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PhD thesis

**CHARACTERIZATION OF NORMAL AND
DEGENERATED HUMAN HYALINE CARTILAGE WITH
THERMAL ANALYSIS**

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Szeged
2009

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PUBLICATIONS

List of full papers directly related to the subject of the Thesis

- 1) **Sohár G**, Pallagi E, Szabó-Révész P, Tóth K: New Thermogravimetric protocol for the investigation of normal and damaged human hyaline cartilage. *J Therm Anal and Calorimetry* 2007; 89(3): 853-56. CIT: 13 IF: 1.483
- 2) Tóth K, **Sohár G**, Pallagi E, Szabó-Révész P: Further characterization of degenerated human cartilage with differential scanning calorimetry. *Thermochimica Acta* 2007; 464: 75-77. CIT: 11 IF: 1.562

List of abstracts directly related to the subject of the Thesis

- 1) **Sohár G**, Tóth K, Pallagi E, Szabó-Révész P: Thermogravimetric investigation of normal and damaged human hyaline cartilage. *Osteoarthritis and Cartilage* 2006; Vol. 14, Supplement B S109-110. IF: 4.017
- 2) **Sohár G**, Aigner Z, Pallagi E, Szabó-Révész P, Tóth K: Complex thermal properties of human cartilage in grade 4 osteoarthritis. *Osteoarthritis and Cartilage* 2007; 15: Supplement C205-206. IF: 3.793
- 3) **Sohár G**, Aigner Z, Szabó-Révész P, Tóth K: Comparing physical properties of osteoarthritic and necrotic human hyaline cartilage. *Osteoarthritis and Cartilage* 2008; 16(4):196-97. IF: 3.793
- 4) **Sohár G**, Mécs L, Wellinger K, Aigner Z, Szabó-Révész P, Tóth K: Physicochemical transformations during thermal degradation of osteoarthritic and rheumatoid hyaline cartilage. 2009; *Osteoarthritis and Cartilage* Vol 17, Supplement 1. S249. IF: 3.793

List of papers and abstracts not related to the subject of the Thesis

- 1) Altorjay A, Juhasz A, Kellner V, **Sohar G**, Fekete M, and Sohar I Metabolic changes in the lower esophageal sphincter influencing the result of anti-reflux surgical interventions in chronic gastroesophageal reflux disease. *World J Gastroenterol* 2005; 11(11): 1623-28. CIT: 3 IF: 2.081
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- 4) Tóth K, Barna I, Nagy G, Kocsis Á, Bender Sr T, **Sohár G**: Level of pain compared to synovial beta-endorphin level in osteoarthritis and femoral head necrosis. *Osteoarthritis and Cartilage* 2007; 15: Supplement C204. IF: 3.793
- 5) Mécs L, **Sohár G**, Tóth K: Development of spine osteoarthritis after posterior lumbar spine interbody fusion. *Osteoarthritis and Cartilage* 2008; 16(4):235-36. IF: 3.793
- 6) Tóth K, **Sohár G**, Aigner Z, Greksa F, Szabó-Révész P: Novel Calorimetric properties of human cartilage samples in rheumatoid arthritis. *J Thermal Anal and Calorimetry* 2009; 95(3): 813–15. IF: 1.483
- 7) Aigner Z, Mécs L, **Sohár G**, Wellinger K, Szabó-Révész P, Tóth K: Novel calorimetric investigation of different degenerative disorders of the human hyaline cartilage. *J Thermal Anal and Calorimetry* 2009; 95(3):801-804. IF: 1.483
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- 11) Tóth K, Wellinger K, **Sohár G**, Bender T, Mécs L: Comparison of synovial -endorphin level in avascular necrosis, rheumatoid arthritis and osteoarthritis of the femoral head and knee. *Osteoarthritis and Cartilage* 2009, Vol 17, Supplement 1. S253-254. IF: 3.793
- 12) Mécs L, Wellinger K, **Sohár G**, Tóth K: Adjacent segment lumbar facet joint osteoarthritis and intervertebral disc degeneration after posterior lumbar interbody fusion. *Osteoarthritis and Cartilage* 2009; Vol 17, Supplement 1. S289. IF: 3.793
- 13) Tóth K, Wellinger K, Greksa F, Mécs L, **Sohár G**: New bone preserving treatment option for young patients with hip osteoarthritis. *Osteoarthritis and Cartilage* 2009 Vol 17, Supplement 1. S290. IF: 3.793
- 14) **Sohár G**, Fiszter I, Kovács T, Tóth K: Egy év alatt végzett szelektív ultrahanggal kiegészített csípőficam szűréseink eredményeinek utánkötése *Magyar Traumatológia, Ortopédia Kézsebészet Plasztikai Sebészet* (in press)

1. ABBREVIATIONS

AVN	avascular necrosis
DMOAD	disease-modifying osteoarthritis drug
DSC	differential scanning calorimetry
DTA	differential thermal analysis
DTG	derivative thermogravimetry
ΔH	enthalpy change
IL-1	interleukin 1
MMP	matrix–metalloproteinases
OA	osteoarthritis
OARSI	Osteoarthritis Research Society International
T	Temperature
TG	thermogravimetry
TGA	thermogravimetric analysis
TNF- α	tumor necrosis factor alpha

2. INTRODUCTION

Osteoarthritis (OA), the most prevalent joint disease, is characterized by the progressive loss of articular cartilage that leads to chronic pain and functional restrictions in affected joints [1-4]. The prior notion of OA as a bland disease related to aging and “wear and tear” of the joint has given way to views of a dynamic system with multiple pathogenic contributors. Traditional views of articular cartilage failure have centered on a variety of genetic [5-7], metabolic [2, 8, 9], and biochemical [10-13] factors. Recent studies has elucidated the importance of local factors [14, 15] as well as crystals [2, 16, 17] and inflammation [2, 18, 19] in contributing to disease progression. The new paradigm of OA considers it a heterogenous disease with numerous factors leading to its pathologic hallmark of cartilage loss and the clinical manifestation of joint pain with movement [3].

Osteoarthritis represents a major therapeutic challenge to medical and health-care providers. In part, this is because osteoarthritis is a chronic condition in which symptoms evolve over long periods of time and in which symptomatic episodes are frequently separated by lengthy asymptomatic periods. It is likely, however, that alterations in joint structure and function continue during these relative periods of clinical quiescence. In addition, limited tools are available for the assessment of the progression of structural changes in joint tissues in association with the progression of osteoarthritis and, importantly, there is not a good correlation between structural alterations and symptoms. Another major challenge with respect to definition of the pathogenic mechanisms associated with the initiation and progression of osteoarthritis is the evidence that osteoarthritis is not a homogeneous disorder. The underlying pathogenic mechanisms differ among individuals and, even in the same individual, the pathologic processes and etiologic mechanisms may differ at specific stages of disease progression. In recent years, much has been learned regarding the specific risk factors that influence the natural history of osteoarthritis. In general, these include genetic factors, the influence of aging, a history of prior injury, abnormal joint mechanics and malalignment, and the presence of inflammation [2].

Over the past years, the pathophysiology of avascular necrosis (AVN) of the femoral head has not been completely elucidated. Whereas some cases of the disease clearly have a direct cause (trauma, radiation, or Caisson disease), the pathophysiology is

uncertain for most cases. Multiple investigators have postulated vascular impairment, altered bone-cell physiology, and other theories [20-23].

An increasing number of publications have been published with the use of calorimetric techniques in the examination of human hyaline cartilage. Previously, thermoanalytical studies were used for the investigation of normal and degenerative human hyaline cartilage. The first paper from this field was the study of P. Than et al. [24]. They have concluded that structural manifestation of osteoarthritis appears as a remarkable change of thermal stability of hyaline cartilage samples. The healthy cartilage samples used in these studies were of cadaver origin as waste material, pathological cartilage was derived as intraoperative tissue fragments. The samples were washed in sterile phosphate-buffered saline and stored in complex solution containing fetal bovine serum, antibiotic, antimycotic solution, and amino acids. The measurements were conducted in 48 hours of sample deriving. The reported data on the calorimetric enthalpy changes proved to be inconsistent. In severely affected osteoarthritis, the ΔH has increased almost twofold, while in an earlier study, enthalpy changes in the intact hyaline cartilage altered from higher to lower levels in some cases [25-27].

