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ORIGINAL ARTICLE

Histological and Molecular Structure Characterization of Annular Collagen after Intradiskal Electrothermal Annuloplasty

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Abstract The mechanism of pain relief of intradiskal electrothermal annuloplasty (IDET) in the treatment of lumbar diskogenic pain is uncertain. Theories include sealing of annular fissures via collagen denaturation and contraction. Prior studies offer conflicting qualitative data on the ability of IDET to denature collagen. The objective of the present study is to evaluate IDET treatment effect on annular collagen using quantitative data supplied by Fourier-transform infrared imaging spectroscopy.

The posterior annulus of disks (n = 3) from an intact human cadaveric spine at room temperature were treated with two different radiothermal catheters using standard intradiskal electrothermal annuloplasty (IDET) heating protocols. Disks were dissected free with catheters in place and fixed in formalin. Channels created by the catheters were marked and catheters were removed. Tissue samples of treated areas adjacent to the channels and internal control areas from the same disk were stained for light microscopy and placed on barium sulfate windows for Fourier transform infrared imaging spectroscopy (FT-IRIS) analysis.

Treated areas showed evidence of disruption in the fibrillar organization of annular collagen by light microscopy compared to intact stroma from control areas. Quantitative FT-IRIS analysis compared ratios of wavenumber regions known to be sensitive to collagen denaturation. Mean values for the ratios amide II/1,338 cm^{-1} (137.21 ± 25.84 treated, 76.94 ± 16.77 control) and 1,640/1,660 cm^{-1} (0.98 ± 0.03 treated, 0.89 ± 0.03 control) were significantly different between treated and control samples ($p < 0.001$), indicating a breakdown in collagen integrity. Separate analysis by catheter type suggests that catheter design may impact treatment effect.

Key words Disk · IDET · annular collagen · FTIR

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Introduction

Chronic lumbar diskogenic pain (CLDP) has been largely attributed to internal disk disruption and/or subsequent degenerative disk disease [1–3]. Intradiskal electrothermal therapy (IDET) provides an alternative therapeutic option to lumbar fusion in patients with CLDP unresponsive to more conservative management. Introduced in 1997, the procedure involves fluoroscopically guiding a flexible heating electrode circumferentially into the posterior annulus of a painful disk [4]. Several long-term prospective studies, including a randomized, placebo-controlled trial (January 2004) [5–9], have demonstrated statistically significant improvement in both pain and function following IDET in a selected subset of patients with CLDP.

Despite published clinical and basic science studies, the precise mechanism of pain relief from annular heating remains elusive. Theories address both the anatomic and biochemical aspects of CLDP. Coagulation of annular nociceptors, sealing of annular fissures via collagen denaturation and contraction, and cauterization of granulation tissue have been proposed mechanisms along with changes in concentrations of intradiskal inflammatory mediators [10–12]. Of these theories, collagen denaturation with subsequent disk shrinkage is supported by the application of thermal coagulation for shrinkage and tightening of capsular collagen in the treatment of shoulder and ankle instability [13–15].

Further investigation of the collagen denaturation theory includes biomechanical and histological studies. To date, studies reporting data on the biomechanical effects of the IDET-treated disk have been concordant. In 2001, Shah et al [16] and Kleinstueck et al [17] found no significant acute destabilizing effect on spinal stability in vitro. However, histological studies of the IDET-treated disk have demonstrated conflicting results. In the aforementioned studies, Shah et al also observed collagen coalescence and stromal disorganization consistent with denaturation in human cadaveric IDET-treated disks. In contrast, Kleinstueck et al [18] did not observe any collagen changes or stiffening of the IDET-treated disk and suggested that IDET did not reach temperatures sufficient to induce collagen denaturation and coalescence.

