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Lavage Solution Temperature Influences Depth of Chondrocyte Death and Surface Contouring During Thermal Chondroplasty with Temperature-Controlled Monopolar Radiofrequency Energy

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Background: Although radiofrequency energy can smooth and contour cartilage surface, it has deleterious effects on chondrocyte viability.
Hypothesis: Monopolar thermal chondroplasty in a 37°C lavage solution, as compared with a 22° lavage solution, will reduce chondrocyte death and result in greater smoothing of the articular cartilage surface.
Study Design: Controlled laboratory study.
Methods: Sixteen chondromalacic samples from patients undergoing total knee arthroplasty were divided into two groups: 22°C and 37°C lavage solution. Each sample was divided into two equal parts and half of each group was treated for 10 seconds and the other half for 15 seconds.
Results: Confocal laser microscopy demonstrated that the depth of chondrocyte death in the 37°C lavage solution group was significantly less (range, 200 to 340 μm) than that in the 22°C solution group for both 10- and 15-second treatment times. Scanning electron microscopy demonstrated that the cartilage surface in the 37°C lavage solution group was smoother than that in the 22°C solution group for the 10-second treatment time. Energy delivery power in the 37°C lavage solution group was significantly lower than in the 22°C solution group for both treatment times.
Conclusions: Thermal chondroplasty with 37°C lavage solution resulted in less depth of chondrocyte death and produced smoother surfaces than with 22°C solution for 10 seconds of treatment.
Clinical Relevance: Less chondrocyte death would permit increased use of thermal chondroplasty.

Thermal chondroplasty with radiofrequency energy has gained widespread use over the past several years.[3,22] Currently, two basic radiofrequency energy systems with temperature-controlled probes/generators are available for clinical application: monopolar and bipolar. In vivo and ex vivo studies investigating the use of radiofrequency energy for thermal chondroplasty have resulted in variable and contradictory results (Refs. 9, 12, 15, 21, 24; B. A. Krenzel et al., unpublished data, 2000). Turner et al.[24] and Kaplan and Uribe[9] reported that bipolar radiofrequency energy smoothed roughened or chondromalacic articular cartilage surfaces without destroying viable chondrocytes. Both research groups concluded that bipolar radiofrequency energy was safe for performing thermal chondroplasty. In contrast, Lu et al.[12,15] reported that use of bipolar radiofrequency energy for chondroplasty

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resulted in significant chondrocyte death, sometimes producing full-thickness chondrocyte death that reached the subchondral bone. In addition, Lu et al.[11] and Edwards et al.[4] compared the effects of thermal chondroplasty by using monopolar and bipolar radiofrequency energy on both bovine and human cartilage and reported that both modalities smoothed cartilaginous surfaces but monopolar energy resulted in less chondrocyte death. The investigators concluded that thermal chondroplasty with radiofrequency energy should be applied cautiously, especially bipolar radiofrequency energy.[3,4,11]

Although radiofrequency energy has the ability to smooth and contour cartilage surface, it is limited in its clinical application because of its potentially deleterious effects on chondrocyte viability. It is well known that chondrocytes have very limited ability to regenerate or repair damaged cartilage.[7] If chondrocyte death can be reduced and controlled when radiofrequency energy is used for chondroplasty, its safety margin for clinical application will be improved.

To date, no studies have been reported investigating methods to limit chondrocyte death during thermal chondroplasty with radiofrequency energy. Monopolar and bipolar radiofrequency energy used for chondroplasty employ different mechanisms and algorithms in their design; therefore, a method to limit chondrocyte death for temperature-controlled monopolar radiofrequency energy was explored first in this study.

We evaluated the thermal penetration and surface smoothing of chondromalacic articular cartilage after monopolar treatment at two lavage temperatures: 22°C and 37°C. On the basis of the monopolar device's temperature-controlled design, we hypothesized that thermal chondroplasty with monopolar radiofrequency energy in a 37°C lavage solution would result in less chondrocyte death as compared with 22°C lavage solution and result in greater smoothing of the articular cartilage surface.