Prior studies have demonstrated the usefulness of calorimetric examination in the characterization of cartilage degeneration [24-27]. We have extended the use of thermal analysis by introducing thermogravimetric investigations. Thereby new information on the physicochemical properties of normal, OA, and AVN tissues has been acquired.

3. CONCEPTUAL BACKGROUND

3.1 Epidemiology

OA is the most common type of joint disease and is one of the 10 most disabling conditions in developed nations. Although OA does not invariably lead to disability in those who have clinical signs of joint damage, its impact is enormous. The prevalence of OA in all joints increases with age [1, 4]. OA is second only to ischemic heart disease as a cause of work disability in men over age of 50 years [28]. OA disables about 10% of people who are older than 60 years of age, compromises the quality of life of more than 40 million Americans, and its economic impact in the United States is greater than 60 billion dollars a year [1, 4]. In some populations, including Hungary, more than 75% of the people over age of 65 years have OA that involves one or more joints [3]. Epidemiologic studies further suggest that there are clear sexspecific differences [3]. Before 50 years of age, the prevalence of OA in most joints is higher in men than in women [1]. After about age of 50 years, women are more often affected with hand, foot, and knee OA than men [28]. Of course, the growing elderly population in addition to the obesity epidemic implies that OA will assume an even greater societal impact in the near future.

3.2 Osteoarthritis

Primary OA is a complex disorder with a largely unknown etiology. Descriptions of the pathogenesis of OA have undergone many revisions since it was first described but, despite the name, the focus in recent decades has predominantly been on the articular cartilage of the synovial joints as the affected tissue, and biomechanics as the causative agent. Changes in other tissues are believed to be secondary; subchondral bone responding to abnormal biomechanics and other tissues to secondary inflammation and enforced inactivity (Fig. 1). Primary OA has to be distinguished from secondary forms of the disease, which are due to traumatic, metabolic or endocrine abnormalities [29].

Until recently, osteoarthritis has received relatively less attention from the clinical and research communities within the field of general rheumatology, in part because of the relative lack of appreciation of the role of inflammation in the pathogenesis of

osteoarthritis and of the additional challenges related to the diagnosis and treatment of this joint disorder. This is somewhat paradoxical, given the data indicating that, in the United States, over 40 million individuals are affected by osteoarthritis and the fact that osteoarthritis is the most common cause of disability in individuals over sixty-five years of age [30, 31].

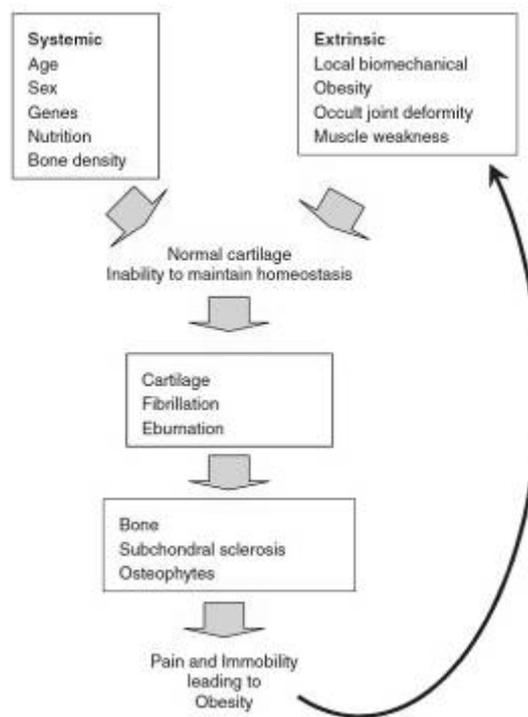


Figure 1. Risk factors for Primary OA and the target tissues

There is a significant difference in the expression levels of cartilage relevant molecules between specimens showing histological alterations and control specimens [32]. A total breakdown in synthesis of matrix molecules leads to the end stage OA with further progression of cartilage loss. Unfortunately, there is huge heterogeneity [6] in OA tissue even from the same species, and even the histological grade within the same specimen can vary [33]. The results also depend on the area of sampling [19] and the cartilage zone analysed [34]. In normal cartilage there seems to be a variation in the distribution of gene expression levels between different areas of the joint [35; 32]. Further, age-related changes might contribute to overall observations. Certainly, the sensitivity of the applied method of analysis plays an outstanding role. Nevertheless, recent studies have thrown new light on the genesis of OA and show an overall consent about the partaking process and the involved molecules.

Conceptualizing OA as phenotypic subsets related to a primary abnormality allows more targeted investigation into disease pathophysiology and treatment. Of course, it must be recognized that this distinction is somewhat artificial, and disease expression is almost certainly a summation of different, interrelated components.

3.3 Avascular Necrosis

Avascular necrosis (AVN) is still poorly understood. It is believed to be a multifactorial disease that is associated in some cases with both a genetic predilection and exposure to certain risk factors. These risk factors include corticosteroid use, alcohol intake, smoking, and various chronic diseases (renal disease, hematological disease, inflammatory bowel disease, post-organ transplantation, hypertension, and gout) [21, 22, 36]. The process of this disease has a huge cost impact on the health system due to surgical treatment. AVN of the femoral head is an increasingly common cause of musculoskeletal disability as well as a major diagnostic and therapeutic challenge. AVN of the femoral head is a pathologic process that results from interruption of blood supply to the bone. AVN of the hip is poorly understood, but this process is the final common pathway of traumatic or nontraumatic factors that compromise the already precarious circulation of the femoral head. Femoral head ischemia results in the death of marrow and osteocytes and usually results in the collapse of the necrotic segment [37].

AVN is extremely rare in healthy individuals. Although initially patients are asymptomatic, AVN usually progresses to joint destruction, requiring total hip replacement in individuals, usually before the fifth decade. No universally satisfactory therapy has been developed, even for early disease. Since joint preservation measures have a much better prognosis when the diagnosis of AVN is made early, in the course of the disease diagnosing of AVN as early as possible is critical. AVN is characterized by areas of dead trabecular bone and marrow extending to involve the subchondral plate. Elderly persons are at decreased risk for developing AVN. Incidence of AVN is increasing. The causes include greater use of exogenous steroids, excessive alcohol intake and an increase in trauma [20, 38, 39]. New pharmacological measures as well as the use of growth and differentiation factors for the prevention and treatment of this disease may eventually alter our treatment approach, but it is necessary to await results of clinical research with long-term follow-up of these patients [40-43].

3.4 Anatomy

Healthy joint cartilage has a smooth surface without fissures or material attached to the surface. It is composed of four layers: in the superficial layer cells are flat and spindle-shaped and lie parallel to the joint surface. Here the collagen fibrils are also arranged parallel to the surface. The cells in the intermediate layer are round and form columns perpendicular to the cartilage surface. They are embedded in extracellular matrix. In the radial zone, the cells are round and the columns open out to the tidemark, the border between non-calcified and calcified cartilage. Beneath this line follows first a zone of calcified cartilage and underneath the subchondral bone (Fig 2).

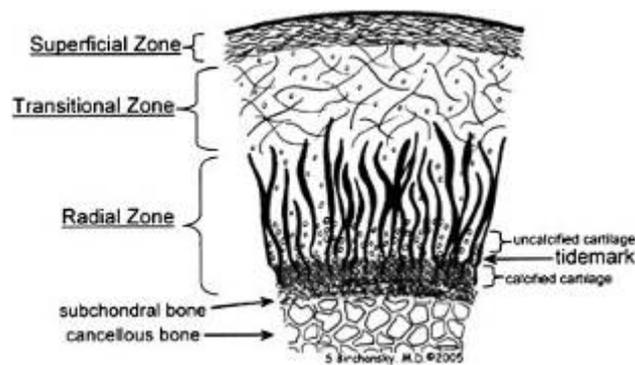


Figure 2. Human hyaline cartilage layers

Cartilage tissue has unique viscoelastic and compressive properties provided by the extracellular matrix, which is mainly composed of collagen type II and the large proteoglycan aggregate (Fig. 3) [7].