In order to further investigate the theory of collagen denaturation and elucidate its role in the IDET-treated disk, this study utilized the technique of Fourier transform infrared imaging spectroscopy (FT-IRIS). FTIR spectroscopy has been shown to be a powerful tool in the evaluation of collagen cross-linking [19] and in the study of molecular changes associated with collagen structure [20]. Although traditional light and electron microscopy techniques are useful for morphological evaluation of tissues and their components, they are limited in their ability to assess molecular changes. In contrast, FTIR analysis is based on monitoring vibrations that originate from molecular components in tissues. Accordingly, evaluation of changes in molecular structure, such as collagen denaturation, can be carried out by direct analysis of spectral absorbances. In the current study, FT-IRIS was used to quantitatively and qualitatively evaluate the effects of IDET on type II collagen structure in a human cadaveric intervertebral disk annulus.

Currently, two types of intradiskal catheters are manufactured for electrothermal therapy: the SpineCATH (Smith and Nephew, Andover, MA, USA) and the electrothermal Decompression catheter (Smith and Nephew, Andover, MA, USA). The SpineCATH applicator has a 5-cm heating segment, whereas the decompression catheter consists of a 3-cm heating segment. Studies comparing the ability of both catheters to denature collagen in the IDET procedure have not yet been published. An additional aim of this study was to use FTIR to generate objective evidence of collagen denaturation using two different IDET catheters.

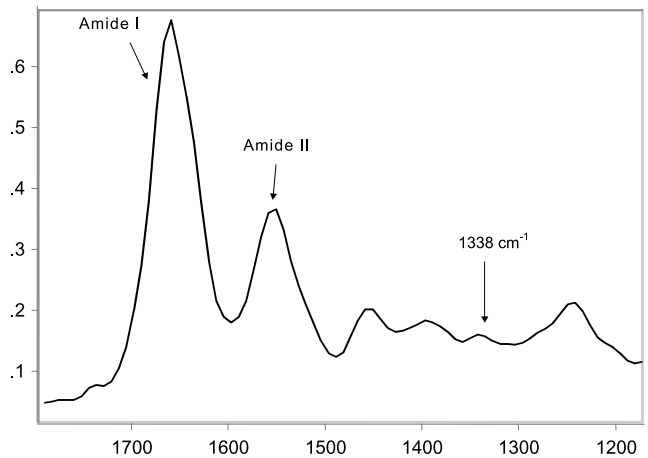

Fig. 1. FTIR collagen spectrum

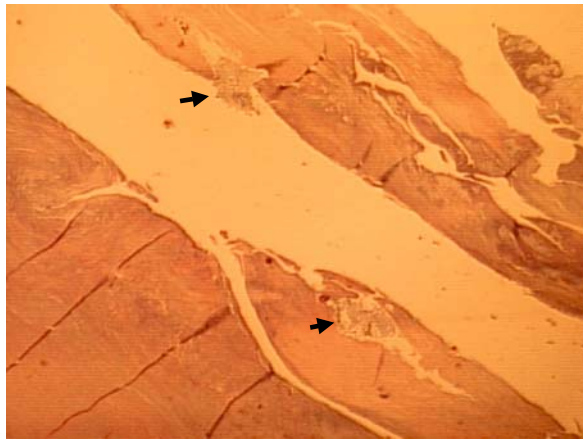

Fig. 2. Posterior annulus with catheter channel marked by nylon threads (black arrows)

Materials and methods

IDET procedure

Five lumbar disks were treated in a single human cadaveric spine. Prior to use the torso was thawed to allow catheter placement within the disk. Generation II 17-gauge introducers were positioned within the ipsilateral anterior disk quadrant under fluoroscopy. Either a SpineCATH or a Decompression catheter (Smith and Nephew, Boston, MA, USA) were threaded through the introducers and placed across the posterior annulus of each disk. Anterior–posterior, lateral, and oblique fluoroscopic projections were taken to guide the catheter placement.

The standard P90 high heating protocol was used for the SpineCATH catheters, starting at 65°C and advanced 1°C every 30 s up to a 90°C maximum over 16.5 min. The AutoTemp mode on the Ora-50™S generator (Smith and Nephew, Boston, MA) was used for the SpineCATH catheters. The Temperature Control mode was used with the Decompression catheters starting at 50°C and advancing 5°C every 30 s up to a 90°C maximum over 13.5 min. SpineCATH catheters were inserted at the L1–L2, L2–L3, and L5–S1 levels. Decompression catheters were inserted at the L3–L4 and L4–L5 levels. Radiopaque marks on each catheter were clearly observed outside each of the introducer needles.