MATERIALS AND METHODS

All procedures were approved by the Institutional Review Board and Human Subjects Committee at the participating universities. Sixteen fresh osteochondral sections from 16 patients undergoing total or partial knee arthroplasty were used in this study. Chondromalacia was graded according to a modified Outerbridge system, as previously described[10]: grade 1, softened cartilage surface; grade 2, softened cartilage with fine fibrillations, grade 3, fibrillated surface with pitting to subchondral bone; and grade 4, fibrillation of cartilage and exposed subchondral bone. To avoid experimental bias, only chondromalacic cartilage of grade 2 was selected for use in this study, and each graded osteochondral section was cut into two sections. One section was treated with monopolar radiofrequency energy in physiologic saline solution (0.15M) at 22°C (room temperature), whereas the other section was treated in physiologic saline solution (0.15M) at 37°C. An area 2 cm distant from the radiofrequency energy-treated area on each specimen served as a control.

For the 37°C lavage solution, 1 liter of physiologic saline solution was placed in a plastic container and heated by a Nuova II stir plate (Thermolyne Corporation, Dubuque, Iowa). A thermometer was used to monitor the temperature, and the saline solution was maintained at 37°C for 60 minutes. After temperature stabilization, cartilage sections were placed in the saline solution and allowed to equilibrate for 20 minutes so that they reached 37°C before the monopolar radiofrequency energy treatment. No fluid flow was used during the treatment because the results from a previous study showed a negative effect of irrigation fluid flow on cartilage matrix temperatures during monopolar radiofrequency energy chondroplasty.[5]

The Vulcan ElectroThermal Arthroscopy System coupled with a TAC-C II probe (Oratec Interventions, Inc., Menlo Park, California) was used to deliver monopolar radiofrequency energy in a light contact fashion over a 1.0-cm^2 area on each section in a paintbrush treatment pattern at a generator setting of 70°C and 15 watts. Treatment times of 10 seconds and 15 seconds were evaluated. For each treatment time/lavage temperature combination, 8 sections were tested, for a total of 32 treatments (4 groups of 8 specimens each). Ten- and 15-second treatment times were selected on the basis of the results of a previous study.[13] In that study, 10 to 15 seconds were required for monopolar radiofrequency energy to smooth a 1.0-cm^2 surface of chondromalacic grade 2 cartilage.

After treatment, each treated area was processed for analysis by vital cell staining/confocal laser microscopy and scanning electron microscopy. A diamond-wafering blade (Isomet 2000 Precision Saw; Buehler, Lake Bluff, Illinois) was used to cut 1.5-mm thick osteochondral sections for confocal laser microscopy. Phosphate-buffered saline solution was used for irrigation to avoid thermal injury during sectioning, as previously described.[11,12,15] Sections were placed in 1.0-ml phosphate-buffered saline solution and maintained at 4°C for 3 hours before staining for cell viability.

Cell viability staining was performed with ethidium homodimer (EthD-1) and calcein acetoxymethylester (calcein-AM) in conjunction with confocal laser microscopy. The 1.5-mm sections were stained by incubation in 1.0-ml of phosphate-buffered saline solution containing 1.0 μl of calcein-AM per 10 μl EthD-1 (LIVE/DEAD Viability/Cytotoxicity Kit [L-3224], Molecular Probes, Eugene, Oregon) for 30 minutes at room temperature. Each 1.5-mm osteochondral section was placed on a glass slide and moistened with several drops of phosphate-buffered saline solution. A confocal laser microscope (MRC-1000, Bio-Rad, Hemel Hempsted/Cambridge, England) equipped with an argon laser and necessary filter systems (fluorescein and rhodamine) was used with a triple-labeling technique. With this technique, the signals emitted from the double-stained specimens can be distinguished because of their different absorption and emission spectra.[4,11,12,15,19] These images were displayed on a monitor in red-green-blue (RGB) mode. All cartilage samples were coded so that treatment time and temperature of the lavage solution were unknown to the examiners.

The depth of chondrocyte death of each section was determined for each treated region in the microscope im-

