The effects of radiofrequency energy probe speed and application force on chondrocyte viability

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Summary
Objective: To determine the thermal effects of monopolar radiofrequency energy (mRFE) on bovine articular cartilage when it was moved at different speeds and using varying application forces.

Methods: Thirty-six fresh osteochondral sections divided into two groups (18 sections/group) were used in this study. The first group was tested at three speed rates of mRFE probe (1 mm/sec, 5 mm/sec and 10 mm/sec) at a constant force (50 g) applied to the probe tip. In the second group, three application forces of the probe tip were tested (25 g, 50 g and 75 g) at a constant speed (5 mm/sec) (n=6/test). All tests were performed using a custom-built jig to control the mRFE (Vulcan EAS™) probe during a 20-mm pass on each section. After treatment, viability of osteochondral sections was determined by confocal laser microscopy (CLM) combined with vital cell staining.

Results: There were not any significant differences in cartilage thickness of tested osteochondral sections among the different speeds or forces. During the mRFE probe treatments at different speeds, CLM demonstrated that probe application at the speed of 1 mm/sec caused significantly greater chondrocyte death than at the speeds of 5 and 10 mm/sec, whereas there were no significant differences in chondrocyte death among the variable application forces (p > 0.05).

Discussion: This in vitro study demonstrated that RFE thermal penetration correlated most closely with probe application speed than application force for this mRFE probe.

Clinical relevance: Improper use of mRFE may cause thermal injury on articular cartilage.

Keywords
Chondrocyte, articular cartilage, radiofrequency energy, confocal laser microscopy, thermal chondroplasty

Original Research
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Introduction
Radiofrequency energy (RFE) has been used for thermal chondroplasty for over 10 years although experimental and clinical studies have demonstrated controversial results regarding the effects of RFE on articular cartilage (1–6). Chondrocyte death and its impact on long-term safety have been significant concerns after RFE thermal chondroplasty. Studies determining the temperature threshold of chondrocyte death have demonstrated that chondrocyte death begins at 52–54°C (7, 8). Currently, RFE manufacturers (Smith&Nephew, Mitek and ArthroCare) have continued to focus their efforts on developing new RFE probes and generator designs trying to limit chondrocyte death to a level less than that caused by mechanical debridement during chondroplasty. Some studies have shown significant progress in RFE probe design resulting in reduction of chondrocyte death following RFE thermal chondroplasty (9–11).

RFE thermal chondroplasty typically is performed through arthroscopy. Proper application of RFE is critical to safely accomplish the surgery and avoid collateral tissue damage. As with any surgical device, improper use can cause injury, especially with RFE. Previous studies have been performed to determine the effects of RFE treatment time and lavage temperature on articular cartilage during thermal chondroplasty (12, 13). However, since each surgeon uses a different RFE treatment pattern (RFE probe movement and force on cartilaginous surface), RFE application may result in variable outcomes with regard to chondrocyte viability during thermal chondroplasty.

In order to determine the optimal RFE probe’s speed and application force to limit chondrocyte death and to provide safety parameters for surgeons who use RFE for thermal chondroplasty, the purpose of this study was to determine the thermal effects of monopolar RFE (mRFE) on bovine articular cartilage when it was moved at different speeds and using varying application forces.

Materials and methods
Thirty-six fresh osteochondral sections from 36 normal bovine patellae (cows were euthanized one day before experiments) were used in this study. Only the central portion of each patella was cut into osteochondral section (length: 3 cm, width: 2 cm, thickness: 2 cm) which was tested in the study. All sections were divided into two groups (18 sections/group). The first group was tested at 3 speed rates of mRFE probe (1 mm/sec, 5 mm/sec and 10 mm/sec) at a constant force (50 gram) applied to the probe tip. In the second group, 3 application forces of the probe tip were tested (25 grams, 50 grams and 75 grams) at a constant speed (5 mm/sec) (n=6/test). All tests were performed using a custom-built jig to control the mRFE probe moving a 20-mm pass on each section at tested speeds and forces. The osteochondral sections were immersed in physiological saline solution (23°C) with a flow rate of 120 ml/min powered by a water pump (Materflex, Cole-Parmer Instrument Co., Chicago, IL) during the mRFE probe treatment. Vulcan EAS™ generator, coupled with an mRFE ceramic probe, which has a flexible tip to facilitate cartilage surface
contact and thus provides good accessibility to the tissue site (Fig. 1) (Smith & Nephew, Endoscopy, Andover, MA) was used to perform all treatments at 80 W in a cutting mode. A personal computer equipped with Vulrec software (Smith & Nephew, Endoscopy, Andover, MA) was used to record actual output powers and impedances of the mRFE generator during the treatment as previously reported (13).

After treatment, osteochondral sections were cut by band saw with phosphate-buffered saline solution irrigation to prevent frictional heat into small osteochondral blocks, including the area treated by RF with 0.5 cm of its associated subchondral bone. A low-speed saw (Buehler, Isomet 2000, Lake Bluff, IL) was used to cut the osteochondral block into slices with a thickness of 1.0 mm for analysis of chondrocyte viability by confocal laser microscopy (CLM) combined with vital cell staining (ethidium homodimer and calcein stains) as previously described (4, 14).

