The Role of Differential Scanning Calorimetry in the Diagnostics of Musculoskeletal Diseases

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Abstract

Background: Despite of the improved biochemical analyzes and imaging technology, diagnosis of different types of musculoskeletal diseases still can be challenging. Therefore, new methods are needed to improve the diagnostic procedures and help to select the most sufficient treatment.

Aims: The purpose of our current review was to demonstrate the usefulness of differential scanning calorimetry in the intraoperative diagnosis of musculoskeletal diseases.

Materials and Methods: Different types of ligaments, connective tissues, joint capsules, arthritic or septic hyaline cartilage were collected intraoperatively from pathologic, or healthy human origin. The thermal denaturation of the samples was monitored by a SETARAM Micro DSC-II calorimeter.

Results: Here we demonstrated a significant difference in the pattern of the thermal denaturation characteristics of the degenerative or inflamed collagen tissue samples, compared with the healthy human connective tissue. The degenerative samples' thermal enthalpy was significantly decreased, while the melting temperature showed an increase. In case of the inflamed samples, we found a significant increase in the enthalpy and a decrease of melting temperature.

Conclusion: These data suggest that DSC analysis could be a clinically relevant, additional method in the intraoperative diagnosis of different types of musculoskeletal diseases.

Keywords: Musculoskeletal System; Degenerative; Inflammatory; Cartilage; Muscle; DSC (Differential Scanning Calorimetry)

Introduction

The different types of musculoskeletal disorders affect millions of people worldwide. Despite of the recent advances in the preoperative diagnostic procedures, to reach the correct diagnosis and establish the stage of certain degenerative and inflammatory diseases still could be challenging. The importance and impact of different musculoskeletal diseases on the health of the world's population is well demonstrated by the fact that WHO (World Health Organization) dedicated the first decade of the 21st century for the scientific research of bone and joint diseases [1-3]. Therefore, there is still a need for new methods to further support the accurate diagnosis of musculoskeletal diseases [1-4].

Of the numerous cellular components of ligaments, joints and muscles, collagens were chosen as a primary target for our experiments. It is known that collagens are one of the elementary components of musculoskeletal system, ubiquitously found in all connective tissues. However, it is important to note, that of the 25 different types collagens, 90% of the collagen is type I, as an abundant component of skin,

tendon, vascular ligature and bone. Additionally, type II collagen composes a dominant part of hyaline cartilage and collagen type III is generally found in granulation tissues [4-10].

Therefore, detection of the level of structural changes of collagens due to degenerative or inflammatory diseases could be a useful diagnostic tool in numerous musculoskeletal disorders [1,4]. Additionally, determination of collagen damage could be helpful in the quantification of the progress of diseases [7,11].

The selection of musculoskeletal diseases included in our study was based on their high incidence and impact on the health condition of a relatively large proportion of population [1,4,11,12]. In the group of degenerative diseases, our primary selection involved ligaments, muscle tendons and hyaline cartilages. Numerous extrinsic and intrinsic factors, including age, body mass, blood supply, diabetes or previous injuries have been implicated in the etiology of the degenerative changes of the collagen structures of tendons, leading to weakening and rupture [5-7]. For special interest, rupture of the Achilles tendon is a relatively common injury of the foot in the middle aged and physically active population (11.3/100,000 per year) [8]. Notably, arthritis is one of the most common orthopedic diseases [13], resulting in cartilage damage and affecting the weight-bearing joints of knee and hip [1].

Importantly, arthritis is the leading cause of disability in the United States: approximately 1 (37.6%) in 3 adults with arthritis reported limitation in their usual activities [13]. An estimated 4.0 million adults in the USA currently live with a total knee replacement, representing 4.2% of the population fifty years of age or older. The annual incidence of total knee replacement is ranged from 1.6% to 11.9% in males and from 2.0% to 10.9% in females [14]. In regard of the hip, more than 1 million total hip replacements are done every year worldwide, and this number is projected to double within the next two decades [15]. Furthermore, the degeneration of the intervertebral discs of the vertebral column is also responsible for a high number of disabilities worldwide [16,17].