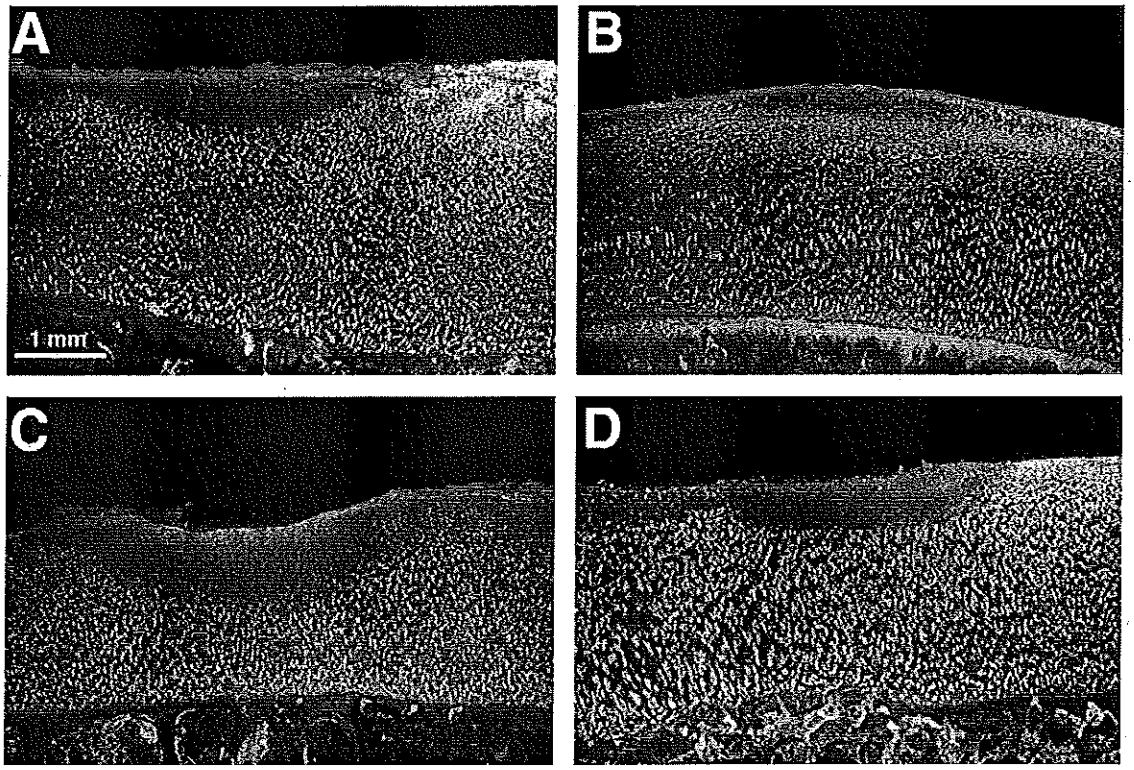

Figure 1. Confocal microscopic image demonstrating radiofrequency energy-treated cartilage surface (top of each image) and subchondral bone (bottom of each image). Specimens were treated in 22°C and 37°C lavage solution for 10 or 15 seconds (original ×20). The green dots indicate viable chondrocytes, and the red dots indicate dead chondrocytes. A, 10-second treatment time, 22°C lavage solution. B, 10-second treatment time, 37°C lavage solution. C, 15-second treatment time, 22°C lavage solution. D, 15-second treatment time, 37°C lavage solution. The depth of chondrocyte death in the 37°C lavage solution was significantly less than that in the 22°C lavage solution in both the 10- and 15-second treatment time groups.

age. The confocal laser microscope was calibrated by using a micrometer measured through the objective lens (2×) used for this project (20× total magnification; objective + eyepiece magnification). The pixel length measured on images was converted to micrometers, as previously described.[4,11,12] The depth of chondrocyte death was determined for each confocal image of the osteochondral sections with Adobe PhotoShop software (Adobe PhotoShop, Version 5.5, San Jose, California).[4,11,12]

After evaluation by confocal laser microscopy, the same cartilage specimens were trimmed (4 × 3 × 1.5 mm) and fixed in modified Karnovsky's solution (2% glutaraldehyde in 0.1 mol/L sodium cacodylate buffer, pH 7.4) for 2 hours and then washed in 0.1 mol/L sodium cacodylate buffer twice at room temperature. These samples were stored in 0.1 mol/L sodium phosphate-buffered saline solution for 8 hours at 4°C. After dehydration in a graded series of ethanol solutions (50%, 70%, 80%, and 100%) and air drying, the samples were coated with gold in a Bio-Rad E5000M gold coater and examined with a Hitachi S570 scanning electron microscope (Hitachi High Technologies Corp., Tokyo, Japan).[11,12,14] The image of each section was coded so that the lavage temperature and treatment time were unknown. The scanning electron microscope images were scored by three investigators independently, using a custom-designed scoring system described in a previous study.[13] Higher scores indicated a smoother cartilage surface.