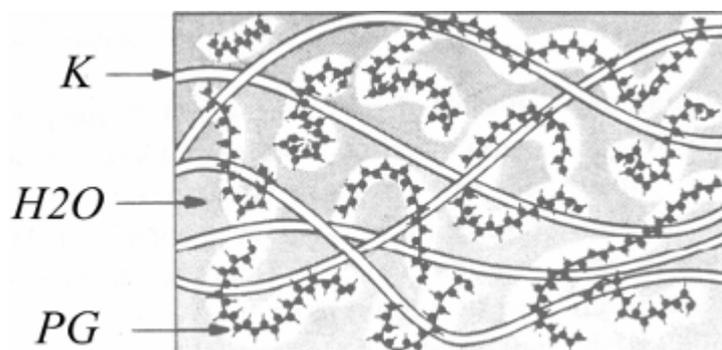


Figure 3. Cartilage matrix (K: collagen type II, H2O: water, PG: proteoglycan)

Articular cartilage lacks blood vessels and is not innervated. Nutrients in the synovial fluid and cellular repair components are transported to the chondrocytes by diffusion from the synovial fluid. Though chondrocytes are metabolically very active, they normally do not divide after adolescence. Only small defects associated with minimal loss of matrix components can be regenerated by hyaline cartilage. More extensive defects exceed the repair capacity and consequently the damage becomes permanent [44-46].

3.5 Pathology

Articular cartilage has been the focus of research into OA for decades and the literature is extensive. The role of the chondrocytes has been reviewed [47] and some key features are also briefly summarized in this thesis. In OA, chondrocytes proliferate (clone) to form multiple cells within a chondron where typically in normal cartilage, even in the elderly, more than 2–3 cells are unusual. There is increased synthesis of the principal matrix molecules [2], including collagen type II [11] and aggrecan [7], but much of this seems to be exported to the synovial fluid rather than incorporated into tissue. Elevated levels of aggrecan fragments in the synovial fluid have been taken to be a marker of increased turnover [48]. Curiously, a splice-variant form of type II collagen, Type IIA, normally expressed during development in chondroprogenitor cells, is re-expressed by adult articular chondrocytes in early and late-stage OA, indicating the potential reversion of the cells to an earlier developmental phenotype [5]. Elevated matrix synthesis is accompanied by increased synthesis of matrix–metalloproteinases (MMP), prostaglandins, and other inflammatory factors in OA tissue. This appears to be related to elevated levels of interleukin-1 (IL-1) and tumornecrosis factor alpha (TNF- α) [47, 49]. Interestingly, an association has been reported between histological severity of OA and lipid accumulation in the cartilage, especially arachidonic acid [50]. As in OA bone, these lipids could provide a reservoir of pro-inflammatory precursors and are another indicator of abnormalities in lipid metabolism (Fig. 4).

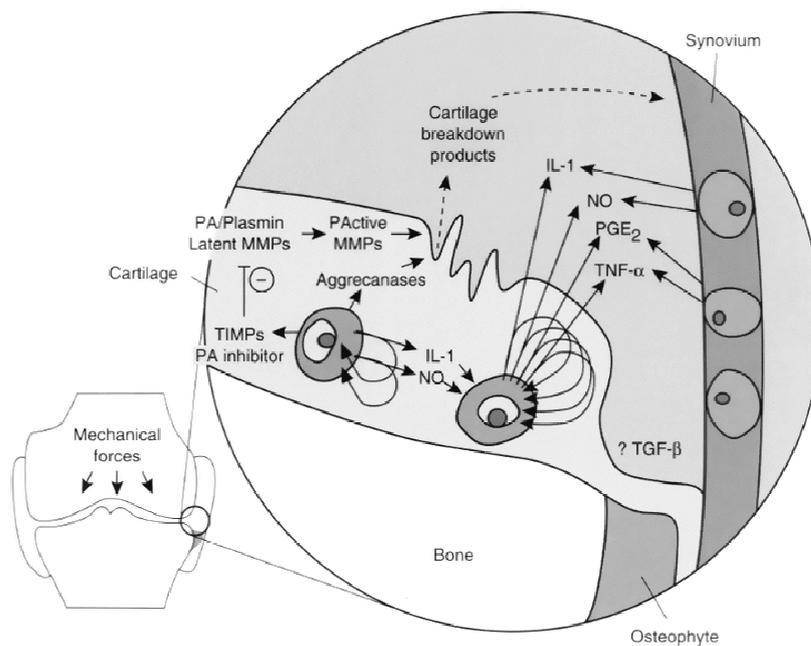


Figure 4. Increased synthesis of the principal matrix molecules in OA

Advancement and duplication of the tidemark [51], which marks the junction between uncalcified and calcified cartilage, are another indicator of renewed growth and tissue formation, though the underlying mechanisms for this are still unknown. Articular cartilage is a highly specialized tissue in which collagen fibrils reinforce a highly hydrated proteoglycan gel [52]; a biological fire-composite material. It is primarily the interactions between collagen and the other components that provide mechanical integrity [9, 53, 54], not simply a stiff collagen ‘network’ and a filler. Both the organization of the fibrils and interactions between the fibrils and the gel are tightly regulated during development to produce the appropriate mechanical properties. Proteoglycan, but not collagen, turnover then maintains the mechanical homeostasis of the adult tissue. A metabolically driven problem leading to excess synthesis of new matrix molecules together with a reversion of the chondrocytes to an earlier developmental phenotype could have the effect of weakening the tissue, either by trying to insert new tissue in the midst of old or by inappropriately trying to remodel the tissue. Such tissue weakening would then make it susceptible to mechanical damage [12].

3.6 Biomechanics

The mechanical properties of cartilage, including viscoelasticity and high resistance against load and shear stress, are controlled through the metabolic balance within the matrix collagen–proteoglycan network. Water is the main composite (60–80%) of the extracellular matrix [10, 55–58]. Proteoglycans induce a high osmotic pressure and have a high water binding capacity. Between 5 and 10% of the cartilage mass are proteoglycans, mostly aggrecan [59]. Collagen (90% collagen type II) is responsible for the high resistance against tensional forces. Collagen type II contributes to up to 60% of the dry weight [60]. During inflammation or slow degeneration, the homeostasis within the collagen–proteoglycan network in the chondral matrix is disordered, mainly due to the activation of MMPs (stromelysin) after stimulation by cytokines like IL-1 and TNF-alpha [10]. Cartilage defects generally are grouped into four stages: softening with intact surface, fibrillations within the superficial cartilage layer, clefts down to the subchondral bone, and complete loss of cartilage [61].

Theoretical and computational analyses of the contact response of cartilage under various loading conditions have predicted that more than 90% of the load transmitted across articular layers is supported by the pressurized interstitial fluid, with the remnant contributed by the collagen-proteoglycan solid matrix. Since this fluid pressure is a hydrostatic stress, and since cartilage has been shown to be nearly incompressible at physiological levels of pressures, it has become evident that the interstitial fluid shields the solid matrix from excessive deformations [62-64].

3.7 Thermal Analysis

Thermal analysis comprises a group of techniques in which a physical property of a substance is measured as a function of temperature, while the substance is subjected to a controlled temperature programme.

Differential scanning calorimetry (DSC) and thermogravimetric (TG) analysis have been widely used for determining physicochemical transformations that occur during thermal degradation [65, 66]. These techniques measure net changes in enthalpy and weight as a result of many reactions taking place simultaneously and are particularly useful for indicating the temperature range and the rate of thermal processes as well as

giving considerable information on physical and chemical changes [67-70]. Relatively little has been published on the thermal properties of human hyaline cartilage.

Understanding the response of drugs and their formulations to thermal stresses is an integral part of the development of stable medicinal products. Thermal analytical methods have thus become important tools for the development of modern medicines. These are precise and accurate techniques with low sample requirements, and can provide detailed information about new chemical entities even at the very earliest stages of drug discovery and development [70, 71].

3.7.1 Thermogravimetry

Thermogravimetric analysis (TGA) is an analytical technique used to determine a material's thermal stability and its fraction of volatile components by monitoring the weight change that occurs as a specimen is heated [72, 73]. The measurement is normally carried out in air or in an inert atmosphere, such as Argon, and the mass is recorded as a function of increasing temperature. Sometimes, the measurement is performed in a lean oxygen atmosphere (1 to 5% O₂ in N₂ or He) to slow down oxidation. In addition to mass changes, some instruments also record the temperature difference between the specimen and one or more reference pans (differential thermal analysis, or DTA) or the heat flow into the specimen pan compared to that of the reference pan (differential scanning calorimetry, or DSC). The latter can be used to monitor the energy released or absorbed via chemical reactions during the heating process.

