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Short communication

Thermal effects of shoulder electrothermal arthroscopic capsulorrhaphy monitored by differential scanning calorimetry—A preliminary study

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Abstract

The shoulder is the most frequently dislocated joint of the human body. The instability of the joint can be caused by ligamentous capsular redundancy. When non-operative management fails for this patient, quality of life is significantly impaired and surgical treatment is required to tighten the ligaments and the joint capsule. Radiofrequency (RF) energy of electrothermal arthroscopic capsulorrhaphy (ETC) represents a relatively non-invasive method to stabilize joints with excessive laxity by thermally shrinking redundant joint capsular tissue. Due to the thermal treatment of joint capsular tissue collagen fibers the capsule shrinks, so the stability of the joint rises. RF energy induces shrinkage, but also significant decline in the structural properties of the collagenous capsular tissue, which is thought to abate the final results of the treatment. The indication of the ETC treatment, the optimal device settings and shrinkage rate are still not clarified. Differential scanning calorimetric (DSC) examination is a validly efficient method for the demonstration of structural changes in biological systems. The purpose of this study was to establish the feasibility of DSC in the field of monitoring the structural alteration of the ETC treated collagenous capsular tissue. We used cadaver tissue samples to establish the thermograms of the thermal treated and the intact capsular joint tissue. Our preliminary findings show that the untreated tissue has a much cooperated transition with about $T_{1/2}$ of 1.5 °C. In case of treated sample this was about 8 °C of course with two superimposed transitions. The lower melting transition stands for the heat-treated part of collagen. This proves, that in the heat-treated part, the collagen transformed into a random coil conformation with less thermal stability and suggest that DSC can be a viable method by monitoring the structural effects of ETC.

Keywords: Collagen shrinkage; DSC; ETC

1. Introduction

The shoulder is the most frequently dislocated major joint of the human body. The primary abnormality causing the instability of the joint is thought to be excessive capsular joint volume and pathologic ligamentous laxity [1].

Radiofrequency (RF) energy-induced heating of electrothermal arthroscopic capsulorrhaphy (ETC) represents a relatively non-invasive method to treat shoulder instability [2,3]. This treatment has numerous advantages compared with open surgery, but the clinical results of ETC do not reproduce the success of open surgery. The negative effect of the thermal shrinkage is that the shrunken capsular tissue loses its mechanical properties, and cannot resist the physiological loading close after the treatment, it can stretch out. This stretching out effect should be aware in the postoperative phase, because it influences hard the result of the treatment. The capsular tissue regains its strength only some week after the injury, through the biological reorganisation, and through new collagen synthesis [4–7].

The biological and biomechanical effect of thermal capsular shrinkage is not been fully understood. The deeper knowledge of this phenomenon could be helpful in the development of medical application of RF energy. Differential scanning calorimetric examination is a validly efficient method for the demonstration of structural changes in biological systems [8–11]. To the knowledge of the authors, there was no previous study performed with the application of DSC in the field of monitoring the effect of ETC on capsular connective tissue. The goal of this preliminary study was to prove whether the ther-

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mal denaturation curve of DSC can represent the structural changes caused by ETC. The authors performed experimental capsulorrhaphies on cadaver shoulder capsule tissue samples to establish the thermograms of the thermal treated and the intact capsular joint tissue. The preliminary findings suggest that DSC can be a viable method by monitoring the structural effects of ETC.

2. Materials and methods

2.1. Sample preparation

Ten cadaver shoulders of five cadavers were used to this experiment (age range 28–33 years, mean age 30 years). We dissected both side the glenohumeral joint capsule carefully, and a 5 mm × 10 mm tissue sample was removed from the anterior part of the capsule. All samples were obtained during autopsy within 24 h postmortem, with standard methods. Five samples were used as a control group (group A), and on five tissue samples experimental capsulorrhaphies were performed (group B). The electrosurgical device used in these capsulorrhaphies was the VAPR System (Mitek Products, Westwood, MA) and thermal electrodes with a bipolar coil tripped probe, with temperature set at 65–67 °C. The conductive irrigating solution was normal saline. RF energy was applied at 20 W for 30 s. After the preparation DSC and histological examination were performed on all samples. Our activities were done under the proper law paragraphs and valid permission.

