak integration.

4. Results and discussion

Thermal denaturation of cadaver samples clearly demonstrated the differences between AF and NP (Figs. 1 and 2) caused by the their different composition. This appeared in the main transition temperatures (62.9 and 58.5°C for AF and NP, respectively) as well as in the total calorimetric enthalpy changes (0.72 and 0.28 J/g).

It can be seen in DSC scans that significant difference (two-sample t-test, supposing that the variances are the same in both case, at level of $P = 0.05$) exists between pathological (cadaver) and standard healthy samples. The degenerated AF proved to be more stable (Fig. 3) from thermal point of view ($T_m = 62.9$°C with $\Delta H = 0.72$ J/g and 64.4°C with $\Delta H = 1.05$ J/g) while the degenerated NP (Fig. 4) was less stable than the healthy sample ($T_m = 58.5$°C with $\Delta H = 0.28$ J/g and $T_m = 59.7$°C with $\Delta H = 0.375$ J/g). These findings could be interpreted in a following way: during ageing and long lasting burdening the AF becomes

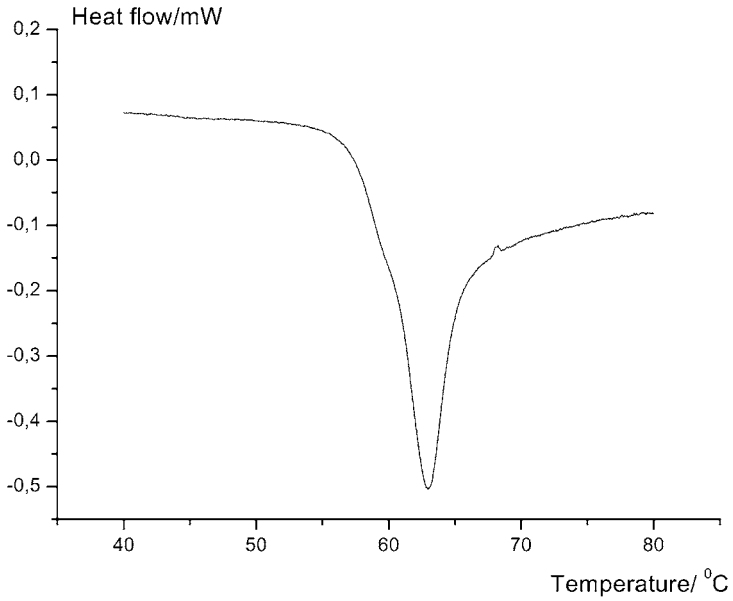

Fig. 1. Thermal denaturation of degenerated anulus fibrosus.

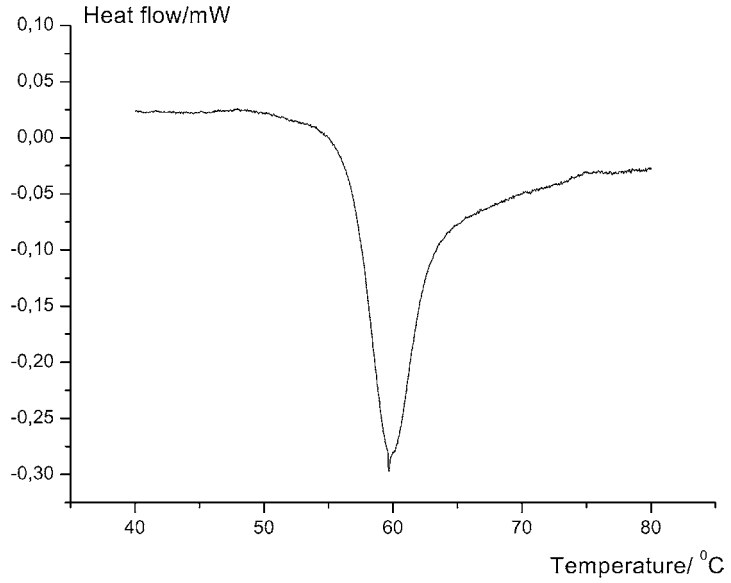

Fig. 2. Thermal denaturation of degenerated nucleus pulposus.

more packed, compressed [8]. It partly looses water and there is a breakdown in the laminas (disorganised fibre structure with extensive mucoid degeneration) as well as a continuous deposition of chondroid substance in the anuluses [10–12]. These structural alterations explain the higher transition temperature and greater enthalpy change of the pathological AF control sample compared to the healthy one (see Table 1). In the degenerated NP during degeneration there is a loss of water and proteoglycan content particularly in the centre of disc [13] with decreased biochemical activity. Therefore, these pathological samples will have less