Preservation of catheter channel

Each disk was dissected free with the catheter left *in situ*. The specimens were fixed in 10% buffered formalin for 1 week. Using a sewing needle and blue nylon thread, loops were thrown around the catheter's distal segments to immortalize the path of the catheter following histologic processing. The needle was inserted perpendicular to the endplate and redirected until contact was made with the

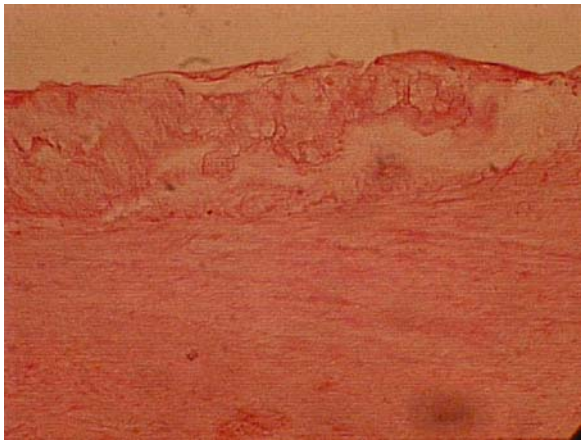

Fig. 3. H/E stain of treated annular tissue adjacent to channel (top)

catheter. The needle with thread was then passed completely through the disk immediately adjacent to the catheter. It was then rethreaded through the disk in a similar fashion adjacent to the opposite edge of the catheter, and the ends of the thread were knotted. The catheters were then pulled from the disks.

Slide preparation

Specimens were decalcified in 10% ethylenediaminetetraacetic acid (EDTA) and embedded in paraffin. Sections, 6 μm thick, from each disk specimen were microtomed and placed onto barium fluoride infrared windows for evaluation by FTIR spectroscopy. Additional specimens were sectioned, placed on glass microscope slides, and stained for histological analysis.

FTIR imaging spectroscopy

Specimens prepared on barium fluoride windows were imaged in transmission mode at 8 cm^{-1} under N_2 purge using a Digilab (Cambridge, MA, USA) UMA 300A FTIR microscope with an FTS-60A step scanning FTIR spectrometer and a 64×64 MCT Focal Plane Array detector (Santa Barbara Focal Plane, Golota, CA, USA). Spectral information was obtained from 400×400 μm^2 regions resulting in 4,096 individual spectra for each scan. Image analysis was performed using Isys Image Analysis software (Spectral Dimensions, Olney, MD, USA).

The spectra were baselined and the collagen absorbances were monitored in the 1,690–1,600, 1,590–1,480, and the 1,338 cm^{-1} spectral regions. The primary molecular vibrations associated with these wavenumber absorbances are the amide I carbonyl stretch (C=O) [21–25], the amide II out-of-phase, in-phase N–H deformation and C–N stretch [24–28], and the CH_2 side chain vibrations [26–28], respectively (Fig. 1). Spectra were evaluated for shifts in the amide I contour and changes in the amide II/1,338 cm^{-1} area ratio. Ratios were taken to avoid errors that could be incurred as a result of concentration-dependent changes in spectral absorbance.