In most cases, TG analysis is performed in an oxidative atmosphere (air or oxygen and inert gas mixtures) with a linear temperature ramp. The maximum temperature is selected so that the specimen mass is stable at the end of the experiment, implying that all chemical reactions are completed (i.e., all of the carbon is burnt off leaving behind metal oxides). This approach provides two important numerical informations: ash content (residual mass, M_{res}) and oxidation temperature (T_o). While the definition of ash content is unambiguous, oxidation temperature can be defined in many ways, including the temperature of the maximum in the weight loss rate (dm/dT_{max}) and the weight loss onset temperature (T_{onset}). The former refers to the temperature of the maximum rate of oxidation, while the latter refers to the temperature when oxidation just begins.

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. Key application areas [72, 74-79] are listed below:

- Thermal Stability: related materials can be compared at elevated temperatures under the required atmosphere. The TG curve can help to elucidate decomposition mechanisms.
- Kinetic Studies: a variety of methods exist for analysing the kinetic features of all types of weight loss or gain, either with a view to predictive studies, or to understanding the controlling chemistry.
- Material characterisation: TG and Derivative Thermogravimetry (DTG) curves can be used to "fingerprint" materials for identification or quality control.
- Corrosion studies: TG provides an excellent means of studying oxidation, or reaction with other reactive gases or vapours.
- Simulation of industrial processes: the thermobalance furnace may be thought of as a mini-reactor, with the ability to mimic the conditions in some types of industrial reactor.
- Compositional analysis: by careful choice of temperature programming and gaseous environment, many complex materials or mixtures may be analysed by selectively decomposing or removing their components. This approach is regularly used to analyse e.g. filler content in polymers; carbon black in oils; ash and carbon in coals, and the moisture content of many substances.

3.7.2 Calorimetry

Differential scanning calorimetry (DSC) is a thermoanalytical technique for measuring the energy necessary to establish a nearly zero temperature difference between a substance and an inert reference material, as the two specimens are subjected to identical temperature regimes in an environment heated or cooled at a controlled rate. In heat-flux DSC, the sample and reference are connected by a low-resistance heat-flow path (a metal disc). The assembly is enclosed in a single furnace (Fig. 5).

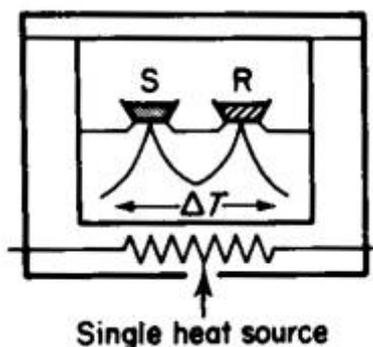


Figure 5. Heat flux DSC (S: substance, R: reference material)

Enthalpy or heat capacity changes in the sample cause a difference in its temperature relative to the reference; the resulting heat flow is small compared with that in differential thermal analysis (DTA) because the sample and reference are in good thermal contact. The temperature difference is recorded and related to enthalpy change in the sample using calibration experiments [69].

DSC is a frequently preferred thermal analytical technique because of its ability to provide detailed information about both the physical and energetic properties of a substance. This information cannot be obtained accurately, easily, or quickly using any other technique. With the development of sophisticated, modulated temperature programs, micro-scale test configurations, robotic systems, and combined DSC spectroscopic instrumentation, it is likely that DSC will retain its place at the forefront of the pharmaceutical thermal analytical sciences for some time to come [71].

Calorimetry can be used to determine most thermodynamic properties; e.g. enthalpy changes for reactions (ΔH), for phase changes, for mixing, etc.; equilibrium constants and thus free energy changes for many reactions; heat capacities and coefficients of thermal expansion and compressibility; entropy and entropy changes. Calorimetry can also be used for qualitative and quantitative analyses. ΔH can often be determined for an unknown reaction in a complex system, and the value of ΔH can then be used to assist in identifying the reaction. Quantitative analyses is done by two methods, either by calorimetric titration or from the ratio of measured heat to ΔH for the reaction. In the analytical literature, the former and latter methods are called thermometric titration and direct injection enthalpimetry, respectively. The same principle used in enthalpimetry is used in thermal analysis with temperature-scanning calorimeters, i.e. where ΔH is the enthalpy change of the process initiated by the temperature change [80-82].

Since calorimetry directly measures the instantaneous rate of the process, calorimetry is a particularly advantageous method for determination of the kinetics of slow processes. Analysis of thermodynamic and kinetic data from calorimetry always involves a model for the system, e.g. a set of chemical reactions, kinetic equations, or a theoretical model for the property as a function of temperature, pressure, or composition. Calorimetric data will be fitted to the model to obtain model parameters, therefore providing a description of the system as a function of the experimental variables. Calorimetric data can also be used to test the predictive power of such models, and thus to gain fundamental insight into a process or property of a material, or to predict failure or hazardous conditions. Such models may require collection of ancillary data simultaneously with the calorimetric measurements [83].

4. AIMS OF THE INVESTIGATIONS

Based on previous studies, we hypothesized that

- thermodynamic findings clearly differentiate normal and degenerated human hyaline cartilage,
- physicochemical transformations may provide information on the role of water content in osteoarthritis and avascular necrosis, and
- enthalpy change of the process, initiated by the temperature change, might represent potential marker of the disease activity.

In order to get answer for the hypotheses above, patients with primary end-stage OA and AVN were chosen for our investigations.

The aim of this study was:

1. to investigate whether cartilage undergoes complex changes in matrix composition (water, proteoglycan, and collagen content) during the late stage of degeneration. These complex deviations develop from the normal matrix composition during the diseases OA and AVN are hypothesized to correlate with changes in thermal analysis;
2. to introduce thermogravimetric examination as part of thermal analysis alongside calorimetry, since water content of the cartilage has not been measured before by thermogravimetry;
3. to establish the kinetic character of the effect of water loss by heating;
4. to find correlation between the enthalpy changes and the severity of OA and AVN;
5. to establish a **new protocol** for sample extraction during live surgery; and
6. the further purpose of this study was to elucidate the importance of water content in contributing to disease progression.

5. PATIENTS AND METHODS

5.1 Patients

In order to conduct the thermoanalytical study, 35 samples were collected from live surgeries of OA patients between October 2005 and April 2006. During hip arthroplasty procedures performed at the Orthopedic Department, University of Szeged, 16 OA and 12 AVN human hyaline cartilage samples and normal cartilage from 7 knee were obtained. There was no clinical meaningful difference in age between OA patients (64 ± 5.2), AVN patients (59 ± 6.4) and controls (61 ± 4.2). There was no considerable sex differences between OA patients (75% females), AVN patients (60% females), and controls (70% females); Chi-square $P = 0.54$.

Usually, in OA of both medial and lateral knee compartments, total knee arthroplasty is performed. When only one compartment is affected and ligamental stability is intact, unicompartmental prosthesis is implanted. We were able to obtain normal cartilage samples from those patients where one compartment of the same knee was degenerated, and the other one was normal. Therefore, the unaffected femoral condyle had to be sacrificed for the procedure because ligamental instability was the indication for total knee arthroplasty.

5.2 Patients grading

Preoperatively, the diagnosis of the patients were established on basis of the patient history, clinical signs, laboratory tests, and radiological findings. The state of the hyaline cartilage was determined intraoperatively. All patients in the osteoarthritic group were considered to be Osteoarthritis Research Society International (OARSI) grade 5-6 articular surface degeneration. OARSI Grade 5-6 OA is characterized by deformation and change in the contour of the articular surface (Fig. 6) [84]. This results not only from articular plate fractures, but also from increased metabolic activity of the articular bone plate, as well as from activation of connective tissue at the lateral and, sometimes, central cartilage/bone interfaces. All patients in the AVN group were classified as Ficat stage 4 [85]. Samples were considered to be normal when hyaline articular cartilage was

uninvolved with OA (OARSI Grade 0). In these results, the cartilage surface is smooth, no enlargement, distortion, and no proliferative changes are observed.

