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Differential scanning calorimetric examination of the human hyaline cartilage A preliminary study

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Abstract

Arthritis of major joints especially osteoarthritis of the knee is a very frequent disease of human beings mainly in the developed countries, the major pathological changes occur in the structure of hyaline cartilage. 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 cartilage. According to the present study the thermograms may prove and follow the changes in the structure of cartilage in different stages of osteoarthritis. The differences were clearly demonstrated between the various anatomical origins of the cartilage as well as intact and osteoarthritic samples with the changes in total enthalpy and heat capacity, as well as by the shape of DSC scans themselves.

Keywords: DSC; Hyaline cartilage; Femoral condyle; Patella; Osteoarthritis

1. Introduction

Osteoarthritis is one of the most common orthopedic diseases, foremost affecting the weight-bearing joints of the lower extremities, i.e., the knee and hip. The etiology of its primary form is unknown, in these cases some biomechanical abnormality of the joints can not be confirmed.

Of all the tissues of the musculosceletal system, the degenerative deformities occurring during osteoarthritis are most common and most definite in cartilage tissue. The derangement occurring in the cartilage leads to the total destruction of the joints over several steps and due to this, to severe disability as well as a decrease in the quality of life. The different pathomorphological changes in osteoarthritis have been the objects of innumerable investigations, the basic histological and biochemical alterations have been clearly described before [1,2,3,4].

The structural changes occurring in the joint cartilage can be described as follows: the amount of chondroitine sulfate, one of the most important components in the surface of the cartilage, is decreasing, the surface shows cracks. There are also significant structural changes in the deeper layers, the number of cartilage cells gradually decreases in certain parts, in

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other parts, irregular cell proliferation can occur. The cell proliferation results in the increased release of proteolytic enzymes which cause a rapidly increased denaturation of proteins. In certain layers, calcium deposits are formed. As a result of all these changes, the integrity of the cartilage tissue gradually weakens. Due to the mechanical forces affecting the articular surface, smaller or bigger parts of the cartilage start coming off and ulcerations start developing. Since the cartilage is destroyed, the bony ends of the joints are left partially or completely cartilage-free [5,6,7].

2. Hypothesis–objectives

Our hypothesis was that in osteoarthritis there is a clear pathological abnormality in the tissue elements building up the hyaline cartilage, which is responsible for the disease. Besides examining healthy cartilage with differential scanning calorimetry (DSC) we planned to carry out investigations of cartilage destruction caused by osteoarthritis. A calorimetric examination of this type has not yet been carried out on international level.

Our aim was to prove with the examinations that there is a definitive difference in the structure of the healthy and pathological cartilage, which can be reproduced.

Objectives of research were:

1. Introduction of the application of a new method in cartilage research.
2. Setting up of calorimetric standards of normal hyaline cartilage.
3. Applying calorimetric methods for the investigation of different samples from clinically proved degenerated articular surfaces.
4. Presentation of the differences in the samples of normal and pathological conditions.

3. Materials and methods

3.1. Sample preparation

The healthy cartilage samples were of cadaver origin. These samples remain as waste materials when several preparates are dispensed for the bone bank of our orthopedic clinic, such as the joint cartilage surface remaining after the preparation of cancellous bone chips from cadaver femoral condyles. The donors taken into our study were all under the age of 40 at their death, we considered these persons to be free of degenerative changes in their joints. We took only samples where degeneration of the cartilage surface could not be verified macroscopically. Law paragraphs and valid permissions control the activity of the bone bank.

The pathologic human samples serving as a basis for research were derived from tissue fragments taken during operations and considered to be waste material. Such were the femoral condyle- and patella pieces removed during knee prosthesis implantations.

The samples were obtained by devices especially designed for this task, from the same anatomic region (medial femoral condyle, patella) by standard methods both in the cadaver and patient samples.