2.2. DSC measurements

For the DSC examination the samples were washed three times in PBS (sterile phosphate-buffer saline, pH 7.4) in order to eliminate tissue remnants. Samples were than put into RPMI-1640 solutions (Sigma) containing 10% foetal bovine serum (Hyclone Laboratories), antibiotic solution (1 U/ml penicillin, streptomycin, gentamycin and fungisone, Gibco Laboratories), non-essential amino acids (Gibco) and sodium carbonate. All the individual samples were stored separately at 4 °C, no longer than 24 h, before they were subjected to calorimetric measurements. The samples were monitored by a SETARAM Micro DSC-II calorimeter. All experiments were conducted between 0 and 100 °C. The heating rate was 0.3 K/min in all cases. Conventional Hastelloy batch vessels were used during the denaturation experiments with 850 μL sample volume (tissue plus buffer) in average. Typical capsular connective tissue wet masses for calorimetric experiments varied between 250 and 400 mg. RPMI-1640 solution was used as a reference sample. The sample and reference vessels were equilibrated with a precision of ±0.1 mg. There was no need to do any correction from the point of view of heat capacity between sample and reference vessels. The scan of RPMI-1640 solution was used as baseline reference, which was subtracted from the original DSC curve. Calorimetric enthalpy was calculated from the area under the heat absorption curve by using two-point setting SETARAM peak integration.

2.3. Histology

For the histological examination the specimens were fixed in 4% buffered formalin for a week. After fixation serial cross-sections were cut and embedded in paraffin, and cut to a thickness of 2 μm. The sections were stained with picrosyrius-red. This staining is useful for demonstration of collagen fibres [12]. The histological analysis was performed with Nikon Eclipse E400 light microscope to visualize the capsular connective tissue structure changes followed by the ETC treatment.

3. Results

3.1. Histological results

The histological examination showed by the intact shoulder capsular tissue samples normal fibrous wavy collagen structure (group A) (Fig. 1). The normal capsular tissue consisted of thick, closely packed collagen bundles, with a periodic climp pattern, and fibroblasts were sparsely scattered between the collagen fibers. ETC treatment caused dramatic alterations of the joint capsular tissue structure (group B) (Fig. 2). In the treated area, we observed fused collagen tissue (hyalinisation) and pycnotic fibroblasts. Between the thermal treated regions the connective tissue showed intact organisation the same as by the control samples. The heated lesions of the capsule were easily identified under light microscopy as well-demarcated areas of collagen damage. The collagen striations were not presented in the treated area.

3.2. DSC results

The thermal denaturation distinguished two shoulder capsular tissue populations in case of treated samples (see Fig. 3). The first part of DSC scan with lower melting temperature seems to

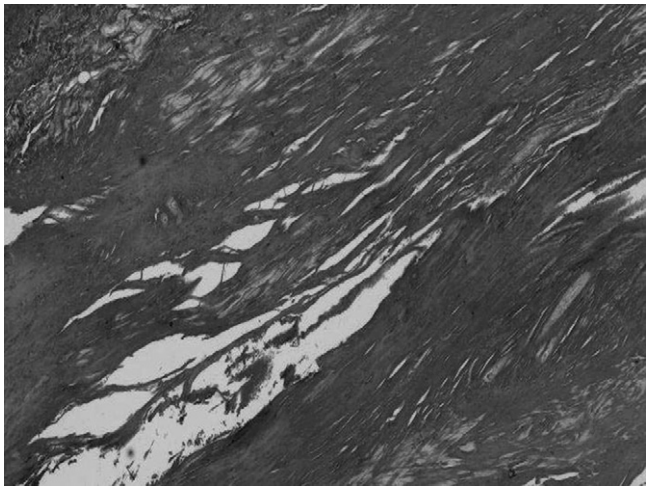

Fig. 1. The histological section showed by the intact shoulder capsular tissue samples normal fibrous wavy collagen structure (group A). The normal capsular tissue consisted of thick, closely packed collagen bundles, with a periodic climp pattern, and fibroblasts were sparsely scattered between the collagen fibers. Picrosyrius-red stained, magnification 40×.