Grade (key feature)	Subgrade (optional)	Associated criteria (tissue reaction)
Grade 0: surface intact, cartilage intact	No subgrade	Intact, uninvolved cartilage
Grade 1: surface intact	1.0 Cells intact 1.5 Cell death	Matrix: superficial zone intact, edema and/or fibrillation Cells: proliferation (clusters), hypertrophy Reaction must be more than superficial fibrillation only
Grade 2: surface discontinuity	2.0 Fibrillation through superficial zone 2.5 Surface abrasion with matrix loss within superficial zone	As above ± Discontinuity at superficial zone ± Cationic stain matrix depletion (Safranin O or Toluidine Blue) upper 1/3 of cartilage (mid zone) ± Disorientation of chondron columns
Grade 3: vertical fissures	3.0 Simple fissures 3.5 Branched/complex fissures	As above ± Cationic stain depletion (Safranin O or Toluidine Blue) into lower 2/3 of cartilage (deep zone) ± New collagen formation (polarized light microscopy, Picro Sirius Red stain)
Grade 4: erosion	4.0 Superficial zone delamination 4.5 Mid zone excavation	Cartilage matrix loss, cyst formation within cartilage matrix
Grade 5: denudation	5.0 Bone surface intact 5.5 Reparative tissue surface present	Surface is sclerotic bone or reparative tissue including fibrocartilage
Grade 6: deformation	6.0 Joint margin osteophytes 6.5 Joint margin and central osteophytes	Bone remodelling, Deformation of articular surface contour (more than osteophyte formation only) Includes: microfracture and repair

Figure 6. OA cartilage advanced grading methodology

5.3 Sample preparation for thermal analysis

After the operation, a disc (5mm in diameter) was removed from the unhealthy and healthy cartilage surfaces. The samples were taken under sterile conditions, excess bone was removed, and only the remaining full thickness cartilage was used. The disc was first washed in sterile saline, then stored in 20 ml saline for transportation at room temperature. Mean storage time was 6 hours (min: 1 hour, max: 26 hour), 29 samples out of 35 were studied within four hours of preparation. Six samples were stored over-night at 5 °C. Preemptive control examinations did not show any change in the calorimetric and thermogravimetric properties after storage for 26 hours at 5 °C.

5.4 Thermal measurements

The success of the thermal experiments depends on the careful preparation of samples and the judicious selection of the appropriate experimental conditions (such as scanning rate and sample size). In general, DSC samples are analyzed in small metal pans, designed for optimal thermal conductivity and minimum reaction with the samples (for example, aluminum alloy, platinum, stainless steel, or silver) [71]. For accurate quantitative work the thermal mass of the sample and reference pans were matched.

All the thermal measurements were conducted at the Department of Pharmaceutical Technology, University of Szeged. The calorimetric properties of samples were determined by DSC method (Mettler-Toledo DSC 821e apparatus, Mettler-Toledo GmbH, Switzerland). Samples were heated from 0 to 80 °C. The heating rate was 0.3 °C/min. Conventional Hastelloy batch vessels were used with 40 µl sample volume. All the DSC measurements were preceded in Ar atmosphere, and the flow rate was 100 ml/min. From the DSC curves, the decomposition temperature (onset temperature), the transition temperature range (endset temperature), and the total calorimetric enthalpy change were calculated. Well-defined standards and calibration procedures are particularly important, therefore high care was taken in calibrating the instrument as close to the transition temperatures of interest as possible.

The thermogravimetric analysis was performed with the use of a MOM Derivatograph (MOM, Budapest, Hungary), and the TG, DTG, and DTA curves were determined. The temperature (T) curve shows the linear increase of temperature during the process. DTG curve represents the first derivative of the mass change curve. The DTA curve shows the same picture as a Differential Scanning Calorimetry examination, in which the temperature change of a sample is compared with the temperature of a thermally inert material in order to give information about the endothermic or exothermic enthalpic transition or other reaction [76-79].

The heating was linear from 25 to 150 °C and the rate of heating was 5 °C/min. Al₂O₃ 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 [86], and it is the first kinetic parameter calculated by the computer:

$$n = 1,26 \cdot S^{\frac{1}{2}} \quad (1)$$

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

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

which is widely used in the literature [70, 87-89].

5.5 Statistical analysis

Fisher LSD method by the Statistica for Windows statistical program was used to compare enthalpy changes in the different groups.

Data are presented as mean \pm SD. Statistical significance was assessed by the Student t test and the Kruskal-Wallis one-way ANOVA on ranks. The results were considered significant, if $p < 0.05$ (*means significant values on the graphs).

5.6 Ethics

All tissues were yielded in accordance to legal regulation, international ethical concerns, and patients' consent. The Human Investigation Review Board of the University of Szeged has decided (2006.09.18.) that the experiments comply with the ethics of research and the declaration of the Medical World Federation.

6. RESULTS

6.1 Thermogravimetry

Normal samples were examined with derivatograph. The thermogravimetric results are presented in Table 1.

Name	Mass loss % (-)	E_{act} kJ/M	Reaction order (n)
cartl2	80.21	50.46	1.15
cartl9	81.32	51.025	1.25
cartl14	74.255	51.915	0.95
cartl17	92.17	62.545	1.1
cartl49	75.43	43.82	0.7
cartl62	94.42	43.63	0.9
cartl63	96.42	55.07	0.9

Table 1. Thermogravimetric measurements of normal samples.

TG, DTG and DTA curves of the normal samples are presented on Fig. 7. Many information can be obtained from the T, TG, DTG and DTA curves.

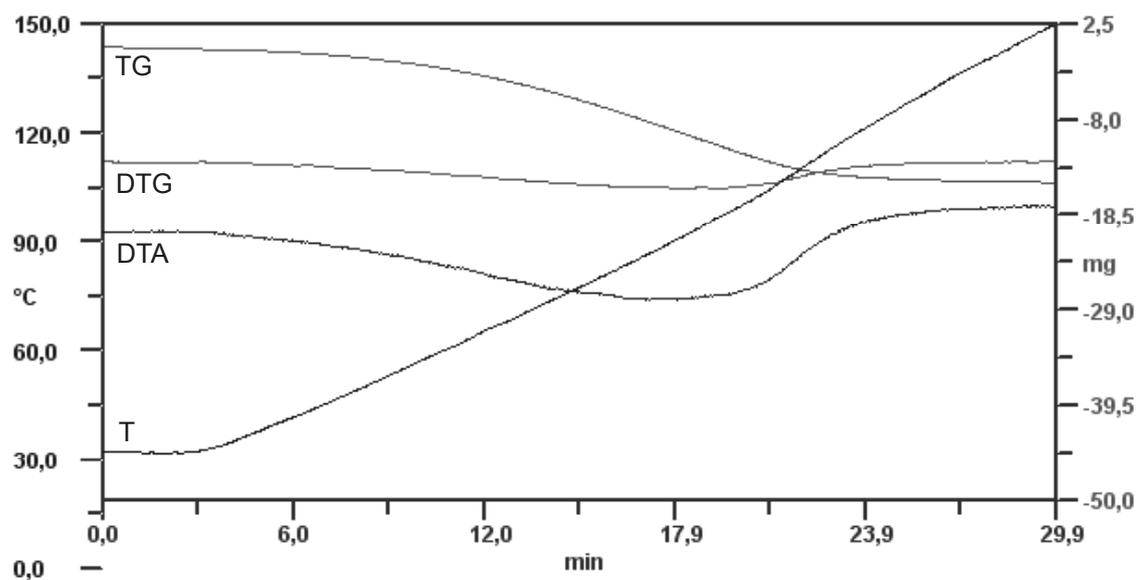


Figure 7. Thermogravimetric curves of a normal sample

It was found, that the average total water content of intact (normal) cartilage is 81%, which was probably the interstitial water, and the difference was supposedly bound to the surface. To remove the cartilage extracellular water content, 52 kJ/M energy was needed.

Total water content of the OA samples was 87%, and 73 kJ/M energy was used for the removal of the fluid content (Table 2 and Fig. 8).