The shape of samples was cylindrical with 3 mm of diameter and 15 mm of length. Most of the samples were identical by size. Samples were washed three times in PBS (sterile phosphate-buffered saline, pH 7.4) in order to eliminate all extracartilaginal tissue remnants. Samples were then put into RPMI-1640 solution (SIGMA) containing 10% fetal bovine serum (HYCLONE laboratories), antibiotic, antimycotic solution (1 U/ml penicilline, streptomycine, gentamycine 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 48 h. Then samples were subjected to calorimetric measurement.

3.2. DSC measurements

The calorimetric experiments were done as they were described earlier [8,9]. The thermal denaturation of different parts of human hyline cartilage samples was monitored by a SETARAM Micro DSC-II calorimeter. All the experiments were performed between 0 and 100°C. The heating rate was 0.3°C/min. Conventional Hastelloy batch vessels were used during the denaturation experiments with 850 μl sample volume, on average. The pieces of different cartilages were stored in RPMI-1640 solution and used for sample, while the pure RPMI-1640 solution served as reference. The sample and reference vessels were equili-

brated with a precision of ±0.5 mg and there was no need to do any correction from the point of view of heat capacity between the sample and reference vessels. The data treatment after ASCII conversion was done by Origin 4.1.

4. Results and discussion

The most important feature of osteoarthritis of major joints is damage of the hyaline cartilage which is a tissue having a complex and active metabolism from the biochemical point of view. It is composed of chondrocytae, collagens, proteins of non-collagen type (e.g. proteoglycans), inorganic materials and in 70–80% of water. The collagen-proteoglycan matrix serves as the mechanical frame of the cartilage and simultaneously determines its mechanical properties [10]. The collagen fibers having a triple helix form are responsible for tensile strength while proteoglycans are responsible for compressibility. There is an increased cellular activity in the arthritic joint at the early stage combined with changes of the matrix and increased water uptake. The homeostasis shifts towards catabolic activity, cartilage is degraded, proteoglycan fragments are liberated which is followed by the fragmentation of collagen fibers and their structural change [1,4].

On the basis of the above mentioned facts we believe that these structural manifestations of osteoarthritis appear as a remarkable change of thermal stability of hyaline cartilage samples prepared from human femoral condyles and patellae. This kind of investigation is a perfectly new approach of this problem.

DSC curves of intact as well as arthritic femoral condyles can be seen on Fig. 1, the same is presented for cartilage samples of the patella on Fig. 2.

DSC scans clearly demonstrate significant differences between the different types and conditions of cartilage samples. The femoral condyle and the patella are parts of the same anatomical but not structural unit, therefore the higher transition enthalpy for samples from the patella could be assigned to the different structure of them (Table 1).

The pronounced heat capacity change between intact and arthritic femur condyle samples can be explained with the structural alterations in osteoar-

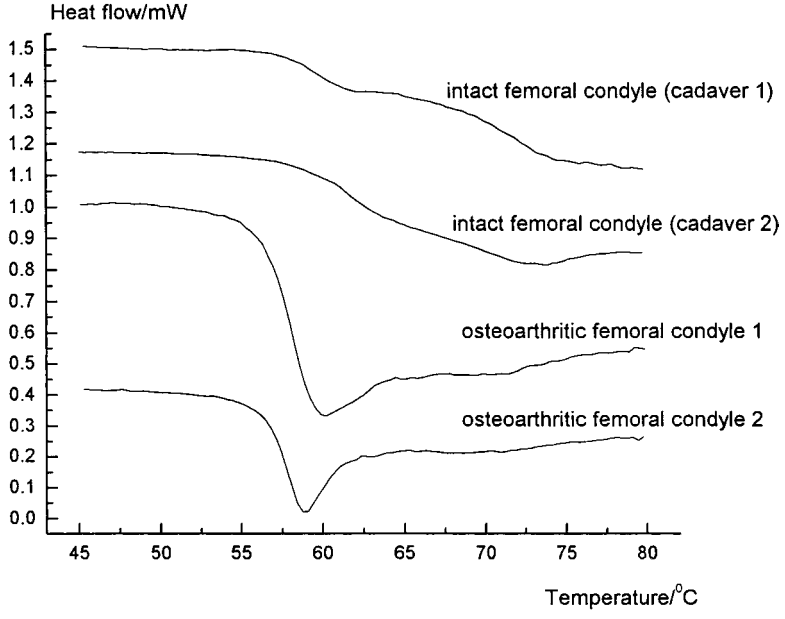

Fig. 1. DSC curves of intact and osteoarthritic femoral condyles. Four different sample sets were measured in each cases and 2–2 representative scans are plot.

