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NEW THERMOGRAVIMETRIC PROTOCOL FOR THE INVESTIGATION OF NORMAL AND DAMAGED HUMAN HYALINE CARTILAGE

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Osteoarthritis, although classically conceived of as a degenerative consequence of aging, is a disease with an increasingly well-characterized molecular pathophysiology. Pathologic changes in cartilage composition and molecular organization, as well as elevated water content, alter the exquisite balance of biomechanical properties. Much of what is known about changes in the extracellular matrix in osteoarthritis comes from animal models.

Previously, thermogravimetric methods have not been used for compositional thermoanalytical study of normal and degenerative human hyaline cartilage. For this reason the research group established a sufficient new thermogravimetric protocol, which proved water content elevation contributing to disease progression.

Keywords: *activation energy, human hyaline cartilage, osteoarthritis, thermogravimetry*

Introduction

Osteoarthritis (OA) is a disease characterized by degeneration of cartilage and its underlying bone within a joint as well as bony overgrowth. The breakdown of these tissues eventually leads to pain and joint stiffness [1]. 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 [2–4].

The specific causes of osteoarthritis are unknown, but are believed to be a result of both mechanical and molecular events in the affected joint. The new paradigm of OA considers it as a heterogeneous disease with numerous factors leading to its pathologic hallmark of cartilage loss [1, 5, 6]. Normal articular cartilage has a unique load-support mechanism, governed by its high water content and its low elastic moduli and permeability. In normal tissues, interstitial water provides over 90% of load support. 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 [7, 8].

The first alteration seen within days after joint destabilization is an increase in cartilage water content. The increase in water content in OA cartilage is due to loss of the collagen network's elastic restraint, enabling the hydrophilic polyanionic proteoglycans to swell more than normal. Very shortly after the increase in cartilage water, newly synthesized proteoglycans are characterized by a higher proportion of chondroitin sulfate and a lower proportion of keratan sulfate, and proteoglycan aggregation is impaired. Once proteoglycan loss reaches a critical threshold, water content, which initially increased, falls below normal [9–13].

Thermoanalytical techniques measure the change in physical or chemical properties of the sample as a function of temperature. Thermogravimetric analysis (TG) is one of the oldest thermal analytical procedures and has been used extensively in the study of polymeric systems. The technique involves monitoring the mass loss of the sample in a chosen atmosphere (usually nitrogen or air) as a function of temperature. The usefulness of thermogravimetry (TG) for analyzing complex systems was greatly enhanced by the introduction of the ability to record simultaneously the first derivative of the mass loss. This is referred to as derivative thermogravimetric analysis (DTA). The ability of TG to generate fundamental quantitative data from almost any class of materials, has led to its widespread use in every field of science and technology. Compositional analysis is a key application: by careful choice of temperature programming and gaseous environment, complex materials or mixtures may be analyzed by selectively decomposing or removing their components. This approach is regularly used to analyze moisture content of many substances. TG is inherently quantitative, and therefore an extremely powerful thermal technique, but

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gives no direct chemical information. The ability to analyze the volatile products during a mass loss is of great value [14–20].

The purpose of this study was to elucidate the importance of water content in contributing to disease progression, and to establish the kinetic character of water loss effect of heating. Previously, thermoanalytical studies were used for the investigation of normal and degenerative human hyaline cartilage, but water content has not been measured. The first paper from this field was the study of Than *et al.* [21], further studies measured the difference between primary arthritis and septic arthritis [22]. Therefore a new thermogravimetric protocol had to be established before the detailed investigation could be performed. Most of the known changes in the extracellular matrix in OA comes from animal models since human samples for investigation are not widely available for experiment.

Experimental

Materials

During arthroplasty procedures performed at the Orthopedic Department, University of Szeged, degenerative human hyaline cartilage was obtained from 28 hip and normal cartilage from 7 knee. 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. We were able to obtain normal cartilage 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, then stored in 20 mL saline for transportation at room temperature. Mean storage time was 6 h (min: 1 h, max: 26 h), 29 samples out of 35 were studied within 4 h of preparation. Six samples were stored over-night at 5°C.

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, 35 samples were collected. Based on the patient diagnosis, seven samples were analyzed as normal hyaline cartilage, 12 were obtained from patients with femoral head necrosis, and 16 were collected from osteoarthritic cartilage.

Methods

Thermal analysis was performed with the use of a MOM Derivatograph (MOM, Hungary), and the TG, DTG and DTA curves were determined. *T* curve shows the linear increase of temperature during the process.

The heating was linear from 25 to 150°C and the rate of heating was 5 K min^{-1}. Al_2O_3 was used as reference material. In the first step, the total water loss and kinetic parameters were calculated. The kinetic parameters calculated by the software are the following: the reaction order (n), the activation energy (E_a) and the pre-exponential factor (A).

The value of n (reaction order) is allocated by the Kissinger method [23] and it is the first kinetic parameter calculated by the computer:

$$n=1.26S^{1/2} \quad (1)$$

where S is the form factor which presents the absolute value of the gradients of DTG curves in the points of min/max. The activation energy (E_a) is determined according to the natural logarithmic form of the Arrhenius-equation

$$k(T)=A\mathrm{e}^{-E_a/RT} \quad (2)$$

which is widely used in the literature [24, 25].

