

Ex vivo investigation of the use of hydrothermal energy to induce chondrocyte necrosis in articular cartilage of the metacarpophalangeal and metatarsophalangeal joints of horses

Florien Jenner, Med Vet; Ryland B. Edwards III, DVM, PhD; Jessica R. Voss, DVM; Louise Southwood, BVSc, PhD; Mark D. Markel DVM, PhD; Dean W. Richardson, DVM

Objective—To evaluate the use of hydrothermal ablation of articular cartilage for arthrodesis in horses through investigation of the effects of joint lavage with physiologic saline (0.9% NaCl) solution (80°C) for various treatment times on chondrocyte viability in the articular cartilage of the metacarpophalangeal and metatarsophalangeal joints of cadaveric horse limbs.

Sample Population—7 pairs of metacarpophalangeal and 8 pairs of metatarsophalangeal joints from 8 Thoroughbreds.

Procedure—The horses were euthanized for reasons unrelated to musculoskeletal disease. On a random basis, 1 joint of each pair underwent intra-articular lavage for 5, 10, or 15 minutes with heated saline solution (80°C); the other joint underwent sham treatment of similar duration with saline solution at 22°C (control). Cartilage samples from the distal articular surface of metacarpus III (or metatarsus III), the proximal surface of the proximal phalanx, and the lateral and medial proximal sesamoid bones were assessed for chondrocyte viability via confocal microscopy and viability staining following enzymatic digestion.

Results—Compared with the control joints, findings of both viability assays indicated that the percentage of sites containing viable chondrocytes in heat-treated joints was decreased. Treatment hazard ratios of 0.048 (confocal microscopy) and 0.2 (digestion assay) were estimated. Histologically, periarticular soft tissues had minimal detrimental effects after heat treatment.

Conclusions and Clinical Relevance—Ex vivo intra-articular lavage with saline solution at 80°C resulted in the death of almost all articular chondrocytes in the joint. This technique may be a satisfactory method for extensive cartilage ablation when performing arthrodesis by minimally invasive techniques. (*Am J Vet Res* 2005;66:36–42)

fusion in the treated joint, the articular cartilage of the opposing bones should be removed.^{1,2} Complete removal of cartilage is important; remaining cartilage islands may persist within the bone bridges across the joint for several years.^{1,2} The most common technique for cartilage ablation has been mechanical removal, which can be achieved via drilling across the joint space or open curettage.^{1,2,15} In most joints, aggressive surgical exposure is needed to completely remove articular cartilage; however, some joints have surfaces that are anatomically inaccessible by use of practical surgical approaches. Chemically induced ankylosis by use of monoiodoacetate (an agent that blocks a specific enzyme pathway in chondrocyte metabolism) has been used as a less invasive approach for arthrodesis of the distal tarsal joints in horses. Although results of initial experiments in the distal intertarsal and tarsometatarsal joints were promising, investigators have since reported complications of the procedure, such as soft tissue necrosis around the injection site, septic arthritis, and necrotic tendonitis.^{3,9} More recently, a new technique involving the use of a neodymium:yttrium-aluminum-garnet laser for arthrodesis of the distal tarsal joints has been introduced, for which good results have been reported.^{5,15} The laser energy is believed to induce synovial fluid vaporization and secondary cartilage heating with subsequent chondrocyte death, but experimental validation of this is incomplete at this time.^{5,15} The effect of thermal energy on chondrocyte viability has been extensively investigated; researchers have evaluated the use of laser and radiofrequency energies for reshaping of cartilage during surgery of the head and neck^{16–18} and for thermal chondroplasty of articular cartilage.^{18–21} The effects of heat on chondrocyte viability are time and temperature dependent^{19,21,22}; however, chondrocyte viability is negatively affected at temperatures as low as 55° to 58°C, and it appears that chondrocyte metabolism is already affected at lower temperatures (45°C).^{19,21,22}

Whereas laser and electrosurgical energies are converted to heat as a result of tissue interaction,

Arthrodesis is a salvage procedure performed in humans and animals to iatrogenically induce joint fusion for relief of pain associated with severe degenerative joint disease or stabilization of a limb after loss of supporting soft tissue structures. To accelerate bony

Received March 18, 2004.