The 1.0 mm thick cartilage slices were stained by incubation in a 1.0 ml PBS containing 0.5 µl calcein (acetoxymethylster)/10 µl ethidium homodimer (Molecular Probes, Eugene, OR) for 30 minutes at room temperature. The method of determining the location of surviving cells was based on the knowledge that viable and nonviable cells differ in their ability to exclude fluorescent dyes. The cell membranes of dead, damaged or dying cells are penetrated by ethidium homodimer to stain their nuclei red. Living cells with intact plasma membranes and active cytoplasm metabolize calcein (acetoxymethyl ester) and show green fluorescence. The method of analysis utilized a 1.0 mm thick cartilage slice that was placed on a glass slide and moistened by several drops of phosphate buffered saline. A CLM (MRC-1024, Bio-Rad, Hemel Hempsted/Cambridge, England) equipped with a krypton/argon laser and the necessary filter systems (fluorescein: 522DF32 and rhodamine: 585EFLP) was employed using the triple-labeling technique. In this technique, the signals emitted from double-stained specimens can be distinguished due to their different absorption and emission spectra (15). All cartilage samples were examined blindly. The CLM was calibrated using a micrometer measured through the objective lens (2x) used for this project. The pixel length measured on images was converted to micrometers. The cartilage thickness of tested sections, maximum, average depths of chondrocyte death and ablated (tissue removal) depth were determined in each osteochondral section with a NIH software (NIH, Bethesda, MA).

Analysis of variance (ANOVA) was used to evaluate the parameters of cartilage thickness, chondrocyte death, ablative depth, power and impedance at different speeds or forces. When differences among the speeds or forces were demonstrated by ANOVA, Duncan’s multiple range tests were used to identify the differences among the cartilage thickness, depth (maximum or average) of chondrocyte death, ablative depth, power, or impedance within each speed or force. P-values less than 0.05 were considered significant. All statistical analyses were performed with a commercially available software program (SAS Version 8e, SAS Institute Inc., Cary, NC).

**Results**

There were not any significant differences in cartilaginous thickness of tested osteo-
chondral sections among the different speeds or forces (p>0.05).

During the mRFE probe treatment at different speeds, CLM demonstrated that the probe at the speed of 1 mm/sec caused significantly greater chondrocyte death (mean±SD, maximum: 272±18 µm, and average: 228±15 µm) than at the speeds of 5 (144±18 µm and 113±16 µm) and 10 mm/sec (120±39 µm and 96±31 µm) (Fig. 2), and also caused greater ablative depth (38 µm) than at the speed of 10 mm/sec (14 µm) (p<0.05). The mRFE output power and impedance at the speed of 1 mm/sec was significantly less and greater, respectively, than the probe with 50 (power: 10.4±0.4 W, impedance: 2625±43 ohms) and 25 g forces (power: 10.3±0.5 W, impedance: 2719±74 ohms) (p<0.05).

**Discussion**

This *in vitro* study demonstrated that chondrocyte death during thermal chondroplasty correlated more closely with probe application speed than application force for the mRFE probe. The tested mRFE probe also displayed a relatively safe treatment profile for thermal chondroplasty (chondrocyte death less than 150 µm) when it was applied at 5 mm/sec and 50 g application force under *in vitro* conditions.

In this study, slowly moving the mRFE probe at 1 mm/sec during treatment caused prolonged treatment time and resulted in greater chondrocyte death as previously reported (12). Some investigators have reported that chondrocyte viability decreased as RFE output power increased (13, 16). However, the mRFE probe used in this study moved at a speed of 1 mm/sec caused significantly greater chondrocyte death and ablation than at the speeds of 5 and 10 mm/sec, although it delivered less power with greater impedance. This result implied that slowing the RFE probe’s speed or extending RFE treatment time had greater effect on chondrocyte death than the output power and impedance.

For the application forces on the mRFE probe tip, 25 g could be considered as light contact of the probe on the cartilage surface whereas 75 g was firm contact. Different application forces may increase contact area between the probe tip and cartilaginous surface depending on probe tip design, especially for non-flat probe’s tip. A previous study demonstrated that larger RFE probe tips caused deeper thermal penetration than small ones (17). However, for the tested mRFE probe, applying different application forces did not cause significantly different thermal penetration. We hypothesized that the increased application force on the probe tip did not increase the contact area between probe tip and cartilaginous surface, since the tip design for this mRFE probe has a flat surface with ceramic insulation around the metal tip. Even with light contact of 25 g application force, the probe tip had a complete contact with the cartilaginous surface. The reason why the mRFE probe with 75 g application force had less output power and greater impedance compared to 25 and 50 g application forces may result from its firm contact, which reduced heat loss and convection during thermal treatment.

In addition, the mRFE probe tested in this study demonstrated less cell death during thermal chondroplasty compared to other mRFE probes tested in previous studies (4, 12, 14). This mRFE probe caused chondrocyte death less than 150 µm when it was applied at 5 mm/sec and 50 g application force. This depth is less or similar to expected depth of chondrocyte loss produced by mechanical debridement and shaving (18–20).

Several limitations in this study should be discussed. First, this *in vitro* study may not precisely reflect the mRFE probe in *in vivo* conditions. Second, the mRFE probe was tested using a jig, hence the results may not exactly reflect the probe used clinically by surgeons. Third, since mRFE device delivers power differently from bipolar RFE probe, the results of this study may not apply to bipolar RFE devices. Fourth, since the cartilage sections tested in this study were...
normal healthy cartilage with 2–3 mm thickness, the results may be different when diseased softened cartilage or cartilages with different thickness were tested.

In summary, the mRFE probe application speed during the thermal chondroplasty should be controlled and applied at a speed of a minimum of 5 mm/sec in order to limit chondrocyte death based on the results of this bovine patellar in vitro model.

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References

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