According to the literature, the connective tissue disorders of the hand have shown a significant increase in the last decades. Therefore, in the group inflammatory diseases, we focused to the carpal tunnel syndrome (CTS), De Quervain tenosynovitis and Dupuytren disease. There is common in their pathomechanism; all of these syndromes are caused by chronic, non-specific inflammation [10]. The carpal tunnel syndrome (CTS) is the entrapment of the median nerve in the tunnel of ligament and bones at the base of the hand, and responsible for neurophysiologic changes of the hand [18,19].

Meanwhile, Dupuytren disease is known as the degenerative shortening of the palmar aponeurosis and one of the most common diseases of the hand in Europe, with the prevalence of approximately 4% to 6% in the Caucasian populations [7,20-23].

Different pathologic conditions of the human musculoskeletal and nerve system often result in consecutive, secondary damages of the skeletal muscles. Therefore, we examined the structural changes of the collagen, collected from muscle tissue, in diseases accompanied with cerebral palsy and congenital clubfoot in childhood [24].

Furthermore, muscle samples collected from lower leg compartment syndrome were also investigated. Compartment syndrome, described with an elevation of the interstitial pressure in closed fascia compartments, could result in micro-vascular compromise and consequentially, permanent muscle damage and loss of function. A diagnostic method that could intraoperatively differentiate the intact muscles from the damaged parts would be a practical help for the surgeon [12,25-27].

The differential scanning calorimetry (DSC) is a well-established thermal analytical method to detect conformational changes of proteins and collagens. Briefly, differential scanning calorimetry measures the amount of heat required to increase the temperature over the time and compares with a standard reference sample. The scans provide further data to determine the investigated tissue samples’ thermal enthalpy and melting temperature, described as the thermal characteristics. The changes of thermal enthalpy and melting temperature can be matched with the structural changes of different proteins [28].

The differential scanning calorimetry has already been proven useful in the detection of structural changes of various tissue samples, mostly in animal models. However, DSC scans have rarely been used to analyze intraoperatively collected human tissue samples [29-34]. Meanwhile, in the past years, our workgroup has successfully utilized differential scanning calorimetry for the intraoperative diagnosis and staging of a wide variety of degenerative and inflammatory musculoskeletal diseases [1,4,6,7,10-12,16,17,25,34-39].

Therefore, the goal of our current review was to provide evidence that comparison of thermal characteristics of human tissue samples based on DSC measurements could be a useful tool in the intraoperative diagnosis of different types of inflammatory and degenerative musculoskeletal disorders. Importantly, the accurate diagnosis and staging could be helpful in the choice of the most sufficient treatment.

Materials and Methods

Sample preparation

Detailed description of sample preparation and storage are described elsewhere [4]. Briefly, the different types of healthy tissue samples were of cadaver origin, taken only where degenerative or posttraumatic changes could not be verified macroscopically. The different types of pathologic tissue samples were derived intraoperatively. All the medical interventions were made according to the ethical regulations and approved by the ethical committee of the University of Pécs.

DSC Measurements

Detailed description of our standardized DSC protocol is found elsewhere [4]. Briefly, the thermal denaturation was measured within 6 hours of sample collection, using a SETARAM Micro DSC-II calorimeter. All the experiments were performed between 0 and 100°C. The heating rate was 0.3 K/min. Conventional Hastelloy batch vessels were used during the denaturation experiments with 850μL sample volume (samples plus buffer) in average. Typical sample wet masses for calorimetric experiments were in between 100 - 200 mg. RPMI-1640 solution was used as a reference sample. Calorimetric enthalpy was calculated from the area under the heat absorption curve by using two-point setting SETARAM peak integration and the data were analyzed with OriginPro 6.0 after ASCII conversion.

Statistical Analysis

For statistical analyzes, unpaired two-tailed Student's t-test was used, with SPSS 13.0 software (SPSS Inc., Chicago, IL). All data are expressed as mean. The statistically significant differences between groups were defined as p < 0.05.