Accepted May 11, 2004.

From the Department of Clinical Studies, New Bolton Center, School of Veterinary Medicine, University of Pennsylvania, Kennett Square, PA 19348 (Jenner, Southwood, Richardson); and the Comparative Orthopaedic Research Laboratory, Department of Medical (Voss, Markel) and Surgical Sciences (Edwards), School of Veterinary Medicine, University of Wisconsin-Madison, Madison, WI 53706.

The authors thank Dr. Rick Birks, Dr. David Galligan, and Virginia Garcia for technical assistance.

Address correspondence to Dr. Jenner.

hydrothermal energy causes tissue damage via direct thermal transfer. This concept has been applied practically in hydrothermal endometrial ablation, a technique involving distension of the uterus with a saline solution at 70° to 90°C for 10 to 15 minutes at a pressure of 26 to 55 mm Hg as a treatment for women with menorrhagia.²³⁻²⁹ The purpose of the study reported here was to evaluate the use of hydrothermal ablation of articular cartilage for arthrodesis in horses through investigation of the effects of joint lavage with physiologic saline (0.9% NaCl) solution (80°C) for various treatment times on chondrocyte viability in the articular cartilage of the metacarpophalangeal and metatarsophalangeal joints of cadaveric horse limbs. Assuming that constant flow of heated saline solution through the joints would maximize heat transfer and provide a rapid thermal equilibrium, we hypothesized that this technique would result in consistent chondrocyte death.

Materials and Methods

Seven forelimb and 8 hind limb pairs were collected from 8 Thoroughbreds (mean age, 10.4 years; range, 2 to 25 years) that were euthanized for reasons unrelated to musculoskeletal disease (the forelimbs of horse 3 were not available for inclusion in the study due to their alternative dedication). Within 6 hours of euthanasia, fore- and hind limbs were severed through the proximal region of **metacarpus III (MCIII)** or **metatarsus III (MTIII)**, respectively, and immediately used for the experiments. One limb of each pair was randomly assigned to the treatment group, and the other was assigned to the sham-operated control group. In both groups, the metacarpophalangeal and metatarsophalangeal joints were allocated to receive treatment of 1 of 3 durations (5, 10, or 15 minutes). In the treatment group, intra-articular lavage of the joints was performed by use of saline solution (80°C) for 5, 10, or 15 minutes; in the sham-operated control group, intra-articular lavage of the joints was performed by use of saline solution at room temperature (22°C) for 5, 10, or 15 minutes.

Intra-articular lavage procedures—After aseptic preparation of the skin, 14-gauge needles were inserted into the palmarolateral (or plantarolateral) and dorsal pouches of the metacarpophalangeal (or metatarsophalangeal) joint and attached to a sterile closed pump circuit, consisting of a 4-L Erlenmeyer flask, 2 segments of tubing^a (outer diameter, 0.33 inches; inner diameter, 0.19 inches) with glued-on Luer-lock adapters, and a roller pump. A large reservoir of sterile saline solution was heated in the 4-L Erlenmeyer flask on a stirring-heating plate to maintain uniform temperature of the circulating fluid. The first 14-gauge needle was inserted into the palmarolateral (or plantarolateral) joint pouch and connected to the ingress tubing system. The second 14-gauge needle was inserted into the dorsal joint pouch and attached to the egress tubing that allowed flow of fluid back into the Erlenmeyer flask reservoir. A thermocouple probe^b connected to a thermocouple thermometer^c measured the temperature of the saline solution immediately before and after its articular passage. A fluid-filled pressure transducer^{d,e} was connected to the lumen of the ingress tubing and mounted at the level of the metacarpophalangeal (or metatarsophalangeal) joint to record the infusion fluid pressure and help maintain the mean pressure at < 70 mm Hg during lavage. The temperature and pressure sensors were calibrated prior to each experiment and connected to a computer equipped with data acquisition software.^f