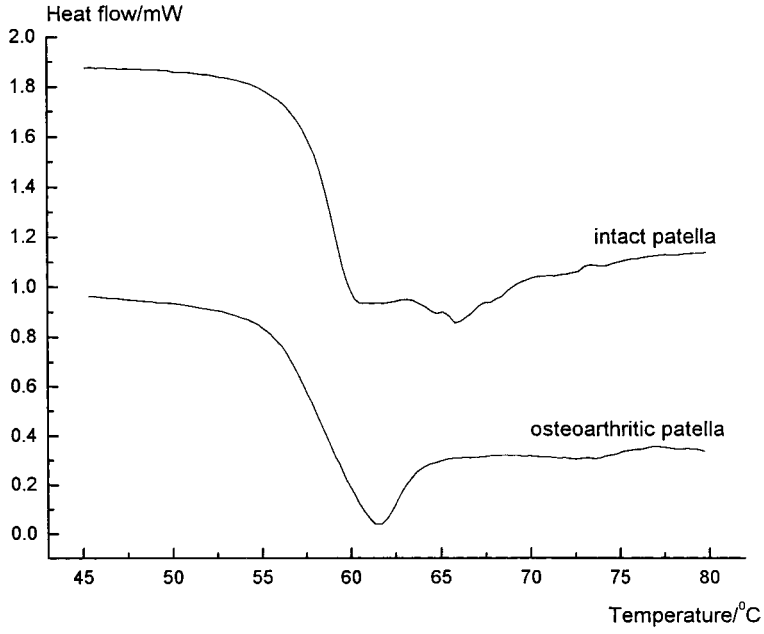

Fig. 2. Characteristic DSC scans (from four different sample sets) for intact (cadaver) as well as osteoarthritic patella.

thritis caused by the biochemical processes. The values of main melting temperature fall into the range (ca. 60°C) of more stable biological macromolecules therefore they could be assigned to the denaturation of collagen and protein compounds of the cartilage.

With our investigations we could demonstrate that DSC is a useful and well applicable method for the investigation of hyaline cartilage. The pathological structural changes of the cartilage can not only be treated by surgical methods but by different substitutional medications too. One of these promising therapies is the use of chondroitin sulfate [11]. Since the goal of using chondroitin sulfate is to achieve structural regeneration of the hyaline cartilage we believe that the effectiveness of the therapy would be well demonstrated with DSC.

We believe that numerous open questions have to be cleared in the future:

1. Which components of the cartilage are mainly responsible for the demonstrated DSC findings? In order to answer it additional separation of different components with biochemical methods is necessary and the establishment of DSC features of each component.
2. What is the effect of varying influencing factors of osteoarthritis in detail? Can the different clinical

Table 1
The melting temperatures and transition enthalpies of different cartilages (average ± standard deviation). S = number of different patient samples, n = number of measurements from the same sample batch

Parameter samples	T_m/°C	H/(J/g)
Intact patella ($s = 5$) ($n = 3$)	64.8 ± 0.9	-1.78 ± 0.12
Osteoarthritic patella ($s = 4$) ($n = 3$)	60.9 ± 0.8	-0.78 ± 0.17
Intact femoral condyle ($s = 3$) ($n = 3$)	72.6 ± 0.7	-0.32 ± 0.07
Osteoarthritic femoral condyle ($s = 6$) ($n = 4$)	58.9 ± 1.2	-0.58 ± 0.16

stages of osteoarthritis be demonstrated on the thermograms as well? Are the thermograms of patients differing from each other by age, gender, activity, previous therapy, duration of symptoms etc. significantly dissimilar? This is an important question because of the great biological variance of the problem, which is complicated a little bit by the fact that the collection of the proper samples is time, confidence of patient as well as clinical hierarchy dependent.

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