Chondrocyte Viability and Metabolic Activity After Treatment of Bovine Articular Cartilage With Bipolar Radiofrequency: An In Vitro Study

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Purpose: Some controversy exists regarding the effects of radiofrequency (RF) probes on articular cartilage. To further elucidate these effects, we examined the chondrocyte viability and metabolic activity after treatment of fresh bovine articular cartilage with bipolar RF probes. Type of Study: In vitro assessment.

Methods: Three fresh bovine knees served as a baseline control for chondrocyte viability, yielding 6 samples (1 from each medial femoral condyle and 1 from each lateral femoral condyle). After the baseline expected chondrocyte viability was determined, 3 additional bovine knees served as the experimental specimens for the study. Under sterile conditions, 2 different bipolar RF probes were used to treat the articular surface in a light contact mode, moving at a linear rate of 3 to 4 mm/s to provide tissue debridement. Full-thickness articular cartilage was then harvested from each of the treatment areas. Six samples per probe were then assessed for chondrocyte viability using fluorescent double-staining followed by confocal microscopy; 6 samples per probe were assessed for metabolic activity using an $^{35}$SO$_4$ incorporation assay; and 12 additional untreated samples were obtained to serve as controls for viability ($n = 6$) and metabolic activity ($n = 6$).

Results: The depth of chondrocyte death (mean ± standard deviation) was 109.4 ± 22.1 μm after treatment with the ACD-50 probe, and was 172.3 ± 34.3 μm after treatment with the 2.5-mm/90° probe. The $^{35}$SO$_4$ uptake (mean ± standard deviation) was 2584 ± 1388 cpm/mg dry cartilage for the ACD-50 probe and 1995 ± 852 cpm/mg of dry cartilage for the 2.5-mm/90° probe. The $^{35}$SO$_4$ uptake for the control was 2647 ± 1380 cpm/mg dry cartilage.

Conclusions: The 2 probes tested created a well-controlled debridement with smooth edges and a defined margin of chondrocyte death that extended approximately 100 to 200 μm deep to the treatment area. There does not appear to be a significant effect on the metabolic activity of the chondrocytes adjacent to the treatment zone, but with the small sample size we lacked sufficient statistical power to definitively determine these effects.

Clinical Relevance: The 2 bipolar radiofrequency probes tested created a well-controlled debridement in normal articular cartilage with smooth edges and a defined margin of chondrocyte death that extended approximately 100 to 200 μm into the treatment area.

Key Words: Bipolar radiofrequency—Cellular viability—Cartilage debridement.
iatrogenic subchondral bone necrosis and chondrocyte death.

More recently, radiofrequency (RF) devices have been used to treat articular cartilage defects. Several studies performed on living or fresh tissue have attempted to evaluate the effects of RF devices on chondral cell viability. When standard histologic techniques were used on acute or chronic specimens, they appeared to have minimal or no detrimental effects on chondrocyte viability. Kaplan and Uribe reported similar appearance and showed no evidence of subchondral model and found that the latter gave a better histologic evaluation to determine if adjacent cells, which are

METHODS

Fresh adult bovine cartilage was used in this study from 1- to 2-year-old animals with closed epiphyses. The animals were killed and the knee joints were harvested en bloc without violation of the joint capsule. The knees were kept at approximately 4°C and delivered to our laboratories 3 to 6 days after death. These delays were necessary because of California regulations and logistic infrastructure.

Model Validation

A model validation study was performed to ensure that adequate chondrocyte viability was maintained during the 3- to 6-day period of storage before arrival at our laboratories. On arrival, the knee joints were opened under sterile conditions using a medial parapatellar arthrotomy. Full thickness cartilage specimens were harvested from the central, weight-bearing portion of the medial and lateral femoral condyles. Six specimens were harvested (one from each medial femoral condyle and one from each lateral femoral condyle). The cartilage specimens were then evaluated for chondrocyte viability using a dual staining protocol followed by confocal microscopy as described subsequently.

Experimental Model

After verifying that an adequate level of chondrocyte viability was maintained during the storage period, we proceeded to the experimental portion of the study. Three additional bovine knees were obtained as described previously. On arrival to our laboratories, each knee joint was exposed under sterile conditions using a medial parapatellar arthrotomy. Using a felt-tipped surgical marker, grids consisting of 6 1-cm² boxes were created on the medial and lateral femoral condyles to demarcate the treatment areas (Fig. 1). The distal femur was then isolated and submerged in a large sterile basin filled with 6 L of normal saline at a temperature of 28°C.