Results

Comparison of the thermal characteristics of degenerative and intact tissue samples

In case of the degenerative tissue samples, we have found a significant decrease in the thermal enthalpy, while the melting temperature showed mostly a moderate, but non-significant increase (Table 1). Based on our results, the Achilles tendon was found to be the least stable of all examined degenerative samples, with the highest level of decrease in the thermal enthalpy ($\Delta H_{\text{cal, intact}}$: 8 Jg$^{-1}$ vs. degenerative: 1.5 Jg$^{-1}$) (Figure 1). The quadriceps tendon showed a moderate decrease ($\Delta H_{\text{cal, intact}}$: 6.27 Jg$^{-1}$ vs. degenerative: 1.53 Jg$^{-1}$). Meanwhile, the decrease of enthalpy was relatively similar in case of the patellar ligament ($\Delta H_{\text{cal, intact}}$: 4.36 Jg$^{-1}$ vs. degenerative: 0.97 Jg$^{-1}$) and the knee cruciate ligament ($\Delta H_{\text{cal, intact}}$: 5.06 Jg$^{-1}$ vs. degenerative: 1.55 Jg$^{-1}$). Notably, the samples from menisci ($\Delta H_{\text{cal, intact}}$: 4.9 Jg$^{-1}$ vs degenerative: 3.6 Jg$^{-1}$, p < 0.05) and annulus fibrosus ($\Delta H_{\text{cal, intact}}$: 0.84 Jg$^{-1}$ vs degenerative: 0.46 Jg$^{-1}$, p < 0.05) showed the lowest decrease in enthalpy.
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<th>Intact</th>
<th>Degenerative</th>
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<tr>
<td></td>
<td>n   Tₘ (°C)  ΔHₘₜ (Jg⁻¹)</td>
<td>n   Tₘ (°C)  ΔHₘₜ (Jg⁻¹)</td>
</tr>
<tr>
<td>1 Glenohumeral joint capsule</td>
<td>4   65.1  6.7</td>
<td>5   65.3  3.7</td>
</tr>
<tr>
<td>2 Quadriceps tendon</td>
<td>4   63.3  6.27</td>
<td>5   64.8  1.53</td>
</tr>
<tr>
<td>3 Patella tendon</td>
<td>4   61.7  4.36</td>
<td>4   63.9  0.97</td>
</tr>
<tr>
<td>4 Achilles tendon</td>
<td>4   59.7  8.5</td>
<td>5   62.8  1.5</td>
</tr>
<tr>
<td>5 Biceps tendon</td>
<td>12  63.4  6</td>
<td>16  62.9  1.28</td>
</tr>
<tr>
<td>6 Knee cruciate ligament</td>
<td>4   64.8  5.06</td>
<td>5  65.1  1.55</td>
</tr>
<tr>
<td>7 Meniscus</td>
<td>13  63.3  4.9</td>
<td>8  62.5  3.6</td>
</tr>
<tr>
<td>8 Anulus fibrosus</td>
<td>14  60.5  0.84</td>
<td>18  62.5  0.46</td>
</tr>
<tr>
<td>9 Femoral head avascular cartilage</td>
<td>5  68.2  2.87</td>
<td>9  70.8  4.02</td>
</tr>
<tr>
<td>10 Femoral condyle arthritic cartilage</td>
<td>3  72.6  0.32</td>
<td>6  58.9  0.58</td>
</tr>
<tr>
<td>11 Patellar arthritic cartilage</td>
<td>3  64.8  1.78</td>
<td>4  60.9  0.78</td>
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*Table 1: The characteristic thermal parameters of the denaturation of different types of intact and degenerative human collagen structures.*

n: Number of Samples; Tₘ (°C): Melting Temperature; ΔHₘₜ (Jg⁻¹): Enthalpy. Unpaired Student’s t-test, * indicates significant difference at p < 0.05 vs. intact collagen samples. Data are expressed as mean, n ≥ 3 in each group.

Figure 1: Thermal denaturation scans of intact and ruptured human Achilles tendon. The endotherm processes are directed downward.