The mean depth of chondrocyte death, the mean monopolar radiofrequency energy delivery power, the time needed to reach the radiofrequency energy preset temperature, and the mean treatment temperature (temperature measured from a thermocouple located within the radiofrequency probe tip) were compared among groups of lavage temperatures and treatment time combinations by using analysis of variance with SAS software (SAS version 7.1, SAS Institute, Cary, North Carolina). Factors included in the analysis were patient age or sex, treatment time, and lavage solution temperature. When differences among groups were demonstrated by analysis of variance, appropriate post hoc tests were employed. Paired *t*-tests were used to compare the effect of lavage solution temperature within treatment time groups. Patient sex was compared by using the Wilcoxon signed-rank test. The inter- and intraobserver precision errors were determined for the scanning electron microscope scores. The Kruskal-Wallis test was used to compare the scanning electron microscope image scores between different lavage temperatures at the same treatment time. When significance was identified with the Kruskal-Wallis test, the Mann-Whitney procedure was used to compare the subjective scores

TABLE 1
The Effects of Lavage Temperature on Chondrocyte Death and Probe Power and Temperature[a]

Variable	Treatment time and lavage temperature group			
	10 seconds		15 seconds	
	22°	37°	22°	37°
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
Depth of chondrocyte death (μm)	620 ± 106	420 ± 219	930 ± 236	590 ± 214
Mean power (watts)	8.5 ± 0.6	5.9 ± 0.9	7.6 ± 0.8	5.1 ± 0.4
Time to set temp (seconds)	1.8 ± 0.5	0.7 ± 0.2	1.3 ± 0.5	1.0 ± 0.5
Mean probe temp (°C)	67.5 ± 1.1	70.1 ± 0.8	68.7 ± 0.6	70.3 ± 0.4

[a] There was a significant difference between lavage temperatures at each treatment time for each variable ($P < 0.05$).

between groups. P values less than 0.05 were considered significant.

RESULTS

There were no significant differences in patient age or sex among treatment groups (mean age, 65 ± 7 years; 7 men and 9 women; $P > 0.05$).

Confocal laser microscopy demonstrated that the depth of chondrocyte death in 37°C lavage solution was significantly less than that in 22°C solution at both 10- and 15-second treatment times (Fig. 1, Table 1). Scanning electron microscopy demonstrated that cartilage surfaces were smoothed in both the 37°C and 22°C lavage solution groups treated for both 10 and 15 seconds, compared with the control specimens (Figs. 2 and 3).

Scanning electron microscopy demonstrated that chondromalacic cartilage surfaces treated by radiofrequency energy in the 37°C lavage solution were smoother than those treated in the 22°C solution for 10 seconds ($P < 0.05$), but that there were no differences in surface smoothing between sections treated in 37°C and in 22°C lavage solution for 15 seconds (Fig. 4). Chondromalacic cartilage surfaces treated by radiofrequency energy for 15 seconds were smoother than those treated for 10 seconds in both 37°C and 22°C lavage solutions ($P < 0.05$). The intra- and interobserver precision errors for scanning electron microscopy scores were 10.8% and 12.9%, respectively.

The mean monopolar radiofrequency energy treatment temperature in the 37°C lavage solution was higher than in the 22°C lavage solution for both the 10-second and 15-second treatment times ($P < 0.05$), whereas radiofrequency energy delivery power in the 37°C solution was lower than in the 22°C lavage solution for both treatment times ($P < 0.05$) (Table 1). The times for monopolar radiofrequency energy to reach the preset temperature were faster in the 37°C lavage solution than in the 22°C lavage solution for both 10 and 15 seconds ($P < 0.05$). Treatment temperatures were more stable in the 37°C lavage solution (coefficient of variation, 6.2%) than in the 22°C lavage solution (coefficient of variation, 7.9%) ($P < 0.05$).

DISCUSSION

Thermal chondroplasty performed with monopolar radiofrequency energy in 37°C lavage solution caused significantly less chondrocyte death than in 22°C lavage solution. Increasing the lavage solution temperature allowed the probe tip to reach a preset temperature more rapidly and resulted in less total power (energy) delivery while still effectively smoothing the cartilaginous surface.