As collagen denatures, order within the triple helix becomes disrupted because of the rearrangement of

Fig. 4. H/E stain of control annular tissue

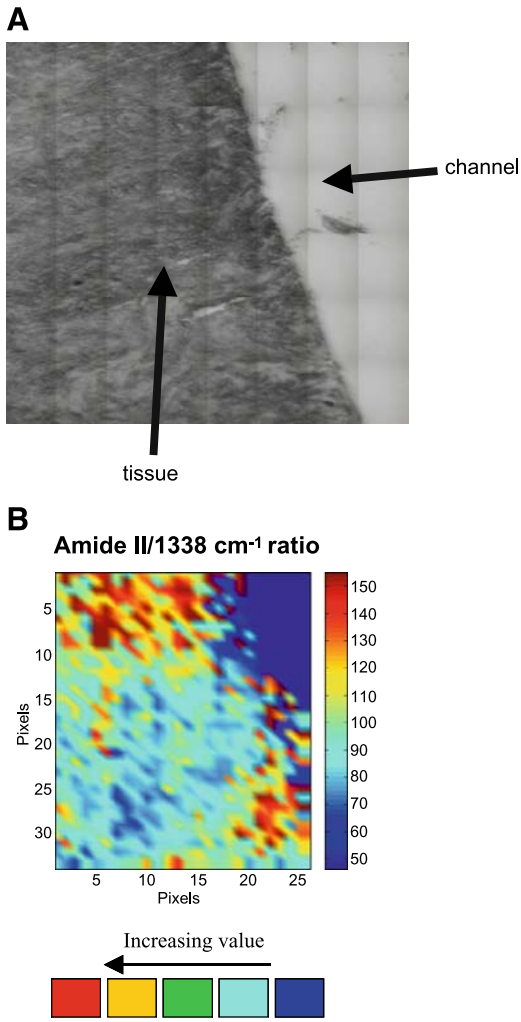

Fig. 5. **A** Light microscopy view of the treated disk sample (channel in upper right corner). **B** Amide II/1,338 cm^{-1} ratio distribution in treated sample (channel dark blue in upper-right corner)

hydrogen bonds and changes in the interactions between amide groups. This results in changes in the conformational arrangement of collagen that can be monitored spectroscopically. Consequently, the structure sensitive peak at 1,338 cm^{-1} decreases [29, 30], resulting in an increased amide II/1,338 cm^{-1} ratio [20]. Also, shifts in the amide I contour from 1,660 cm^{-1} to lower wavenumbers have been known to accompany collagen denaturation [31] resulting in an increase in the 1,640/1,660 cm^{-1} intensity ratio.

Tissues immediately adjacent to the channels created by the catheter were chosen as treatment areas for analysis. Internal controls were taken from each disk in areas in the anterior annulus farthest removed from these channels in the posterior annulus as well as from tissue within several millimeters of the channels. Internal controls were chosen so that localized changes due to heating could be easily assessed adjacent to the channels and away from the channels.

Statistical analysis

Mean values and standard deviations of the peak area ratios (amide II/1,338 cm^{-1} and 1,640/1,660 cm^{-1}) were calculated for treatment and controls. Student's *t*-test was applied to the two sets of data with statistical significance determined at $p < 0.05$. SPSS version 9.0 software was utilized for data analysis.

Results

Annuloplasty

Placement of the catheters within the disk was made with difficulty as a result of the degenerative nature of the cadaveric torso at nonphysiologic temperature. Navigation of the catheter around obstructions in the disk was necessary to attain adequate position. Catheter placement spanning the entire posterior annulus in an optimal position for heating was obtainable in three out of the five disk levels.

Cadaveric spine

The specimen, dissected free of the torso, confirmed the fluoroscopic and procedural impression of moderate to severe lumbar disc degeneration. Diffuse posterior and lateral disk bulges were noted at all levels with the more severe degenerative changes at L3–L4 and inferior levels. There were osteophytic projections of the superior and inferior endplates of L3–L4, L4–L5, and L5–S1 with hypertrophy of the zygapophoseal joints bilaterally at each level.

Disk morphology

Due to these technical difficulties, only disks at the L1–L2, L2–L3, and L4–L5 levels were included in the analysis. SpineCaths were used at the two higher levels, whereas a decompression catheter was used at the L4–L5 level. Disk preparation was successful in preserving the channels created by the catheter at each level. Channels in the posterior annulus bordered by nylon thread clusters were evident on light microscopy (Fig. 2).

Histology

Tissues in the posterior annulus bordering the channels exhibited loss of organization in the stromal structure.