On each medial and lateral femoral condyle, the articular surface was treated as follows: Treatment areas 0a and 0b were treated with an ArthroCare (Sunnyvale, CA) 2.5-mm/90° probe; all 0a specimens were analyzed for chondrocyte viability (n = 6), and
all 0b specimens were analyzed for \(^{35}\text{SO}_4\) uptake \((n = 6)\). Treatment areas 1a and 1b were treated with an ArthroCare ACD-50 probe; all 1a specimens were analyzed for chondrocyte viability \((n = 6)\) and all 1b specimens were analyzed for \(^{35}\text{SO}_4\) uptake \((n = 6)\).

Treatment areas 2a and 2b served as control specimens in Fig 1. These areas were swept by an inactive ACD-50 probe in the identical fashion as the treated specimens. All 2a specimens were analyzed for chondrocyte viability \((n = 6)\) and all 2b specimens were analyzed for \(^{35}\text{SO}_4\) uptake \((n = 6)\). Additionally, one full thickness cartilage specimen measuring \(1 \times 0.2\) cm was harvested from each medial and lateral femoral condyle \((n = 6)\) and used as a negative control for \(^{35}\text{SO}_4\) uptake as described subsequently.

Radiofrequency Probes

The 2.5-mm/90° probe is a bipolar multi-electrode arthroscopic probe consisting of 7 active electrodes inserted inside an alumina ceramic spacer, placed inside a stainless steel return electrode \((304)\ 2.2\) mm in diameter. The tip of the device is oriented at 90° to the 2.4-mm stainless steel shaft. Each active electrode consists of a titanium wire 0.38 mm in diameter and 0.4 mm in length. The 2.2-mm diameter ceramic spacer separates the active electrodes from the return electrode by a 2-mm gap.

The ACD-50 probe is a bipolar arthroscopic probe consisting of a single active electrode separated from the return electrode by a silicone polymer spacer. The active electrode is made of a Molybdenum wire, 0.150 mm in diameter and configured in a reverse U shape 1.5 mm wide and 0.5 mm high. The silicone spacer separates the active electrode from the stainless steel return electrode \((2.2\) mm in diameter\) by a distance of \(0.9 \pm 0.3\) mm.

**Treatment of the Chondral Surface**

Treatment of the articular surface with the 2 RF probes was performed in a free-hand manner patterned after the technique of the senior orthopedist \((J.P.T.)\). The probe is used at a power setting of 4. It is oriented at approximately 60° to the articular surface and used in a light contact mode passing at a linear rate of 3 to 4 mm/s across the surface to affect volumetric debridement of the surface. Clinically, this is the technique used by the senior orthopaedist author to debride loose cartilage flaps or large fibrillations. The articular surface was kept entirely submerged in the saline bath during treatment. No fluid flow was used, in contrast to the usual arthroscopic environment in which these probes are used. Although the method was kept constant from sample to sample, ultimately the only control over variability in the amount of pressure, angle of operation, and duration of treatment of each area lies in the hands of the surgeon, just as in the clinical setting.

After each area had been treated, the distal femur was removed from the basin and returned to the sterile drape. Osteochondral blocks from each treatment area were isolated and extracted using an oscillating saw, and then full-thickness cartilage was carefully dissected free from the subchondral bone using a No. 20 scalpel blade. For the specimens designated for \(^{35}\text{SO}_4\) uptake testing, the cartilage was trimmed down to only the line of treatment and a margin of 2 mm circumferentially surrounding it. This margin was chosen to standardize the findings and to minimize variability that would be created if too much “healthy” cartilage were included that could mask the true effects of the RF probes on chondrocyte metabolic activity. Two millimeters was chosen based on previous
literature that suggests that up to 2 mm of cartilage surrounding the treatment zone may suffer chondral cell death.5-7