Traditionally, mechanical shaving has been used to debride, contour, and smooth chondromalacic articular cartilage. However, this method has several drawbacks: 1) adjacent normal cartilage is often removed while focal lesions are debrided, 2) it is difficult to completely smooth the cartilage surface and not leave fine fibrillated regions, and 3) it is a challenge to create a completely smooth cartilage surface with mechanical shaving. After treatment, normal loading typically causes continued degradation that results in further fibrillation and degradation.[2,6,8,17,18,23]

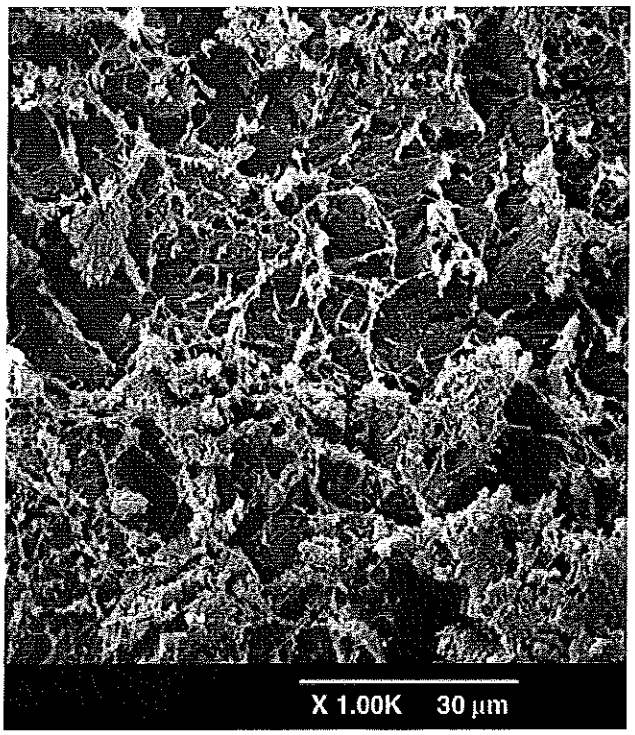

Figure 2. Scanning electron microscope image demonstrating sham-operated control cartilage with fibrillated and rough surface (original magnification ×1000).

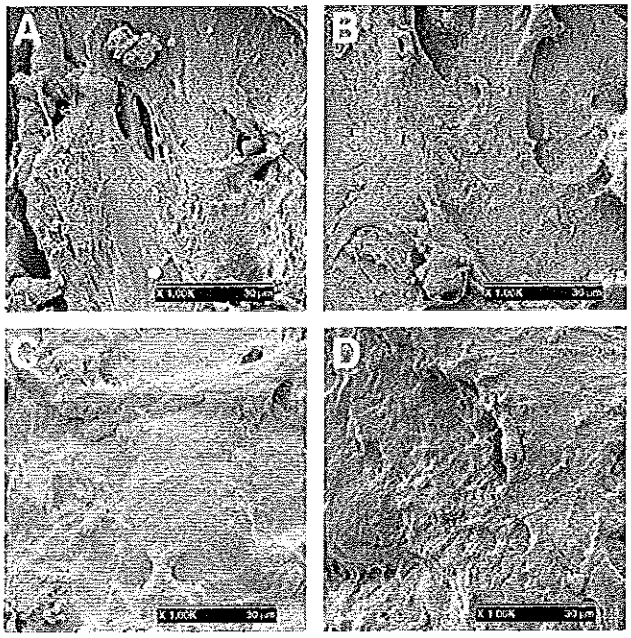

Figure 3. Scanning electron microscope images of the radiofrequency energy-treated cartilage surface. A, 10-second treatment time, 22°C lavage solution B, 10-second treatment time, 37°C lavage solution. C, 15-second treatment time, 22°C lavage solution. D, 15-second treatment time, 37°C lavage solution (original magnification, ×1000). Radiofrequency energy melted down the cartilaginous fronds and smoothed the chondromalacic cartilage surface in each lavage temperature/treatment time combination compared with the control. This result is verified by previous studies performed in our laboratory.[11,13]