Chondrocyte viability assay of cartilage samples after enzymatic digestion—Within 1 hour of the completion of the lavage, cartilage samples were aseptically excised by use of a 6-mm trephine from the lateral and medial aspects of the distal articular surface of MCIII (or MTIII), the lateral and medial aspects of the proximal surface of the proximal phalanx, and the lateral and medial proximal sesamoid bones. Each cartilage sample was minced and placed in 1.5 mL of a solution of collagenase D (0.5%; in Dulbecco's modified Eagle's medium). Samples were placed in an incubator at 37°C with 5% carbon dioxide and 100% humidity and constantly gently stirred until the extracellular matrix had been completely removed. The digestion process required 24 to 36 hours to complete. The resultant cell suspension was filtered through a 70- μ m nylon mesh cell strainer to eliminate any undigested tissue. To remove collagenase, the cells were washed twice with PBS solution containing penicillin and streptomycin (1% vol/vol) and centrifuged for 5 minutes at 400 \times g. The cell pellet was resuspended in 200 μ L of PBS solution. A 25- μ L aliquot was mixed with an equal amount of trypan blue, and the mixture was transferred to the chambers of a hemocytometer. Viable (unstained) and nonviable (stained) chondrocytes were counted. The percentage of cell viability was calculated by use of the following equation: number of viable cells (unstained)/total number of cells (stained + unstained) \times 100.

Chondrocyte viability assay of osteochondral specimens by use of confocal microscopy—Osteochondral specimens were aseptically excised by use of a 6-mm trephine from areas adjacent to the cartilage excision sites. These samples were placed in Dulbecco's modified Eagle's medium with 10% fetal bovine serum and 50 μ g of gentamicin/mL and placed on ice until confocal microscopy was performed. Staining to assess chondrocyte viability was performed by incubation of an undecalcified osteochondral section (5 \times 1.5 \times 2 mm) in 1 mL of PBS solution containing 1 μ L of calcein-acetoxymethylester and 10 μ L of ethidium homodimer-1^g for 30 minutes at 22°C. A confocal microscope^h equipped with an argon laser and fluorescein-rhodamine filter systems was used with a triple-labeling technique to allow signal distinction of the double-stained specimens. The images were displayed in red-green-blue mode. The number of dead (red) and live (green) chondrocytes in each specimen was determined by use of image analysis software.^{i,j} The percentage of cell viability was calculated by use of the following equation: number of viable cells (green)/total number of cells (red + green) \times 100.

Histologic evaluation of soft tissue specimens—For treated and control joints, full thickness samples of skin, subcutaneous tissue, ligaments, tendons, and joint capsule were obtained from several locations, including the palmarolateral (or plantarolateral) and the dorsal aspects of each joint at which the ingress and egress needles had been located. The samples were fixed in 4% buffered formaldehyde solution, dehydrated, embedded in paraffin, sectioned, and stained with H&E. Histologic analysis was performed by use of light microscopy.

Data analyses—The dependent variables were the percentage of viable chondrocytes calculated from results of confocal microscopic evaluation and digestion assay. For the digestion assay, the percentage of surviving chondrocytes was calculated for each site (6 sites/joint) on the basis of the cell counts of viable and nonviable chondrocytes (mean number of hemocytometer chambers evaluated, 10). For the confocal microscopic evaluation, the percentage of surviving chondrocytes was calculated for each site on the basis of the number of dead (red) and live (green) chondrocytes detected by use of image analysis software on digitized confocal

microscopy images. The percentage of surviving chondrocytes at each site constituted 1 observation and was included as such in the statistical analyses.