The DSC curves represent significant alterations of the structure of ruptured Achilles tendon, indicated by the increased half width of denaturing temperature and decrease of calorimetric enthalpy.
The cartilage samples collected from avascular necrotic femoral head and arthritic femur condyle demonstrated a moderate, but significant increase in the thermal characteristics. ($\Delta H_{\text{cal}}$ intact: 2.87 J g$^{-1}$ vs arthritic: 4.02 J g$^{-1}$, $p < 0.05$; $\Delta H_{\text{cal}}$ intact: 0.32 J g$^{-1}$ vs arthritic: 0.58 J g$^{-1}$, $p < 0.05$, respectively). In this set of experiments, cartilage samples were collected from patients who developed avascular necrosis of the femoral head (ANFH) after medial femoral neck fracture and screw fixation. At different time points following the primary surgery and depending on the onset of ANFH, the samples were taken intraoperatively during hip replacement. Interestingly, instead of the single thermal domain of the intact hyaline cartilage, we obtained a wide endothermic transition on the DSC curves of the pathologic cartilages (Figure 2). In the cases of progressive ANFH (6 months after the fracture), two different thermal domains appeared on the DSC scans [11]. It could be the sign of severe damage of the cartilage, the denaturation of proteins or the formation of calcium deposits.

![Figure 2: Thermal denaturation scans of hyaline cartilage collected from patients with avascular necrosis of the femoral head (ANFH) at different time-points after primary surgery. The endotherm processes are directed downward.](image.png)

The DSC scans clearly demonstrate significant differences between the samples collected at different time-points after primary surgery. In cases of severe avascular necrosis (6 months after the fracture), two different thermal domains appear on the DSC scans.

However, arthritic patellar cartilage showed a significant decrease in both melting temperature and thermal enthalpy; $T_m$ intact: 64.8°C vs arthritic: 6.90°C, $p < 0.05$; $\Delta H_{\text{cal}}$ intact: 1.78 J g$^{-1}$ vs arthritic: 0.78 J g$^{-1}$ ($p < 0.05$).

Comparison of the thermal characteristics of inflamed and intact tissue samples

In contrast to the degenerative samples, here we have demonstrated a significant increase of the thermal enthalpy, comparing the inflamed and intact human tissue samples of patients with Dupuytren-contracture, De Quervain tenosynovitis and carpal tunnel syndrome.

The highest increase in the thermal enthalpy was found in case of carpal tunnel syndrome ($\Delta H_{\text{cal, int}}$: 4.0 J g$^{-1}$ vs inflamed: 5.9 J g$^{-1}$, $p < 0.05$) and De Quervain tenosynovitis ($\Delta H_{\text{cal, int}}$: 3.84 J g$^{-1}$ vs inflamed: 5.39 J g$^{-1}$, $p < 0.05$). Additionally, we found a less marked, but still significant increase in the enthalpy of samples from Dupuytren-contracture ($\Delta H_{\text{cal, int}}$: 4.1 J g$^{-1}$ vs. inflamed: 5.2 J g$^{-1}$). The acquired DSC scans clearly demonstrated significant changes between the different stages of the disease (Figure 3), further supporting the notion that DSC analyzes could be practical in the determination of the progress of degenerative and inflammatory diseases. These changes were accompanied with a moderate, but not significant decrease in the denaturation temperature of samples collected from Dupuytren-contracture ($T_m$ intact: 63.0°C vs. inflamed: 61.7°C) and also with a non-significant increase in samples from De Quervain tenosynovitis ($T_m$ intact: 61.3°C vs. degenerative: 62.1°C) and carpal tunnel syndrome ($T_m$ intact: 61.3°C vs. degenerative: 61.8°C), respectively.

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<th>Intact</th>
<th>Inflamed</th>
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<tr>
<td></td>
<td>$T_m$ (°C)</td>
<td>$\Delta H_{\text{cal}}$ (J g$^{-1}$)</td>
</tr>
<tr>
<td>1 Dupuytren contracture</td>
<td>4 63.0 4.1</td>
<td>10 61.7 5.2*</td>
</tr>
<tr>
<td>2 Carpal tunnel syndrome</td>
<td>4 61.3 4.0</td>
<td>10 61.8 5.9*</td>
</tr>
<tr>
<td>3 De Quervain tenosynovitis</td>
<td>4 61.3 3.84</td>
<td>10 62.1 5.39*</td>
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Table 2: The characteristic thermal parameters of the denaturation of different types of intact and inflamed human collagen structures.