Name	Mass loss % (-)	E_{act} kJ/M	Reaction order (n)
cartl14	72.19	136.7	1.8
cartl17	73.15	80.23	1.25
cartl19	83.69	61.09	0.75
cartl23	89.64	53.21	0.7
cartl28	85.09	55.70	0.8
cartl29	88.90	99.21	1.15
cartl30	89.32	77.73	1
cartl31	80.28	73.22	1
cartl34	73.92	105.61	1.3
cartl35	94.59	66.50	1.15
cartl36	95.29	51.09	0.8
cartl37	88.34	60.25	1.1
cartl38	93.28	44.72	0.7
cartl41	90.36	78.68	1.15
cartl42	98.62	49.12	0.9
cartl50	90.64	70.49	1

Table 2. Thermogravimetric measurements of osteoarthritic samples.

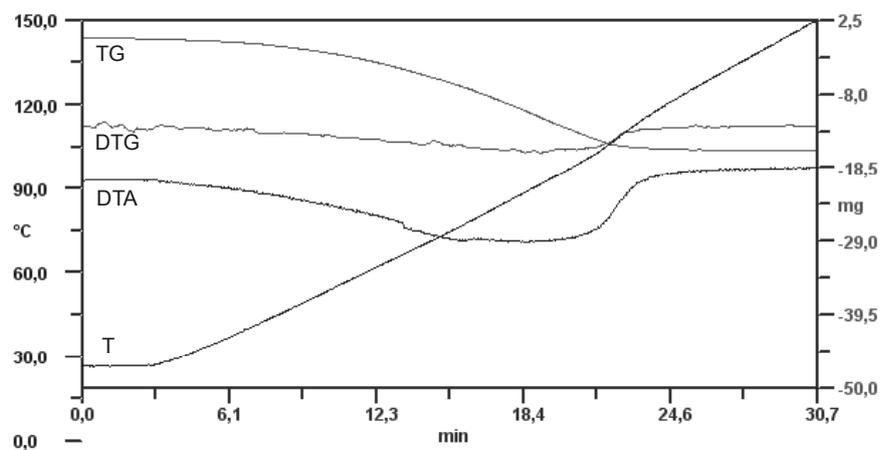


Figure 8. Thermogravimetric curves of an osteoarthritic sample

Cartilage obtained from necrotic femoral head (Table 3. and Fig. 9.) had a higher water content of 88% than the normal samples. Extraction of the cartilage fluid content needed 70 kJ/M energy.

Name	Mass loss % (-)	E _{act} kJ/M	Reaction order (n)
cartl1	89.09	68.36	0.8
cartl16	72.5	38.305	0.6
cartl25	71.205	120.655	1.15
cartl27	87.54	81.42	1
cartl33	82.78	80.03	1
cartl39	89.105	64.555	1.1
cartl40	84.445	52.845	1
cartl44	88.68	61.87	0.8
cartl46	84.52	52	0.85
cartl52	94	63.5	1
cartl53	99.64	65.1	1.2
cartl54	96.04	103.29	2
cartl55	84.34	43.36	0.7
cartl59	95.88	83.86	1.1
cartl64	97.26	72.71	0.9

Table 3. Thermogravimetric measurements of necrotic samples.

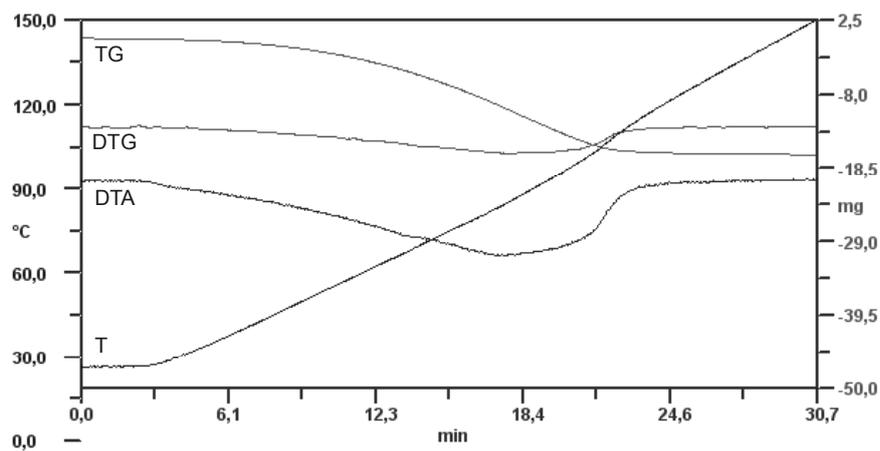


Figure 9. Thermogravimetric curves of a necrotic sample

Loss of water content in all three groups are presented with a sharp step on the TG curve, starting on average temperature of 37 °C and ending at 116 °C. Linear part of the TG curve begun at around 57 °C and ended at around 104 °C (Table 4). 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 5). The slope of the linear region correlated in all three groups.

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
OA	16	36.4-121.9	86.71 SD: 7.84	72.72 SD: 23.46
AVN	12	37.4-116.2	87.80 SD: 8.06	70.25 SD: 21.71

Table 4. Average mass loss and activation energy of normal and degenerated samples.

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. In the osteoarthritic samples (average mass: 17.02 mg), 0.242 mg decrease was measured which represents 1.4% °C⁻¹ mass reduction. Necrotic samples (average mass: 15.51mg) released 0.262 mg of water with the same increase of temperature, so 1.7% °C⁻¹ decrease in mass was observed. The resulting amount of mass lost in the linear region was recounted from these results (Table 5).

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
OA	16	58.0-104.6	-65.24	1.03 SD: 0.27	-0.048
AVN	12	57.6-102.0	-75.48	1.03 SD: 0.32	-0.042

Table 5. Reaction kinetic parameters of normal and degenerated samples.

6.2 Calorimetry

With the rise of temperature, an endothermic reaction was observed in all of the cases (Tables 6, 7, and 8).

Name	ΔH J/g (-)	Peak °C	Onset °C	Endset °C
cartl2	867.99	50.44	26.5	59.09
cartl9	815.61	49.75	31.83	58.15
cartl14	727.58	47.74	34.62	53.62
cartl17	837.96	52.38	36.25	57.25
cartl49	808.34	54.11	35.72	62.47
cartl62	611.95	43.72	30.05	47.02
cartl63	848.99	53.15	32.3	62.03

Table 6. Calorimetric measurements of normal samples.

Name	ΔH J/g (-)	Peak °C	Onset °C	Endset °C
cartl4	424.97	45.55	32.92	50.53
cartl6	542.53	52.72	27.3	57.67
cartl7	427.73	49.07	42.1	58.19
cartl10	509.96	46.02	35.4	55.1
cartl19	550.37	50.73	31	51.76
cartl23	695.55	52.78	31.15	57.73
cartl28	492.43	48.85	34.77	53.05
cartl29	511.59	46.66	30.9	55.36
cartl30	516.24	49.35	37.81	55.95
cartl31	496.11	54.11	34	57.07
cartl35	649.92	50.24	27.38	58.82
cartl36	558.65	52.14	31.71	58.55
cartl37	440.68	46.56	28.92	50.53
cartl38	576.2	50.72	39.84	54.27
cartl41	682.5	55.74	36.76	52
cartl42	590.79	52.26	30.54	55.64

Table 7. Calorimetric measurements of osteoarthritic samples.

Name	ΔH J/g (-)	Peak °C	Onset °C	Endset °C
cartl1	507.56	45.24	31.84	53.04
cartl16	399.44	36.84	28.33	44.74
cartl27	525.22	53.19	31.74	57.63
cartl33	444.95	43.85	29.7	48.44
cartl39	469.35	53.33	34.8	57.24
cartl40	483.84	47.11	36.14	49.79
cartl44	780.95	48.33	31.78	53
cartl46	748.74	58.01	44.87	60.94
cartl52	536.23	51.19	38	53.73
cartl53	713.81	56.7	38.19	57.22
cartl55	545.34	45.51	33	51.65
cartl64	649.56	47.95	31.6	53.1

Table 8. Calorimetric measurements of necrotic samples.