Viability Assessment

The cartilage tested for viability was first sliced into full thickness coronal sections approximately 500-μm thick using a No. 20 scalpel blade. The slices were then placed into staining solution that contains 2’,7’-Bis(2-carboxyethyl)-5(6)-carboxyfluorescein, acetoxymethylester (BCECF-AM), and propidium iodide. BCECF-AM is a fluorescein derivative that stains viable cells green. Propidium iodide is a cell nucleus stain that stains dead cells red. After approximately 40 minutes of incubation in the staining solution, cartilage slices were removed and evaluated with a Zeiss LSM 510 Laser Confocal Scanning Microscope equipped with a krypton and argon laser (Carl Zeiss, Thornwood, NY). During the validation phase of the study, cartilage was evaluated for overall chondrocyte viability. This was performed by randomly obtaining images from the superficial and deep regions of the cartilage. Seven images were obtained from each region in each specimen. Viable green cells and dead red cells were counted, and viability was then calculated for the superficial region, the deep region, and then overall per specimen.

In the experimental phase of the study, the cartilage specimens were evaluated under lower (5 ×) magnification to allow complete visualization of the treatment area and the adjacent zone of cell death. The Zeiss LSM 510 imaging software was used to superimpose an imbedded micrometer onto each image which allowed a calculation of the depth of cell death. Again, 7 images were obtained from each specimen, and the depth of chondrocyte death was measured and averaged. The point of greatest depth of chondrocyte death below the surface treatment was chosen in each image for our calculations.

Glycosaminoglycan Synthesis (35SO4 Uptake)

Glycosaminoglycan synthesis was quantified by the amount of 35SO4 incorporation in the sample tissue. As described previously, the specimens designated for 35SO4 uptake were first trimmed to include only the treatment zone and a circumferential margin of 2 mm surrounding this zone. These specimens were then incubated in tissue culture media containing 5 μCi/mL of 35SO4 for 48 hours at 37°C. After incubation, the tissue samples were washed with distilled water to remove unincorporated 35SO4, freeze-dried, and weighed. The tissue was hydrolyzed in 1N NaOH for 2 hours at 65°C, and the hydrolysate analyzed in a liquid scintillation spectrometer for quantitation of 35SO4 incorporation.9 The results were calculated and reported as counts per minute (cpm) per milligram dry weight. Six specimens per probe plus 6 positive and 6 negative control specimens were evaluated. The negative control specimens consisted of full-thickness cartilage slices measuring 1 × 0.2 cm (roughly the same size as the experimental specimens). These specimens were submerged in liquid nitrogen for 30 seconds to cause complete chondrocyte death. The negative control provides the background for zero uptake of 35SO4 because of cell death.

Statistical Analysis

Sample results are presented in the text as mean ± standard deviation. All values were subjected to statistical analysis using analysis of variance (ANOVA) with the level of significance at P = .05. The number of specimens in this study needed to ensure recognition of statistical significance was calculated10 and found to be 6.2. We have assessed 6 sites for each modality tested for statistical evaluation.

RESULTS

Control Study

The percent viability of the chondrocytes within the bovine articular cartilage is given, with the viability broken down by sample (animals 1, 2, and 3), location (medial or lateral femoral condyle), and cartilage region (superficial or deep) (Table 1). All samples were immersed for a minimum of 2 hours in culture media before staining.

The average cell viability varied from 69% to 96%. In all animals, the cell viability was higher in the superficial layers than in deeper chondral layers. This

<table>
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<th>Table 1. Viability of Fresh Bovine Articular Cartilage</th>
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<tr>
<td>Animal 1</td>
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<tr>
<td>Medial femoral condyle (MFC)</td>
</tr>
<tr>
<td>MFC superficial zone</td>
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<tr>
<td>MFC deep zone</td>
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<tr>
<td>LFC (lateral femoral condyle)</td>
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<tr>
<td>LFC superficial zone</td>
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<tr>
<td>LFC deep zone</td>
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<tr>
<td>Overall viability (both condyles)</td>
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<tr>
<td>Overall superficial zone</td>
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<td>Overall deep zone</td>
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may be explained by the fact that, although the animals were killed several days before the study, the superficial layers were still in direct contact with synovial fluid and the deeper layers were not. This study shows that use of freshly harvested bovine cartilage is a valid model to study the effect of RF devices. The confocal microscopic images in Fig 2A through C illustrate the superficial zones of articular cartilage of bovine knee samples (A) 1, (B) 2, and (C) 3, respectively. Panels D through F represent the deep zones of the articular cartilage of bovine knee samples (D) 1, (E) 2, and (F) 3, respectively. (Original magnification ×10).