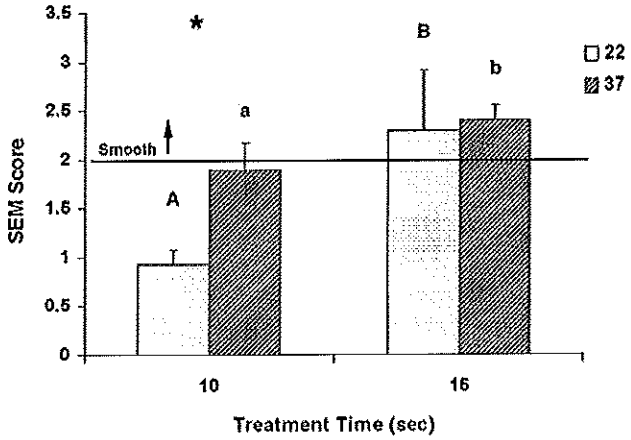

Figure 4. Scanning electron microscope scores of monopolar radiofrequency energy-treated surfaces at different lavage temperature/treatment time combinations. Higher scores indicate smoother surface. Score values represent the means of three observers plus or minus the standard deviation. Means with different letters are significantly different from each other at different treatment time intervals ($P < 0.05$). Means with an asterisk are significantly different from each other at different lavage temperatures ($P < 0.05$). Scores above the transverse line at score 2 mean that cartilage surfaces were smoothed. SEM, scanning electron microscope.

The use of radiofrequency energy for thermal chondroplasty began approximately 4 to 5 years ago. Turner et al.[24] reported that bipolar radiofrequency energy resulted in smoother cartilage surfaces than conventional mechanical debridement without detrimental effects on chondrocyte viability. Later, Lu et al.[15] demonstrated that monopolar radiofrequency energy created a smoother cartilage surface than mechanical shaving but also resulted in extensive chondrocyte death in a sheep model. Recently, Lu et al.[11] and Edwards et al.[4] reported that both monopolar and bipolar radiofrequency energy effectively smoothed chondromalacic cartilage surfaces; however, both modalities caused significant chondrocyte death during thermal chondroplasty treatment.

The thickness of human articular cartilage in the region of the femoral condyles is approximately 2 to 4 mm.[1] Traditional mechanical debridement with shaving systems usually removes 200 to 400 μm of cartilage, including both diseased cartilage and the underlying normal cartilage, if the shaver is well controlled during the treatment. After mechanical debridement, further chondrocyte death between 100 and 400 μm deep from the surface occurs within the first 2 postoperative weeks.[2,6,8,17,18,23] Therefore, mechanical debridement with a shaver results in 300 to 800 μm of chondrocyte loss from tissue removal and subsequent chondrocyte death, with the cartilaginous surface still microscopically rough after the treatment.

The goal of this study was to determine whether use of normothermic lavage solution (37°C), as compared with room-temperature lavage solution (22°C), would limit the depth of chondrocyte death when temperature-controlled monopolar radiofrequency energy was used to perform thermal chondroplasty. This hypothesis was based on the design and temperature control algorithm of monopolar radiofrequency energy. The Vulcan monopolar system evaluated uses delivered power to control the tissue temperature reflected by a thermocouple within the probe tip. At the beginning of treatment, the radiofrequency generator delivers full preset power to cause tissue heating. The thermocouple within the monopolar probe tip is subsequently heated, reaching the preset temperature relatively quickly. After reaching the preset temperature, the monopolar algorithm reduces the power to decrease tissue/probe-tip temperature and then uses minimum power output to maintain the tissue temperature near the preset temperature. This results in the monopolar radiofrequency energy generator delivering mean power that is significantly less than the preset power (34% to 57% of preset power in this study) to maintain the preset temperatures.

The results of this study demonstrated that thermal chondroplasty performed with monopolar radiofrequency energy in 37°C lavage solution caused significantly less chondrocyte death than that in 22°C lavage solution. The explanation for this decreased cell death is that less delivered power (energy) resulted in less chondrocyte injury.

The delivered power in 37°C lavage solution was approximately 40% less than that in 22°C lavage solution during both 10- and 15-second treatment times. Delivered power equals the electric current multiplied by electric voltage. Organ[20] reported that radiofrequency energy current intensity had a very strong influence on the size of the lesion generated. The lesion size increased as the intensity was squared. The temperature-controlled monopolar device tested in this study was able to maintain the probe-tip temperatures equivalent to the preset temperature at a lower mean power in 37°C compared with the 22°C lavage solution.