$n$: Number of Samples, $T_m$ (°C): Melting Temperature, $\Delta H_{\text{cal}}$ (J g$^{-1}$): Enthalpy. Unpaired Student's t-test, * indicates significant difference at $p < 0.05$ Vs. intact collagen samples. Data are expressed as mean, $n \geq 3$ in each group.

Figure 3: Thermal denaturation scans of normal, clinically Grade II, and Grade IV, palmar fascia changes in Dupuytren disease. The endotherm processes are directed downward. The DSC scan indicates the presence of a single thermal domain with a weak water release after the denaturation. In Grade II, the presence of a second thermal structural unit can be observed that becomes more pronounced in Grade IV. The DSC scans clearly demonstrate the significant differences between the different stages, indicating the progression of the contracture.
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The chronic non-specific inflammation, which is responsible for the development of De Quervain disease, CTS and Dupuytren contracture, may affect regulatory pathways that interfere with extracellular matrix synthesis and fibroblast proliferation. The production of irregular bundles and changes in the balance of different types of collagens could provide a possible explanation for the changes of the detected changes in the thermal characteristics.

Comparison of the thermal characteristics of injured and intact human muscle samples

Here we compared the standard calorimetric properties of the healthy human skeletal muscle with human skeletal muscles in primary peripheral leg deformities (congenital clubfoot) and secondary deformities caused by the malfunction of the central nervous system (cerebral palsy) (Table 3).

<table>
<thead>
<tr>
<th>n</th>
<th>Thermal parameters</th>
<th>Healthy control (adductor hallucis muscle)</th>
<th>Cerebral palsy (gastrocnemius muscle)</th>
<th>Clubfoot (adductor hallucis muscle)</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>T&lt;sub&gt;m&lt;/sub&gt; (°C)</td>
<td>56.5</td>
<td>60.5</td>
<td>57.7</td>
</tr>
<tr>
<td></td>
<td>ΔH&lt;sub&gt;cal&lt;/sub&gt; (Jg&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>0.54</td>
<td>0.64*</td>
<td>0.85*</td>
</tr>
</tbody>
</table>

Table 3: The characteristic thermal parameters of the denaturation of different types of intact and pathologic human muscle structures.

n: Number of Samples; T<sub>m</sub> (°C): Melting Temperature; ΔH<sub>cal</sub> (Jg<sup>-1</sup>): Enthalpy (-stands for endothermic reaction). Unpaired Student’s t-test, * indicates significant difference at p < 0.05 vs. healthy control muscle samples. Data are expressed as mean, n ≥ 3 in each group.

The acquired data have clearly demonstrated definitive changes in the thermal enthalpy, comparing healthy controls to samples collected from patients with cerebral palsy or clubfoot deformity (ΔH<sub>cal</sub> intact: -540 J Kg<sup>-1</sup>, cerebral palsy: -640 J Kg<sup>-1</sup>, clubfoot: -850 J Kg<sup>-1</sup>). It is important to note, that healthy samples were collected during surgical correction of hallux valgus deformity; these samples were not from cadaver origin.

With special clinical relevance, we have also evaluated the changes of thermal characteristics caused by compartment syndrome-induced tissue ischemia. In case of the compartment syndrome, the DSC analysis have demonstrated significant differences between the different types and grades of the collected samples (control: T<sub>m</sub>: 55.5; 59.9°C, ΔH<sub>cal</sub>: 0.52 Jg<sup>-1</sup>, Gr. I.: T<sub>m</sub>: 58.1; 62.2°C, ΔH<sub>cal</sub>: 0.28 Jg<sup>-1</sup>, Gr. II.: T<sub>m</sub>: 57.45; 61.5°C, ΔH<sub>cal</sub>: 0.24 Jg<sup>-1</sup>, Volkmann’s ischemic contracture: T<sub>m</sub>: 57.75; 61.8; 65.8°C and ΔH<sub>cal</sub>: 0.74 Jg<sup>-1</sup>). The two different T<sub>m</sub> indicates the presence of two thermal domains, likely caused by the melting and separation of myosin and actin components (Table 4 and Figure 4) [40,41].