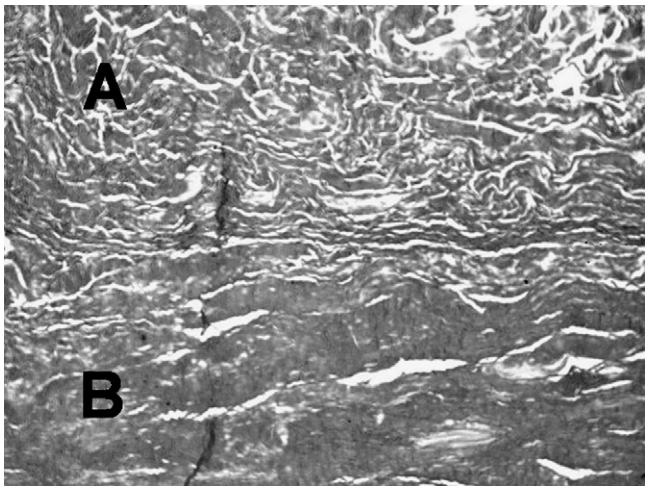

Fig. 2. Histological section from a ETC treated shoulder capsular tissue (group B). In the treated area, we observed fused collagen tissue (hyalinisation) and pycnotic fibroblasts (A area). Between the thermal treated regions the connective tissue showed intact organisation the same as by the control samples (B area). The heated lesions of the capsule were easily identified under light microscopy as well-demarcated areas of collagen damage. The collagen striations were not presented in the treated area. Picrosyrius-red stained, magnification 40×.

be the RF treated, while the high melting component should be the denaturation of the intact tissue. According to the different thermal parameters of denaturation (see Table 1) the RF treatment has significant effect on thermal stability of shoulder capsular tissue: the starting temperature at least by 3 °C is smaller than in case of intact tissue, the half width of transition is wider, so the system became less cooperative with smaller calorimetric enthalpy change. The unaffected part of shoulder capsular tissue saved its melting temperature and its cooperativity too (see Fig. 3).

4. Discussion

The primary abnormality causing the instability of the shoulder is thought to be excessive capsular joint volume and pathologic ligamentous laxity [1]. The treatment of shoulder instability starts with a conservative therapy. If conservative rehabilitative program fails surgery may be an option. The capsular laxity can be reduced by open reconstruction or by ETC [1,13–15].

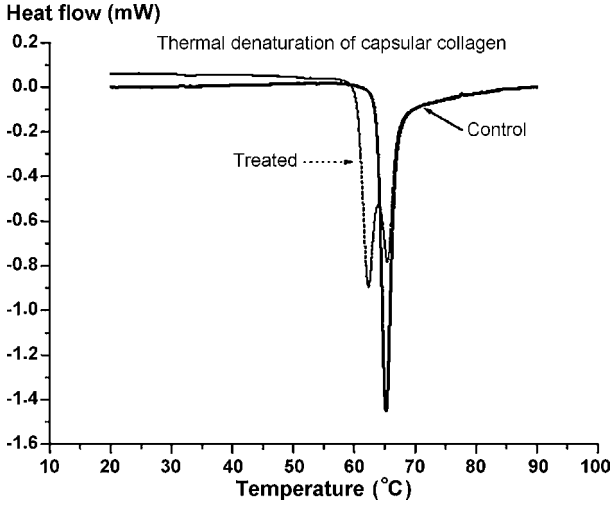

Fig. 3. Thermal denaturation curve of the intact control (group A) and the treated (group B) shoulder capsular tissue.

Table 1
Calorimetric results of the shoulder capsular tissue

Group	No. of samples	Thermal parameters
A	5	T_1 (°C): 59.7 ± 0.2 T_2 (°C): 79.9 ± 0.2 T_m (°C): 65.1 ± 0.2 $T_{1/2}$ (°C): 1.5 ± 0.1 ΔH (J/g): 6.7 ± 0.3
B	5	T_1 (°C): 56.6 ± 0.2 T_2 (°C): 79.9 ± 0.2 T_{m1} (°C): 62.3 ± 0.2 T_{m2} (°C): 65.3 ± 0.2 $T_{1/2}$ (°C): 8.0 ± 0.2 ΔH (J/g): 3.7 ± 0.2

Group A: intact samples, Group B: treated samples, T_1/T_2: starting/end points of transition, and T_m: main transition or melting temperature, $T_{1/2}$ is the half width of denaturation scan, ΔH: calorimetric enthalpy change normalised for total wet sample mass.

Open surgical joint capsular procedures are generally successful in the prevention of luxation, but there are numerous complications associated with the injury, including nerve damage, contractures, joint degeneration, persistent pain, and loss of range of motion. Arthroscopic approaches to the human shoulder have numerous advantages compared with the open repair, including lower postoperative morbidity, shorter hospital stay and rehabilitation, and minimal to no loss of range of motion [16].

ETC represents a relatively non-invasive method to stabilize the shoulder joint with excessive laxity by thermally shrinking collagen bundles building up the redundant joint capsular tissue [2,3]. This treatment involves using an arthroscopic approach with RF energy to the synovial surface to shrink redundant capsular tissue. The goal of this RF energy application is to produce a well-defined area of tissue heating that results volume reduction of the redundant capsular tissue, which results an increase of the joint stability. The effect of volume reduction of the capsular tissue can be interpreted by the phenomenon of collagen shrinkage.