The enthalpy change of the process initiated by the temperature change showed marked difference between the normal and pathological groups. (Table 9)

Sample group	Sample number	ΔH (J/g) (-)	DSC peak (°C)	Beginning (°C)	Ending (°C)
Normal	7	788.346 SD: 83.181	50.18 SD: 3.31	≈32.5 SD: 3.45	57.09 SD: 5.35
OA	16	543.838 SD: 88.572	50.34 SD: 2.937	≈33.8 SD: 4.3	≈33.8 SD: 4.3
AVN	12	567.083 SD: 120.17	48.93 SD: 5.93	≈34.16 SD: 4.37	53.38 SD: 4.28

Table 9. Thermal parameters of denaturation (mean \pm SD) of normal and degenerated samples.

Greater change in the enthalpy was observed in normal cartilage: 788.346 J/g (SD = 83.18). In case of osteoarthritis 543.838 J/g (SD = 88.57), while in the necrotic samples 567.083 J/g (SD = 120.17) was measured (Table 9). Therefore, denaturation caused by heating was largest in the normal human hyaline cartilage. Consequently these samples required the largest amount of energy for decomposition. Statistical tests proved these calculations to be significant (Fisher LSD method, $p < 0.05$). Denaturation peak in

normal cartilage was at 50.18 °C (SD = 3.31), in necrotic samples it was lower at 48.93 °C (SD = 5.93) however in osteoarthritis 50.34 °C (SD = 2.93) was similar to control samples which had normal hyaline cartilage (Fig. 10, 11).

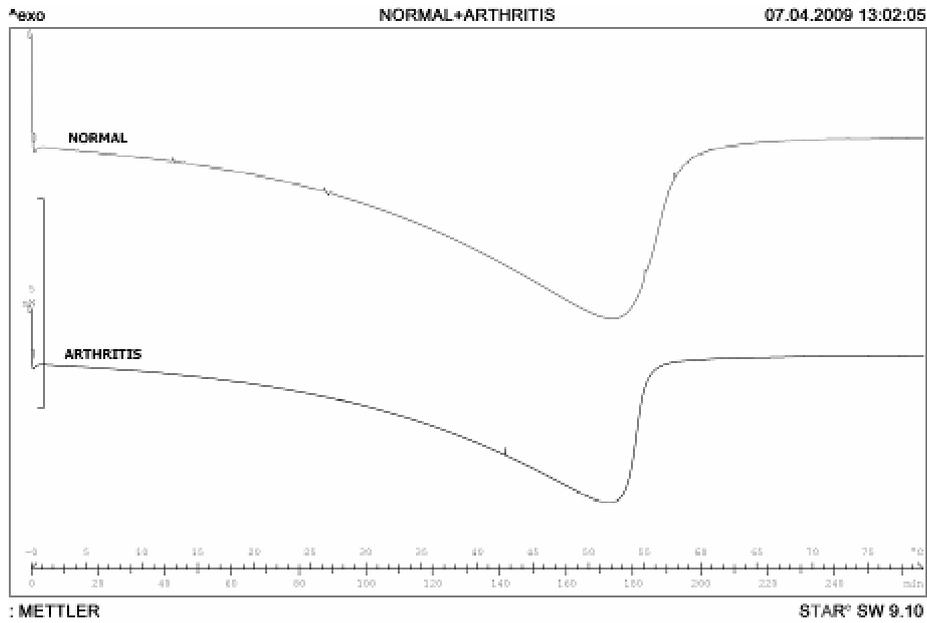


Figure 10. DSC curve of normal and osteoarthritic human hyaline cartilage samples. (the downwards deflection means endothermic effect)

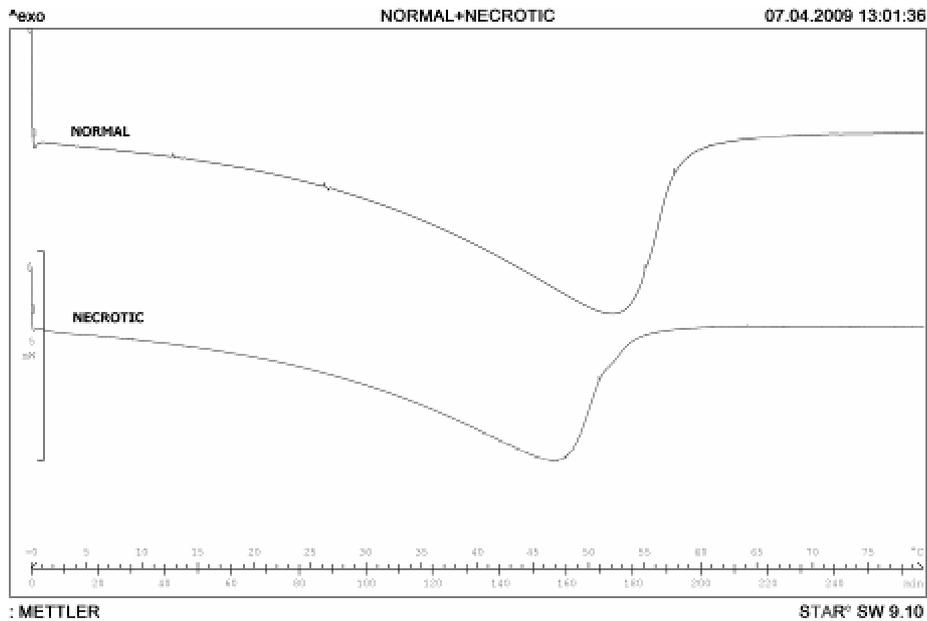


Figure 11. DSC curve of normal and necrotic human hyaline cartilage samples. (the downwards deflection means endothermic effect)

7. DISCUSSION

OA development takes place in consecutive steps of breakdown and attempted regeneration. Though the comprehension about OA has grown enormously over the last years, there is still need to extend our knowledge about the basic context of OA genesis and development. Several biochemical and biomechanical factors are considered for the pathogenesis [14, 90]. The data up to date show, however, that OA is a very complex disease procedure, and it can be speculated, that the context leading to the progressive process is not finally resolved. There might still be molecules involved in the process, which molecules have not yet been studied or even identified [91].

OA is widely believed to result from local mechanical factors acting within the context of systemic susceptibility [8, 11, 15-17, 47, 92]. Molecular pathology of osteoarthritis is under intense investigation [6, 7, 18, 93-97] since biomechanical factors result in chemical alteration within the joint [19, 98, 99]. Rearrangements of intra- and intermolecular bonds in collagen molecule and disaggregation of proteoglycans and their elimination from OA cartilage found to be responsible for water accumulation [13]. It was also shown that the most part of water is free water and its quantity is increased in the osteoarthritis of the hyaline cartilage [100].

We observed increase in water content of the cartilage matrix in all cases of the investigated degenerative cartilages [101]. Based on our results, it can be stated that water content is higher in impaired samples, meanwhile water interstitial bonding was stronger in the OA and AVN 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. Denaturation caused by heating was larger in the normal cartilage than in the diseased ones, therefore normal samples required the largest amount of energy for decomposition [102].

The purpose of our study was also to clarify the previously reported studies in the literature [24-27]. By acquiring normal cartilage from live surgery and by performing the investigation in a relatively short period of time compared to the earlier reports [24-27, 101, 102], similar sample environment was provided as with the degenerative samples. This way, we minimized the extracorporeal degeneration. All samples we used showed a clear denaturation peak on the calorimetric curve, therefore volume of the curve was easily calculated giving the enthalpy change of the sample. These changes correlated with

the water content of the samples. Due to the increased number of samples acquired for our studies, the results were much better reproducible than results in the literature [24, 102], and the difference between the normal and necrotic samples was significant [101, 102].

The newly established thermogravimetric protocol that we used was sufficient for compositional thermoanalytical study of normal and degenerative human hyaline cartilage. Water content elevation contributing to disease progression was observed in both OA and AVN. Previously, this method has not been used for this type of investigations. The main goal of the thermogravimetric measurements was to identify the nature and quantity of water molecules in the investigated samples. Water molecules' binding mode may have an important consequence in pharmacokinetics. The reaction order turned out to be approximately 1 in all three cases (normal, OA, and AVN), and the standard deviation was low (Table 5). The TG curve's slope of the linear region showed, that the rate of water loss depends on the water amount remaining in the tissue. Comparing the data in the presented tables (Tables 4, 5) (Total mass loss: normal: 80.79%, OA: 86.71%, and AVN: 87.80%), it can be concluded that the higher water content in the degenerative samples bound 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 the water amount remaining in the tissue, namely if the amount of water decreases in the tissue, the rate of loss also decreases.