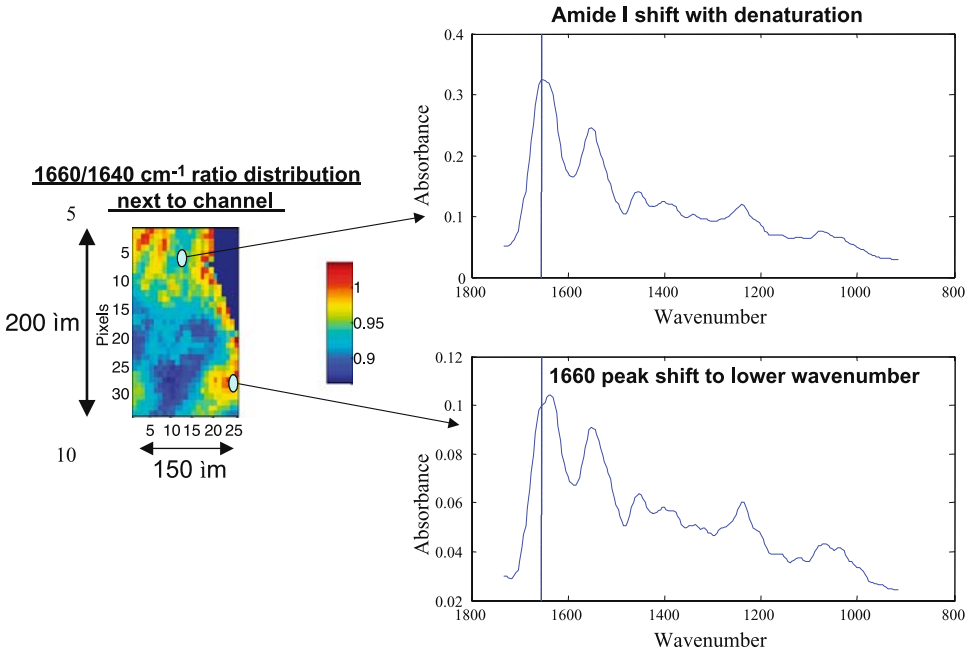

Fig. 6. 1,640/1,660 cm^{-1} ratio distribution. Channel dark blue in upper-right corner

Table 1. Wavenumber ratio mean values, treatment vs control

Ratio	Treated	Control
Amide II/1,338 cm^{-1}	137.21 ± 25.84	76.94 ± 16.77*
1,640/1,660 cm^{-1}	0.98 ± 0.03	0.89 ± 0.03*

*p value < 0.001

Picrosirius red stain, specific for collagen, and hemotoxylin and eosin staining revealed hyalinization of collagen, globular forms, and apparent vacuole formation (Fig. 3). These changes are consistent with what might be expected as a result of thermal injury to nonvital tissue. Control specimens from the anterior annulus, by contrast, showed no histological evidence of damage. A normal annular appearance and stromal organization with a nondisrupted fibrillar arrangement of collagen was observed in all specimens (Fig. 4).

FT-IRIS analysis

Quantitative FT-IRIS analysis was performed on treated sections immediately adjacent to the channels. Figure 5 is an FT-IR image showing the amide II/1,338 cm^{-1} ratio distribution in this area. The mean value of the ratio of the treated areas was 137.21 ± 25.8, whereas the value for control areas away from the channels was 76.94 ± 16.77. Similarly, Figure 6 shows the FT-IRIS image of the 1,640/1,660 cm^{-1} intensity ratio shift and distribution. Again, higher values indicating greater degradation were observed in areas next to the channels (0.98 ± 0.03 vs 0.89 ± 0.03). The increase in values of the treated areas compared to controls was highly significant for both outcome ratios ($p < 0.001$) (Table 1). This treatment effect was seen at distances up to 1 mm from the channel. The mean values for both ratios dropped to control levels at greater distances.

Catheter type

Findings were consistent in histology among levels using the SpineCath and the single level at which a decompression catheter was used. Evidence for stromal disorganization was similar in quality in areas bordering the channels.