Experimental Study: Radiofrequency Treatment

Gross Appearance: Both the ACD-50 and the 2.5-mm/90° RF probes provided tissue debridement. As seen in Fig 3, a shallow, narrow trough was created with each probe. The articular cartilage before treatment had a normal appearance (no surface fibrillations), and after treatment, the troughs had a smooth border in all samples, with no cracks or fissures.

Viability Assessment: Representative confocal microscopic images for the 2 probes tested are illustrated in Fig 4. These images show the margin of chondrocyte death surrounding the defects by the probes. In all samples an obvious area of superficial debridement and a surrounding rim of red-stained cells that represents the margin of chondrocyte death were seen. As in previous studies, the depth of chondrocyte death was calculated as the deepest margin seen in a given image. As seen in Fig 4, a peripheral rim of chondrocyte death extending to each side of the trough is also seen.

The ArthroCare ACD-50 probe had the smallest margin of chondrocyte death, which measured 109.4 ± 22.2 μm. The depth of chondrocyte death for the 2.5-mm/90° probe was 172.3 ± 34.3 μm (Table 2). The margin of cell death did not approach the subchondral bone in any of the samples tested with either probe (Fig 4). In the control viability specimens, no cell death or surface trough was seen, as would be expected.

Glycosaminoglycan Synthesis

The $^{35}$SO$_4$ uptake indicating glycosaminoglycan synthesis (metabolic activity) was assessed after debridement of the bovine articular cartilage with the 2 probes. Table 3 illustrates the mean values found after treatment with each probe, as well as the $^{35}$SO$_4$ uptake of the control, untreated cartilage.

$^{35}$SO$_4$ uptake for the control, untreated cartilage was 2,647 ± 1,380 cpm/mg of dry tissue. $^{35}$SO$_4$ uptake for the cartilage treated with the ACD-50 probe and the 2.5-mm/90° probe was found to be 2,584 ± 1,385 cpm/mg and 1,975 ± 852 cpm/mg of dry cartilage, respectively. The negative control specimen (cartilage submerged in liquid nitrogen for 30 seconds to cause complete chondrocyte death) was measured as 22 ± 12 cpm/mg dry cartilage.

No statistical significance at the level of $P < .05$ was detected when compared with the control. However, the higher than expected standard deviations resulted in a lower statistical power than our ad hoc calculations accounted for. Therefore, we are unable.
to conclude with certainty that the probes had no significant effect on chondrocyte metabolic activity.

DISCUSSION

Since their introduction, RF probes have become very popular with arthroscopists. This is largely because of their relative ease of use, efficiency in debridement, and the macroscopically aesthetic effects they have on the treated tissues. However, over the past few years, the safety of these devices has been questioned.

Initially, Turner et al. reported the effects of bipolar RF probe treatment on articular cartilage in an in vivo sheep model. These authors created a roughened articular surface that was then treated with either an arthroscopic shaver or an RF probe. After 0, 6, 12, and 24 weeks, the treated cartilage was evaluated histologically using a modified Mankin scale, and the authors reported significantly better results in the RF probe group at all time points. They noted no pathologic changes in the subchondral bone. Subsequently, Kaplan and Uribe reported similar benign findings. In this study, radiofrequency energy was used to treat the articular cartilage of human knees that were undergoing total knee arthroplasty. Histologically, the surface fibrillations of the cartilage were smoothed out, the chondrocytes appeared viable, and no deleterious change in the tissue architecture was observed. These authors concluded that RF energy was safe for use on articular cartilage.

However, in a subsequent series of studies, large margins of chondrocyte death were seen after treatment of the articular surface with RF probes. These authors used a viable staining technique followed by confocal laser microscopy to assess chondrocyte viability. The depth of cell death below the surface treatment penetrated as deep as 2 to 3 mm, even extending to the subchondral bone in some specimens. These studies were well designed and highly controlled. The probes were mounted on mechanical jigs that provided precise pressure application and duration of treatment along the articular surface. In reviewing these studies, the published confocal images showed a smooth surface with no evidence of tissue debridement, suggesting that the devices were used for surface “annealing” rather than debridement.

In the current study, the 2 probes tested (ACD-50 and 2.5-mm/90°) showed a well-defined zone of ablation with a consistent margin of chondrocyte death (100 to 200 μ; Fig 4). This is approximately one-tenth the depth of penetration seen in the previous studies.