The results of these investigations were also statistically evaluated by Microsoft Excel. A Paired t-test was used to compare mean water loss of normal and degenerative cartilage during thermal analysis. The linear range of the first step of TG curve in water loss was analyzed to establish its slope. The determination of linear parts of TG curves and fitting linear straight line on the curve was performed by the statistical program.

Results and discussion

TG and DTA curves of a normal and degenerated samples are presented on Fig. 1.

It was found, that the total water content of intact (normal) cartilage is 81%, which was probably the interstitial water and the difference was supposedly bound on the surface. To remove the cartilage

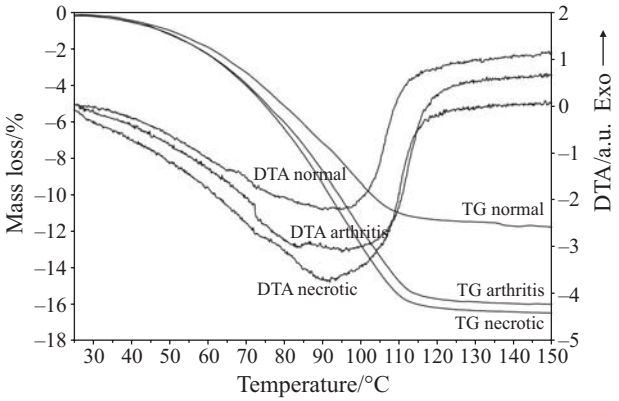

Fig. 1 TG and DTA curves of a normal and degenerated samples

extracellular water content, 52 kJ M^{-1} energy was needed.

Cartilage obtained from necrotic femoral head had a higher water content of 88%. Extraction of the cartilage fluid content needed 70 kJ M^{-1} energy. Total water content of the osteoarthritic samples was 87%, and 73 kJ M^{-1} energy was used for the removal of the fluid content (Table 1).

Loss of water content in all three groups are presented with a sharp step on the TG curve, starting on average temperature of 37 and ending at 116°C. Linear part of the TG curve begun at around 57 and ended at around 104°C (Table 1). Placing a line on this portion of the curve, the slope of the curve can be calculated which represents the speed of the water content loss (Table 2). The slope of the linear region correlated in all three groups.

In case of the normal hyaline cartilage, 0.196 mg of fluid content release was observed (average mass of the normal samples was 15.48 mg) with increase of temperature by 1°C, therefore 1.3% $°C^{-1}$ loss was detected. Necrotic samples (average mass: 15.51 mg) released 0.262 mg of water with the same increase of temperature, so 1.7% $°C^{-1}$ decrease in mass was observed. In the osteoarthritic samples (average mass: 17.02 mg), 0.242 mg decrease was measured which represents 1.4% $°C^{-1}$ mass reduction. The resulting amount of mass lost in the linear region was recounted from these results (Table 2).

Molecular pathology of osteoarthritis is under intense investigation since biomechanical factors result in chemical alteration within the joint. Increase in the cartilage matrix water content in all cases of degenerative cartilage was observed. Based on the results it can be stated that water content is higher in impaired samples, meanwhile water interstitial bonding was stronger in these cases. Rise in water adherence was well distinguishable since higher energy was needed for removal. Activation energy correlated considerably with water content in the samples.

The main goal of the thermogravimetric measurements was to identify the nature and quantity of water molecules in the obtained samples. Water molecules' binding mode may have an important consequence in pharmacokinetics. The reaction order was in all three cases (normal, necrotic, osteoarthritis) approximately 1 and the standard deviation was low (Table 2). The TG curve's slope of the linear region showed, that the rate of water loss does not depend on the water amount remaining in the tissue. Comparing the data in the two presented tables it can be concluded that in the degenerative samples higher water content binded stronger to the matrix. However, the reaction order and the slope of the linear region correlated in all three groups. This first order kinetic means that the rate of water loss depends on

Table 1 Mass loss and activation energy of normal and degenerated samples

Sample group	Sample number	TG step/°C	Total mass loss/%	E_{act}/kJ M^{-1}
Normal	7	39.1–113.8	80.79 SD: 7.09	52.33 SD: 6.68
Necrotic	12	37.4–116.2	87.80 SD: 8.06	70.25 SD: 21.71
Arthritis	16	36.4–121.9	86.71 SD: 7.84	72.72 SD: 23.46

Table 2 Reaction kinetic parameters of normal and degenerated samples

Sample group	Sample number	TG step linear region/°C	Mass loss/%	Reaction order/n	Slope of linear region
Normal	7	62.67–102.25	–51.45	1 SD: 0.203	–0.039
Necrotic	12	57.60–102.00	–75.48	1.03 SD: 0.32	–0.042
Arthritis	16	58.00–104.60	–65.24	1.03 SD: 0.27	–0.048

the water amount remaining in the tissue, namely if the amount of water decreases in the tissue, the rate of loss also decreases.

The newly established thermogravimetric protocol was sufficient for compositional thermoanalytical study of normal and degenerative human hyaline cartilage. Water content elevation contributing to disease progression was observed in both osteoarthritis and aseptic necrosis. Previously, this method has not been used for this purpose.

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. With better understanding the exact amount of matrix water content and binding characteristics, preventive measures can be developed. These therapeutic steps can be adequately tested and monitored with thermogravimetric measurements. Further investigation is needed to examine the effectiveness of currently used medications (Glucosamin, Chondroitin) for resolving cartilage matrix degeneration.

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