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<table>
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<tr>
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<th>Compartiment pressure</th>
<th>$T_m$ (°C)</th>
<th>$\Delta H_{cal}$ (J/g)</th>
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<tbody>
<tr>
<td>1</td>
<td>Healthy leg muscle</td>
<td>4 &lt; 30 mmHg</td>
<td>55.5 59.9</td>
</tr>
<tr>
<td>2</td>
<td>Compartment syndrome Gr. I. (mild)</td>
<td>4 30 - 35 mmHg</td>
<td>58.1 62.2</td>
</tr>
<tr>
<td>3</td>
<td>Compartment syndrome Gr. II. (severe)</td>
<td>4 &gt; 30 mmHg</td>
<td>57.45 61.5</td>
</tr>
<tr>
<td>4</td>
<td>Volkmann’s ischemic contracture</td>
<td>3 N/A (chronic)</td>
<td>57.75 61.8 65.8</td>
</tr>
</tbody>
</table>

Table 4: The compartment pressure and characteristic thermal parameters of intact and ischemic human skeletal muscle samples.

- $n$: Number of Samples
- $T_m$ (°C): Melting Temperature
- $\Delta H_{cal}$ (J/g): Enthalpy

Unpaired Student’s t-test, * indicates significant difference at $p < 0.05$ vs. healthy muscle samples. Data are expressed as mean, $n \geq 3$ in each group.

Figure 4: Thermal denaturation scans of muscle samples from normal, mild and severe clinical stages of compartment syndrome. The endotherm processes are directed downward.

The DSC scans showed no difference in the changes of heat capacity of native (healthy) and the denatured states of proteins. In case of control, we obtained a wide endothermic transition with two different thermal domains with $T_m$ 58.1 and 62.2 °C, which could be the melting of myosin and actin. In group I (mild stage), a significant change of the shape and melting temperatures ($T_m$ 55.5 and 59.9 °C) of denaturation were observed. The separation of myosin and actin contributions is more pronounced with a greater myosin enthalpy contribution, compared to the controls (indicated by the smaller half with of its thermal transition). In group II (severe stage), further transition temperature change can be observed ($T_m$ 57.45 and 61.5°C) with increased myosin damage (smaller $\Delta H_{cal}$), as a consequence of marked necrosis of the muscle.

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Discussion

Here we have demonstrated that analyzes of human musculoskeletal tissue samples by differential scanning calorimetry can clearly show significant differences between the pathologic and healthy groups. These data confirm that the thermal stability and protein conformation of the examined degenerative, ischemic or inflamed pathologic samples are significantly different from the intact tissue samples.

According to the literature, differential scanning calorimetry has widely been used for the detection of conformational changes of different types of proteins [29-32,40,42]. On the other hand, DSC scans have rarely been utilized for the evaluation intraoperatively collected human samples. Therefore, we have introduced this method as part of the diagnostic procedure, using ligament, connective tissue, joint capsule, arthritic or septic hyaline cartilage samples affected by various types of musculoskeletal disorders [1,4,6,7,10-12,16,17,25,34-39].

In our experiments, we have found a significant difference between the intact and degenerated tendons or joint capsules. The significant reduction in the enthalpy is likely due to depolymerization and denaturation of the collagen fibers. It is possible, that the overextension may result in an intermolecular and intrafibrillar sliding of the collagen. The collagen components then became re-organized, parallel with an increase of the level of type III collagen [43]. Therefore, the injured tissue became less compacted and cooperative, resulting in the overall decrease of the thermal enthalpy [4,6]. Taken together, the changes of thermal characteristics could be explained by the structural changes of the collagen (irregularity, scars), as a result of microtraumas and degeneration [7].

Recently, Chaudhury, et al. has published data about the changes of thermal properties after human rotator cuff injuries. Consistently with our data, they found that the small and massive rotator cuff tears had significantly higher $T_{onset}$, $T_{peak}$ and $\Delta H$ ($\sim 4 \text{ Jg}^{-1}$ difference), compared to controls [44].