Confocal laser microscopy demonstrated chondrocyte death in the treated regions with red staining and a clear demarcation of thermal injury. Some investigators have questioned whether chondrocytes are actually dead after thermal treatment or whether their function has been temporarily impaired.[25] There are many reports that support the validity of the confocal laser microscopy findings and the fact that thermal treatment results in irreversible chondrocyte death.[12,15,16,19] In an in vivo ovine study investigating treatment of partial-thickness articular cartilage defects with radiofrequency energy, chondrocyte death was identifiable at time 0 and persisted until the termination of the project 6 months later.[15] In no case did the investigators observe return of cell viability in areas where previous cell death had been demonstrated. In fact, a greater depth of chondrocyte death was identified in samples analyzed 14 days after treatment, compared with those evaluated immediately afterward, indicating that chondrocyte death may result both from immediate thermal necrosis and from continued necrosis or induction of apoptosis.

In addition, the results of this study demonstrated that the time required to reach the preset temperature at the initiation of treatment in the 37°C lavage solution was significantly faster than in the 22°C lavage solution in both 10- and 15-second treatment groups. This difference likely occurred because the temperature difference between the lavage solution and the preset radiofrequency energy temperature was 33°C for the 37°C group and 48°C for the 22°C group.

In this study, scanning electron microscopy demonstrated that there were no significant differences in cartilage surface smoothing and contouring between the 37°C lavage solution and the 22°C lavage solution for the 15-second treatment group. However, monopolar radiofrequency energy treatment of the cartilage surface in 37°C lavage solution for 10 seconds resulted in a significantly smoother surface than did the same treatment time in a 22°C lavage solution. This difference probably was caused by the faster time to achieve the preset temperature in the 37°C lavage solution group (0.7 versus 1.8 seconds) and the higher mean temperature reached with the 37°C group (70.1°C versus 67.5°C). The major reason why the mean treatment temperature in the 37°C lavage solution was significantly higher than that in the 22°C lavage solution during monopolar radiofrequency energy treatment was that the temperature fluctuation in the 37°C lavage solution was less than that in the 22°C lavage solution. The monopolar generator is able to maintain probe-tip temperature closer to the preset temperature in 37°C lavage solution than in 22°C lavage solution, with lower delivered power.

Several limitations in this study deserve to be discussed. First, this ex vivo study may not precisely reflect monopolar radiofrequency energy use in in vivo conditions; therefore, in vivo studies need to be performed to determine the significance of these results. Second, the depth of chondrocyte death was measured acutely in the ex vivo condition. This treatment condition may not accurately reflect chondrocyte viability in long-term in vivo studies. A previous study demonstrated progressive chondrocyte death over time in a sheep model.[15] Third, use of 37°C lavage solution to reduce chondrocyte death may only be beneficial for this temperature-controlled monopolar device. It may not be beneficial and could be detrimental when used with other radiofrequency energy systems. Fourth, the tested treatment times (10 and 15 seconds) and cartilage surface scoring system were selected based on the previous study performed in our laboratory.[13] Longer radiofrequency energy treatment application times may eliminate the beneficial effects of the 37°C lavage solution demonstrated in this study. Fifth, samples were harvested from patients with a mean age of 65 ± 7 years. Although not demonstrated in previous animal studies, it is possible that chondrocytes from older patients are more susceptible to thermal injury than those in younger patients. We randomized samples among treatment groups to minimize or eliminate any effect age may have had on chondrocyte death.

CONCLUSIONS

This ex vivo study indicated that thermal chondroplasty with monopolar radiofrequency energy in 37°C lavage solution significantly reduced chondrocyte death compared with use of the standard room temperature (22°C) lavage solution. During 10- and 15-second treatment times over a 1 cm^2 area of grade 2 chondromalacic cartilage (with an average patient age over 65), the mean depth of chondrocyte death ranged from 420 to 590 μm. This depth is similar to the expected depth of chondrocyte loss produced by mechanical debridement and shaving. Compared with mechanical debridement with a shaver, monopolar radiofrequency energy has several advantages: 1) a smoother surface may be produced, 2) injury to adjacent and untreated regions may be more easily avoided, and 3) rapid and easy contouring is achieved that may result in a shortened operative process.

On the basis of the results of this study, we believe that lavage solution temperature may have a significant influence on chondrocyte viability when thermal chondroplasty with monopolar radiofrequency energy is performed. Further in vivo studies investigating the long-term effects of lavage solution temperature need to be conducted.

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