Because the percentage of viable chondrocytes was zero in 52% of confocal microscopic assay and 40% of the digestion assay observations, the variables were recoded to a dichotomous variable (presence or absence of viable chondrocytes) and the data were analyzed categorically. Each site (lateral and medial aspects of the distal articular surface of MCIII [or MTIII], the lateral and medial aspects of the proximal surface of the proximal phalanx, and the lateral and medial proximal sesamoid bones) in each joint (left forelimb, right forelimb, left hind limb, and right hind limb) was classified as having viable chondrocytes (> 0% viable cells) or having no viable cells (0% viable cells). The total number of sites with viable or nonviable chondrocytes in each treatment group (treatment and control) at each time (5, 10, and 15 minutes) was recorded and expressed as a percentage of sites with viable cells.

Statistical analyses were performed by use of computer software.^k A Cox proportional hazard model was used to access the effect of treatment (treatment vs control) over time on the presence or absence of viable cells. This analysis involved comparison of the change in chondrocyte viability over time (at 5, 10, and 15 minutes) between the 2 groups (treatment vs control). The analysis was stratified on horse to control for horse effects.³⁰ Limb (left vs right) and axis (forelimb vs hind limb) were randomly assigned to treatment group and time, and an assumption was made that there was no association between cell viability and limb or axis. The hazard ratio was used as a measure of treatment effect and was effectively the ratio of the percentage of sites with viable chondrocytes in treated joints to control joints. In this application, a hazard ratio of 1 would indicate that heat treatment had no effect on cell viability, compared with the effects of the sham treatment; a ratio < 1 would indicate that heat treatment had a lower percentage of sites with viable chondrocytes over time. A Pearson's correlation was performed between the percentage of viable cells calculated from findings of the confocal microscopic and digestion assays. A value of $P < 0.05$ was considered significant.

Results

In the treatment group, mean \pm SD temperature of the saline solution used for intra-articular lavage was $79.8 \pm 7.22^\circ\text{C}$; in the sham-operated control group, mean temperature of the saline solution used for intra-articular lavage was $25.5 \pm 4.87^\circ\text{C}$. The mean fluid pressure was maintained at < 70 mm Hg during lavage of all joints. Five limbs (2 fore- and 3 hind limbs) were lavaged for 5 minutes, 6 limbs (3 fore- and 3 hind limbs) were lavaged for 10 minutes, and 4 limbs (2 fore- and 2 hind limbs) were lavaged for 15 minutes; in the latter treatment group, no further

experiments were performed because of evidence of subchondral bone damage and the significant results following assessment of the data collected.

By use of confocal laser microscopy as well as viability staining after enzymatic digestion, viable chondrocytes were readily distinguished from dead chondrocytes (Figure 1). The percentage of viable chondrocytes in the treated joints as determined via confocal microscopy was $21.70 \pm 8.34\%$, $14.44 \pm 5.36\%$, and $6.55 \pm 3.29\%$ after 5, 10, and 15 minutes of intra-articular lavage, respectively. The percentage of viable chondrocytes in the sham-operated control joints as determined via confocal microscopy was $44.29 \pm 7.01\%$, $41.47 \pm 14.19\%$, and $43.85 \pm 6.09\%$ after 5, 10, and 15 minutes of intra-articular lavage, respectively. The percentage of viable chondrocytes in the treated joints as determined via digestion assay was $4.38 \pm 5.22\%$, $0.00 \pm 0.00\%$, and $0.00 \pm 0.00\%$ after 5, 10, and 15 minutes of intra-articular lavage, respectively. The percentage of viable chondrocytes in the sham-operated control joints as determined via digestion assay was $35.95 \pm 17.06\%$, $29.86 \pm 18.70\%$, and $37.34 \pm 23.80\%$ after 5, 10, and 15 minutes of intra-