Contrary, the significantly increased thermal enthalpy of femoral head and condyle cartilage could be a sign that during the degenerative processes, the structure of the cartilage has become more densely packed [11,34].

In contrast to the degenerative diseases, the thermal enthalpy of the inflamed tissue samples was significantly increased, compared to the healthy origins. The chronic non-specific inflammation, which is responsible for the development of De Quervain disease, carpal tunnel syndrome (CTS) and Dupuytren contracture, may affect regulatory pathways interfering with extracellular matrix synthesis and inducing fibroblast proliferation. The production of irregular bundles and changes in the balance of different types of collagens could provide a possible explanation for the detected changes in the thermal characteristics [4,7,10].

It is noteworthy, that both of cerebral palsy and the abnormal structural change of the muscle caused by clubfoot showed an increased global thermal stability, compared to the healthy controls. It could be caused by an impaired inter-domain communication of the different subunits of muscle proteins, resulting in muscle dysfunction or the different ‘packaging’ of the affected parts [24]. Nevertheless, DSC scans clearly demonstrated the global conformational changes of muscle proteins in cases of the functional muscle impairments, caused by either cerebral palsy or passive structural constrain (clubfoot) [24].

Compartment syndrome occurs when the pressure in a certain muscle compartments is higher than the pressure in the capillaries, which leads to progressive muscle ischemia, hypoxic degeneration, edema and ultimately, infarction and necrosis of the compartment contents. Since compartment syndrome requires immediate surgical interventions, it has a particular importance to differentiate accurately the affected components from the healthy parts [12,25-27]. The DSC scans clearly demonstrated the significant differences of the different stages of compartment syndrome (Table 4), supported by the calorimetric enthalpy values (control $\Delta H_{cal}: 0.52 \text{ Jg}^{-1}$, in group I (mild) 0.28 Jg$^{-1}$, in group II (severe) 0.24 Jg$^{-1}$ and in Volkmann’s ischemic contracture: 0.74 Jg$^{-1}$, respectively). These observations could be explained by the structural alternations caused by the biochemical processes and the higher compartment pressure. The thermal parameters of the healthy and the pathologic muscles were absolutely different. These findings clearly indicate the efficacy of DCS in the determination of the progress of muscle damage due to ischemic insult.

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Moreover, differential scanning calorimetry could be a useful tool not only in the diagnosis of musculoskeletal disorders, but also in the determination of other human diseases. For example, Könczöl F., et al. has introduced DSC measurements for forensic investigation: in context with chemotherapy induced neuropathy, they found that the thermal denaturation of the affected muscle and nerve samples decreased, while the calorimetric enthalpy increased, depending on the therapeutic cyclophosphamide doses [45]. They also found that nerves were more sensitive to chemotherapy, compared to the muscles, concluding that the dose-dependent toxic effects of cyclophosphamide on peripheral nerves and muscles can be measured and analyzed by calorimetry, further supporting the findings of histological examinations [45].

In New Zealand rabbit model of Staphylococcus aureus induced experimental septic knee arthritis, Sillinger., et al. demonstrated that the development of infective structural destruction of the femoral cartilage could be confirmed by the means of DSC measurements [37]. The DSC scans showed first a decrease, then a marked increase in the thermal enthalpy with increasing melting temperatures.

Utilizing human tissue samples, Naumov., et al. found clear differences, comparing interfacial membranes collected after aseptic and septic loosening of hip prostheses (aseptic membrane: \( T_m = 62.2 \) °C and \( \Delta H_{cal} = 2.13 \) Jg\(^{-1}\); septic membrane: \( T_m = 60.2 \) °C and \( \Delta H_{cal} = 3.22 \) Jg\(^{-1}\) ) [35]. These findings support the notion that DCS could be a useful tool in the clinical settings for determining the prognosis of not only sterile, but also septic inflammations.