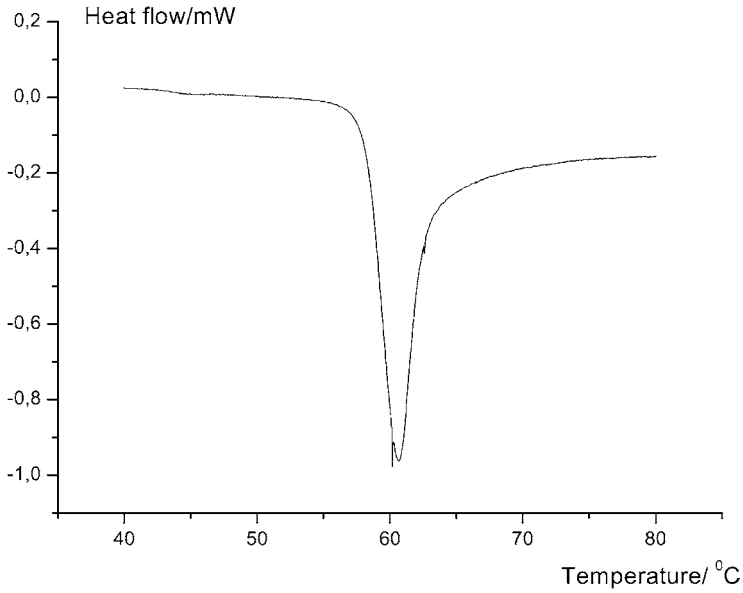

Fig. 3. Thermal denaturation of healthy anulus fibrosus.

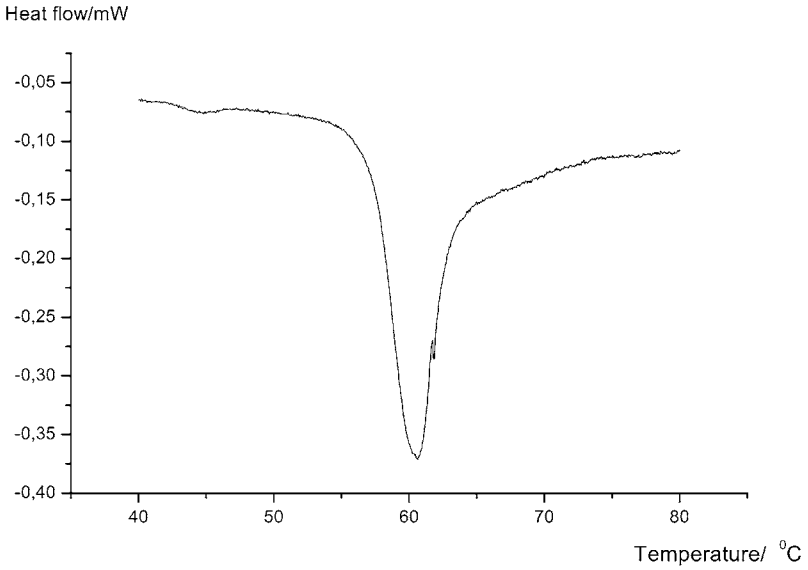

Fig. 4. Thermal denaturation of healthy nucleus pulposus.

Table 1
The melting temperatures and transition enthalpy changes of degenerated (cadaver) and healthy discs (average ± S.D.)[a]

Parameter samples		T_m (°C)	ΔH (J/g)
Degenerated L4–L5 AF	AF $s = 6$, $n = 3$	62.9 ± 0.4	0.72 ± 0.08
	NP $s = 6$, $n = 3$	58.5 ± 0.3	0.28 ± 0.04
Degenerated L5–S1	AF $s = 4$, $n = 3$	64.4 ± 0.5	1.05 ± 0.12
	NP $s = 4$, $n = 3$	59.7 ± 0.4	0.375 ± 0.05
Healthy L4–L5	AF $s = 5$, $n = 3$	60.5 ± 0.3	0.89 ± 0.1
	NP $s = 5$, $n = 3$	60.6 ± 0.4	0.44 ± 0.06

[a] s = number of different patient samples and n = number of measurements from the same sample batch.

stable thermal parameters ($T_m = 58.4$°C and 59.7°C, $\Delta H = 0.28$ and 0.375 J/g, respectively) compared to the healthy ones ($T_m = 60.6$°C, $\Delta H = 0.44$ J/g). (The thermal parameters of AF and NP are changing in the same direction in the same disc (L4–L5 IVD or L5–S1 IVD)).

With our preliminary work we could demonstrate that DSC is a useful and applicable tool for the investigation of intervertebral disc. The structural changes in pathological samples are manifested in characteristic thermal deviation. We believe that components of AF and NP responsible for the demonstrated DSC findings can be identified in the future by application of additional biochemical and histological methods. Thus, the better knowledge of the pathomechanism of degeneration procedures can be helpful in the prevention and in the development of new pharmaceutical products and surgical methods.

Acknowledgements

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