Amide II/1,338 cm^{-1} ratios were significantly increased relative to controls in all treated specimens regardless of catheter type. However, analysis of 1,640/1,660 cm^{-1} ratios by catheter type revealed treatment areas in the SpineCath disks to be significantly increased relative to controls ($p = 0.01$), whereas no significant difference was found in the disk treated with the Decompression catheter ($p = 0.67$).

Discussion

In this study, qualitative visual evidence of collagen denaturation by light microscopy following IDET annuloplasty in human cadaveric disks is supported by the quantitative evidence of alteration in collagen structure as determined by FT-IRIS analysis. The spectral peak area ratios in treated areas were significantly increased relative to control areas indicative of disruption in collagen secondary structure and conformational changes accompanying denaturation. A separate analysis of the quantitative data suggests that catheter design may affect the efficiency in heating annular collagen to temperatures sufficient to cause denaturation of its secondary structure.

The effect of annuloplasty on collagen was noted only in the tissues immediately adjacent to the channels created by the catheters. Several factors in the present study worked to mitigate the size of this effect. These include the partially frozen nature of the specimen and the advanced native degeneration of the cadaveric disks.

Reduced disk temperatures have an obvious impact on the thermal treatment of annular collagen. The standard heating protocols were not altered for the study; there was no increase in temperature or prolongation of time of application. Temperature within the specimens was above freezing but lower than room temperature and far below physiologic temperatures of the in vivo disk. It is reasonable to assume that the time required to reach the maximum temperatures within the disks were prolonged thus shortening the heating time and possibly reducing the maximum temperatures attained. More than the other factors, this would greatly attenuate the IDET treatment effect on collagen both at the immediate interface with the catheter as well as in adjacent annular tissue. Nevertheless, temperatures created by the catheter were sufficient to effect conformational change in collagen structure however blunted the treatment may have been by the nonphysiologic temperatures in the disk at the time of procedure.

The degenerative condition of the cadaveric spine used for the study may also have reduced the potential treatment effect. These factors may have contributed to pretreatment collagen denaturation in the disks sufficient to raise baseline values of the spectral peak ratios sensitive to such changes thus narrowing the difference in ratio values between treated and control areas. In other words, healthier, less-degenerated disks at baseline may have shown a greater treatment effect because they had more intact collagen to denature.

The main difference in design of the two catheter types used in this study is the length of the distal heating coil. The SpineCath catheter is longer at 5 cm compared with 3 cm in the decompression catheter. As a result, the effective channel length created by each catheter differs as well. During catheter preservation, every effort was made to mark the annulus in the most distal portion of the channels. However, there is an increased margin for error with a smaller effective channel length. Mismarking the decompression catheter channel proximal to the heating filament could have adversely affected the FTIR data.

More pertinent than catheter design may be the differences in the associated heating protocols. Both reached maximum temperatures of 90°C but the SpineCath protocol begins at a higher temperature (65 vs 50°C) and lasts longer (16.5 vs 13.5 min). Greater thermal energy applied

for a greater period of time may equal greater efficiency in altering collagen secondary structure.

It is also important to note that the sample size, two disks compared with one, in the analysis of the SpineCath and decompression catheters, may simply have been too small for an accurate comparison of type.

Findings of this study confirm that both catheter types denature the collagen of the annulus as confirmed by FT-IRIS analysis. The clinical relevance of these findings lies in the alteration of intervertebral disk annular structure. Although they do not establish the mechanism of pain relief obtainable through IDET in patients with lumbar diskogenic pain, the data support the proposed anatomic theories mentioned previously. Neither do they exclude possible biochemical changes induced within the disk that may also contribute to the clinical effect of the procedure.

The significance of this study is that FT-IRIS allows for a quantitative analysis of the thermal effects on disk collagen of these treatment methods. Further studies with larger sample sizes are needed to determine which catheter may have a better potential therapeutic effect. In addition, FT-IRIS analysis can be used to evaluate not only the effects of different catheter designs, but also of different heating protocols.

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