Joint capsules primary element is type l collagen, which is made up of three polypeptide chains in a triple helix arrangement and stabilized by intermolecular crosslinks. These molecules aggregate to form collagen fibrils. Heating damages the intramolecular crosslinks and causes the protein to become disorganised. The shrunken and coiled collagen fibril configuration is maintained by heat-stable intermolecular crosslinks. The degree of tissue shrinkage is influenced by intrinsic qualities such as age, collagen content, number and stability of crosslinks, and direction of collagen fibers [7,17,18].

The recommended treatment temperature is in the range of 65–75 °C [19,20]. Any temperature which exceeds the shrink temperature is unnecessary and will cause unnecessary ther-

mal damage. In addition variables with regard to RF thermal shrinkage technique including duration of energy application, area of energy application, source of RF energy, and RF generator power settings affect the thermal modification of collagen. Optimal values for these variables have not been clearly defined [21].

Several investigators have reported that bipolar RF device have less depth penetration [22]. However, there is no evidence that the bipolar device offers any clinical advantages or ensures safety compared with monopolar device.

Many in vivo and in vitro basic studies have been conducted to clarify whether RF energy produces significant shrinkage of joint capsular tissues [2,6,19]. Increase in the RF wattage and time produced progressively greater shrinkage [23]. Hayashi et al. also found a correlation between the wattage and the amount of shrinkage obtained, and the work of Shellock et al. confirms that the treatment power and tissue temperature are directly related to the tissue shrinkage obtained [4,24].

Thermal modification of the collagen results in shortening but also in substantial weakening of treated tissue [3,5,6,20]. For successful clinical outcomes after joint capsular thermal shrinkage using RF, the shortened state must be maintained while tissue healing occurs. During the reparative process, fibroblastic infiltration of affected collagen results in reorganisation, which may allow maintenance of the shortened state [2,6,24]. The time required to allow such weakened tissue to regain strength and more normal biomechanical properties is variously reported. A period of anywhere from 6 weeks to 7 months is required to allow the tissue to regain its biomechanical properties [4–7]. It seems evident that a most conservative rehabilitation protocol is indicated for patients having thermal shrinkage [21]. Whether mechanical properties reliably or ever return to pre-treatment level remains unknown.

Multiple authors have demonstrated examples of failure of the use of thermal capsular shrinkage for shoulder instability [25–29]. These complications are thought to be based on the substantial weakening of the thermal shrunken joint capsule. No consensus has been reached about optimal amount of shrinkage, optimal postoperative rehabilitation protocol, or long-term effects on the biomechanical properties of capsular tissue [30].

Based on the previous mentioned data, further investigations are needed to clarify the effect of this method on the structural properties of the collagenous capsular tissue. It was performed in this preliminary study an experimental capsulorrhaphy on cadavers with RF electrodes. Intact and ETC treated capsular tissue samples were put through histological and DSC examinations, to prove whether the thermogram of a DSC calorimeter can indicate the effect of the RF electrode created structural alteration. The histological sections of the ETC treated samples showed a typical view of a heat-treated connective tissue.

On the thermal denaturation curves (Fig. 3) of the intact and the ETC treated capsular tissue we can clearly separate the native and the denaturated state with a small heat capacity decrease (caused by the loosening of bound water). The untreated tissue showed a much cooperated transition with about $T_{1/2}$ of 1.5 °C. In case of treated sample this was about 8 °C of course with two superimposed transitions. The lower melting transition stands for the ETC treated part of collagen. This proves, that in this treated part, the collagen transformed into an intermediate conformation with less thermal stability. Its transition enthalpy was about 55% of the total calorimetric enthalpy.

5. Conclusion

The role of ETC for the treatment of shoulder instability has to be further defined. Our preliminary findings suggest that DSC would be a viable method in the monitoring of the thermal consequence of RF electrodes in in vitro circumstances. Further studies are needed to analyse the thermograms of ETC treated tissue samples with different device power setting, action duration and electrode type (monopolar versus bipolar). We suppose, that the healing process of the treated capsular tissue could be followed on an animal model, the different steps of the structural reorganisation could be demonstrated on the DSC thermograms too. The results of these studies could contribute to the development of this medical treatment. Of course, the limitation of the thermal analysis in this field is that DSC cannot be used in in vivo human studies.

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