Ischemia-reperfusion injury could significantly affect the thermal characteristics of different tissue samples. Nedvig K., et al. have recently demonstrated that DSC analyzes were helpful in the detection of thermal characteristic changes of the small bowel structure, in a rabbit model of ischemia-reperfusion injury [46]. Melling., et al investigated the degenerative shortening of palmar aponeurosis of diabetic and non-diabetic patients with DSC measurements [47]. Furthermore, other authors utilized DCS analyzes for the measurement of the biostability of hernia meshes by assessing local immunological reactions and collagen formation [48]. DSC was also used for the investigation of vascular graft failures, measuring the decrease of crystallinity [49]. The DSC analyzes of hip joint capsule of patients with degenerative or inflammatory hip disorders have also been applied in the evaluation of surgical techniques [34].

Importantly, the workgroup of Sohár., et al. has recently published a series of important results, using DSC scans for analyzes of human cartilage and fat tissue samples. In particular interest, Mécs L., et al. demonstrated the changes of thermal characteristics of human degenerative cartilage, in correlation with altered metabolism in spondylolosthesis: the samples of intervertebral disc, facet joint and vertebral end-plate were obtained intraoperatively and DSC analyzes showed the greatest change in the enthalpy in case of the intervertebral disc samples. While the tendency correlates with our results, interestingly, the dimension of the measured values differs, which may reflect to differences in the utilized protocols [50].

Furthermore, Aigner., et al. reported about the calorimetric properties of cartilage samples from femoral head necrosis and osteoarthritis, collected intraoperatively. All the examined samples showed a clear denaturation peak on the calorimetric curve. Additionally, cartilage obtained from necrotic femoral head required the lowest amount of energy for decomposition [51]. Furthermore, they concluded differential scanning calorimetry as a reliable method for the differentiation between healthy and degenerated human cartilage of the shoulder joint or for the study of cartilage damages in rheumatoid arthritis [52,53].

Differential diagnosis of septic and non-septic arthritis could also be supported by DSC examination of human synovial fluid samples. Dandé., et al. have demonstrated a specific pattern of DSC curves of synovial fluid samples inoculated with three different bacterial strains [54].

Recently, Pintér., et al. has reported marked differences in thermal characteristics of subcutaneous fat tissues obtained during total hip arthroplasty (THA) from patient with avascular femoral head necrosis (ANFH) due to alcohol abuse, comparing with healthy patient who underwent surgery due to traumatic hip fracture. DSC revealed, that in case of non-necrotic samples as a reference, two separable transitions were found with \( T_m = 5.7 \) and 9.9°C, total \( \Delta H = -20.8 \) Jg\(^{-1}\) vs alcohol-induced ANFH with: \( T_m = 7.3 \) °C, total \( \Delta H = -26.9 \) Jg\(^{-1}\). Importantly,
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only DSC scan, but not histological examination could reveal detectable differences [55]. These results, in part, are opposite to our findings with cartilage collected of ANFH, where $T_m$ showed a non-significant increase (68.2 vs 70.8°C), meanwhile, thermal enthalpy significantly increased (2.87 Jg$^{-1}$ vs 4.02 Jg$^{-1}$). It could be explained by the fact that different tissue samples (cartilage vs subcutaneous fat) may react in a different way to toxic or degenerative agents.

Nowadays, there are several surgical techniques available to the joint surface reconstruction, including the use of human cartilage stored at -80°C. Utilizing DSC analyses of human cartilage samples, Patczai, et al. have shown that after the sixth week, both the enthalpy and the transition temperature decreases, compared to the control samples. These finding clearly indicates that the duration of cryopreservation interferes with the morphology of human cartilage samples after 6 weeks of storage time [56].

Conclusion

Summarizing our experiences with the utilization of differential scanning caloriometry, we have successfully demonstrated that a recognizable pattern could be detected in the thermal denaturation characteristics of the intraoperatively collected pathologic and healthy human tissue samples. Therefore, beside the preoperative diagnostic procedures, differential scanning caloriometry could provide useful, additional information to achieve a more accurate intraoperative diagnosis and prognostic grading of different types of musculoskeletal diseases.

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Conflict of Interest

There is no conflict of interest to report.

Bibliography


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