The pathogenesis of OA progression likely revolves around a complex interplay of numerous factors. The major contributors include chondrocyte regulation of the extracellular matrix [11, 12, 95], genetic influences [5-7], local mechanical factors [14, 15, 55], and inflammation [2, 18, 19]. DSC as part of thermal analysis was a reliable method for differentiating normal hyaline cartilage from degenerated samples. The available calorimeter proved to be adequate for these measurements.

DSC techniques are still developing and many new variants and applications are reported each year. Combined techniques [103-105] with microscopic or spectroscopic instruments are of obvious value to the pharmaceutical scientist, although commercially available units are not widely used and have limited pharmaceutical applications. With the rapid development of atomic and molecular scale microscopy, hyphenated micro-thermal analysis techniques, such as atomic force microscopy-DSC, are also becoming commercially available. There may be many future applications of micro-DSC

measurements to pharmaceutical problems, although these are likely to be limited to basic research applications in the next few years until the full potential of the technique has been demonstrated.

AVN of the femoral head is a common cause of painful hip in young adults. The natural course of this disease has steady progression with eventual collapse of the femoral head, followed by secondary osteoarthritis in the hip joint. Molecular pathology of AVN is under intense investigation since biomechanical factors result in chemical alteration within the joint [23, 106-108]. In AVN of the femoral head, interference with blood results in infarction of a segment of the head leading to drastic early degeneration of the surface hyaline cartilage [108, 109]. Patients in our study undergoing arthroplasty procedure showed clinically and radiologically end-stage degradation of the cartilage, with very little resemblance to normal cartilage.

Our study has had several limitations, as many other studies on OA and AVN. First, the sample size was not large enough to arrive at definitive conclusions. Additional measurements are needed to affirm the results of our study. Secondly, we investigated those patients for normal cartilage samples of the knee, who underwent surgery for the other compartment OA. This was the only ethical and technical way of acquiring normal tissues from living persons for our experiments. Previous thermoanalytical studies used cadaver samples for the investigation as normal human hyaline cartilage. All samples that were extracted for our studies were obtained during live surgeries. and were macroscopically intact [101, 102]. There is no previous report in the literature of examining normal cartilage from live surgery. Only full thickness cartilage was used for the normal analysis. Prior results indicate that early OA is primarily characterized by the changes in collagen orientation and proteoglycan content only in the superficial zone, while collagen content does not change until OA has progressed to its late stage [110]. A new protocol had to be established before the detailed investigation of human tissues could be performed. Most of the known changes in the extracellular matrix in OA come from animal models in the literature since human samples for investigation are not widely available for experiments.

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 its binding characteristics, preventive measures can be developed. These therapeutic steps can be adequately tested and monitored with thermogravimetric measurements. The use of this method can also determine the effectiveness of currently used medications (Glucosamin, Chondroitin) for resolving cartilage matrix degeneration.

Further understanding of the initiating events in cartilage destruction, the relationship between the different pathologic influences, and the role of the chondrocyte in maintaining extracellular matrix homeostasis will be necessary to reveal potential targets of therapy. Clinical trials are currently underway for a number of potential disease modifying agents that may significantly change the treatment approach for OA [31, 99]. With the possibility of disease-modifying OA drugs (DMOADs), the necessity for instruments that are sensitive to changes has become very apparent in clinical trials [111].

With regard to the clinical management of patients with OA, efforts should be made wherever possible to influence modifiable risk factors. At present, there are a number of modifying therapeutic options that may be used to alter the rate of disease progression. While these therapies are currently underutilized, they might play a greater role if modification of disease progression has to be demonstrated. However, the role of these therapies is unclear, given the paucity of long-term, well-designed controlled trials [112-114].

Finally, we have to mention that common histopathologic assessment methods (Grade) under both clinical and experimental conditions poorly reflect mild phases of the disease, and are very non-linear over the range from mild to advanced stages of the disease. In the OARSI (OsteoArthritis Research Society International) Scoring System grade is defined as OA depth progression into cartilage irrespective of its horizontal extent. Stage is defined as the horizontal extent of cartilage involvement within one side of a joint compartment irrespective of the underlying grade. Therefore, a detailed thermal examination is needed on the same joint surface with samples taken from different grades of degeneration within the same joint.

The promise of biomarkers has yet to be fulfilled in OA. Type II collagen, cartilage oligomeric matrix protein, hyaluronan, and aggrecan have been some of the many biomarkers investigated so far [45, 115-117]. Although numerous clinical studies have suggested that specific biomarkers or their combinations can have predictive value in terms of the presence and severity of the disease [118]. The wide variability in these values limits their use for individual patients. Whereas, the use of thermal analysis could

be a simple and effective method for controlling the relationship between these markers and disease progression. The revised protocol for sample taking during live surgeries eliminates the presence of disturbing substances during the examination. A detailed thermal examination is also needed on the same joint surface with samples taken from different grades of degeneration within the same joint.

8. SUMMARY AND CONCLUSIONS

In summary, we examined the water content of human hyaline cartilage of normal origin and in patients with OA and AVN. We were the first ones who used normal samples that were extracted from live surgeries for the investigations.

A newly established thermogravimetric protocol was used for our experiments. This method proved to be suitable for compositional thermoanalytical study of normal and degenerative human hyaline cartilage.

Our results showed clear evidence that:

- complex deviations from the normal matrix composition during the late stage of degeneration correlated significantly with changes in thermal properties;
- patients with primary OA and AVN had significantly higher levels of water content in the degenerative samples and water bound stronger to the matrix than in controls;
- the kinetic character of the effect of water loss by heating was established, the reaction order was approximately 1 in all cases;
- correlation was found between the enthalpy changes and the severity of OA and AVN;
- a **new** protocol was established for sample extraction during live surgery using simple saline solution instead of the previously used phosphate buffer;
- this **new** method proved to be suitable for the thermoanalytical investigations; and
- the introduction of thermogravimetric examination as part of thermal analysis alongside calorimetry might be a useful method for determining the severity of OA and AVN.

One of the possibilities of getting fast information is the use of thermoanalytical methods. The simple new protocol we established might also be used for gaining information about the healthy or sick state of other human tissues.

Additional investigation will be needed to fully understand how water content affect cartilage degradation. Further studies are in progress to elucidate the contribution

of physicochemical properties of water in cartilage to the pathogenesis of the degenerative process of OA.

We need to strive to develop these methods and make them available in the clinical settings since without the means to monitor the effectiveness of DMOADs, we will never know if we can control and perhaps even prevent osteoarthritis.

From the work described in this thesis, a model can be proposed whereby the level of injury to cartilage within the joint can be monitored by a simple thermoanalytical method. A deeper knowledge of the pathways in the development of degenerative cartilage diseases might lead to the development of newer therapies for arthritis in the future.

We hope that our data provide further evidence for the importance of cartilage physicochemical properties in developing cartilage degeneration.

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10. ACKNOWLEDGEMENTS

I would like to express my sincere gratitude to **Professor Kálmán Tóth** who was my supervisor and tutor, for his guidance, enthusiasm, and inspiration to make this work possible. His extensive knowledge of this field formed the bases of my clinical and scientific work. He has always guided me with his valuable advice and instructions.

I am thankful for the thermal measurements performed in the laboratory of **Professor Piroska Szabó-Révész** at the Department of Pharmaceutical Technology, University of Szeged. Her inspiring ideas, useful advice, and constructive criticism have helped me to accomplish my dissertation.

I greatly acknowledge to **Professor Tamás Mészáros** providing me the possibility to work at the Department of Orthopedics under his leadership.

I am indebted to **Dr. Edina Pallagi** and **Dr. Zoltán Aigner** for their help in managing the laboratory measurements and analyzing all the data gathered for this work.

I wish to thank **Klára Kovács** and **Piroska Lakatosné** for their excellent technical assistance at the laboratory work of the Department of Pharmaceutical Technology.

I am grateful for all the members of the Department of Orthopedics for their help and friendship.

I am grateful to my **Family** for their endless and valuable help and support in my work, and efforts.