The described study shows a number of differences from the previous studies. First, the RF probe treatment conditions were very different. The probes were used with a free-hand technique, simulating actual clinical use in contrast to the mechanical jig set up. Theoretically, this could affect variability, because obviously the only control over duration of application and amount of pressure lies in the hand of the operator. However, this potential limitation exists in the everyday use of these devices during arthroscopy. Furthermore, we actually found relatively little variability from sample to sample, as evidenced by the
relatively low standard deviations (109.4 ± 22.2 μm for the ACD-50 probe and 172.3 ± 34.3 μm for the 2.5-mm/90° probe), and this speaks to the reproducibility of our treatment method.

Secondly, in the current study, the probes were used to affect debridement of the articular surface as would be done for debriding a loose cartilage flap. In previous studies, published images show no clear debridement of the surface. This suggests that the probes were tested as annealing devices. If large margins of chondrocytes are killed, the smooth surface that is created by such treatment will degrade without cells remaining to maintain the extracellular matrix.13

Finally, the probes tested in the current study have

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<th>TABLE 2. Depth of Chondrocyte Death</th>
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<td><strong>2.5 mm/90°</strong></td>
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<tr>
<td>Animal 1 MFC</td>
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<td>Animal 1 LFC</td>
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<td>Animal 2 MFC</td>
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<td>Animal 2 LFC</td>
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<td>Animal 3 MFC</td>
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<tr>
<td>Animal 3 LFC</td>
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<td>Mean value ± SD</td>
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NOTE. Six sites were tested.

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<th>TABLE 3. ³⁵SO₄ Uptake for Glycosaminoglycan (GAG) Synthesis</th>
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<td><strong>Control</strong></td>
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<tr>
<td>LFC</td>
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<tr>
<td>MFC</td>
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<td>LFC + MFC</td>
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NOTE: Metabolic uptake of ³⁵SO₄ chondrocytes from articular cartilage treated with the 2.5-mm/90° probe and the ACD-50 probe, and adjacent control tissue (see Fig 1). Six sites were tested. Results expressed as mean values ± standard deviation.

*All results are expressed as counts per minute (cpm) per mg of dry cartilage.
not been assessed in previous studies. Any or all of these differences may account for the smaller margin of cell death seen in the present study.

With respect to the metabolic activity of the chondrocytes adjacent to the treatment zone, the RF probe treatment does not appear to cause a deleterious effect. However, as noted previously, because of the small sample size and the relatively large standard deviations seen with $^{35}$SO$_4$ uptake, we lack sufficient statistical power to express the results at the 95% level of confidence.

Given the various findings of all of the studies assessing RF devices, it is important to point out some limitations in applying these laboratory studies directly to the clinical setting. First, a wide range of RF probes exist, and individual probes clearly have different characteristics. Furthermore, a number of methods of application can be used (contact v noncontact, linear sweep v paintbrush, surface annealing v debridement). If one is to draw conclusions from this or any other RF probe study, these conclusions should only be applied to the probes tested and the conditions under which they were tested. For example, the probes assessed in the current study may have different effects if used in a different manner (such as a paintbrush stroke with a goal of annealing the surface). Further laboratory study is clearly warranted to further define the role of RF probes in arthroscopy and the methods with which they should be used.

Finally, we noted a paucity of clinical literature regarding the outcomes after RF probe treatment of articular cartilage defects of the knee. We found only one prospective study of RF probes. In a 2-year prospective analysis of RF versus mechanical debridement of isolated patellar chondral lesions in 2002, Owens et al,14 reported superior clinical outcomes after debridement of patellar grade II and III chondral lesions with a bipolar radiofrequency probe (20 patients) as compared with treatment with a mechanical shaver (19 patients). The patients were assessed using the Fulkerson-Shea Patellofemoral Joint Evaluation score.

In summary, further basic science studies are needed to assess the multitude of RF probes that are available and to better define the most appropriate use of such devices in arthroscopic surgery and in the treatment of chondral defects. Long-term, prospective studies should also be undertaken with magnetic resonance imaging or second-look arthroscopic evaluation to appropriately assess the effects on the articular cartilage. In the meantime, these devices should be used with caution in the clinical setting.

REFERENCES