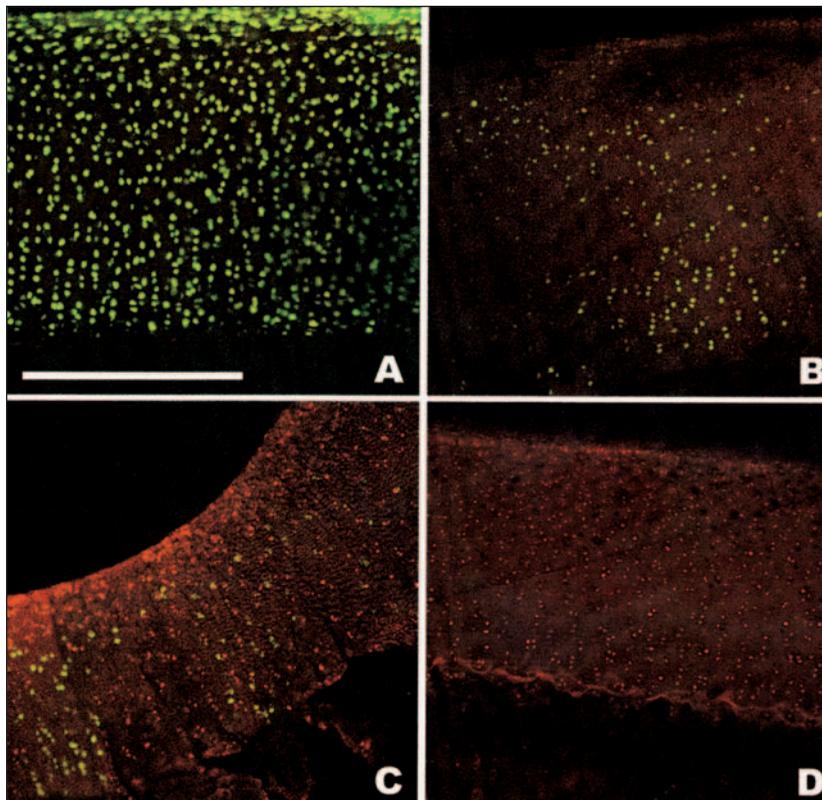


Figure 1—Photomicrographs illustrating the results of chondrocyte viability assessments via confocal microscopy of preparations of cartilage of the lateral and medial aspects of the distal articular surface of metacarpus III (or metatarsus III), the lateral and medial aspects of the proximal surface of the proximal phalanx, and the lateral and medial proximal sesamoid bones obtained from the metacarpophalangeal and metatarsophalangeal joints of cadaveric horse limbs after intra-articular lavage with physiologic saline (0.9% NaCl) solution at 80° or 22°C (control) for various treatment times. Dead chondrocytes appear red and live chondrocytes appear green. A—Cartilage preparation obtained from a sham-operated control metacarpophalangeal joint (treatment duration, 10 minutes). B—Cartilage preparation obtained from a metatarsophalangeal joint that was heat-treated for 5 minutes. C—Cartilage preparation obtained from a metatarsophalangeal joint that was heat-treated for 10 minutes. D—Cartilage preparation obtained from a metacarpophalangeal joint that was heat-treated for 15 minutes. Calcein-acetoxymethyl ester and ethidium homodimer-1 stain; bar = 500 μm (applies to all panels).

articular lavage, respectively. The correlation between the percentage of viable cells assessed via confocal microscopy and the digestion assay was good ($r^2 = 0.7$).

With both outcome measures, heat treatment decreased the percentage of sites with viable cells over time, compared with the effect of the control treatment. There was a significant association between treatment group and the percentage of viable cells determined via confocal microscopy and digestion assay.

Because the percentage of viable chondrocytes was zero in 52% of confocal microscopic assay and 40% of the digestion assay observations, the data could not be normalized and therefore were analyzed categorically with each site being categorized as having viable cells or having no viable cells. There was a significant association between treatment group and the percentage of sites containing viable cells determined via confocal microscopy and digestion assay. For the confocal microscopy and digestion assay outcomes, treatment hazard ratios of 0.048 ($P < 0.001$) and 0.2 ($P < 0.001$) were estimated, respectively. These indicated that the heat treatment resulted in a decrease in the percentage of sites containing viable cells by use of either assay method. The hazard ratio was not influenced by horse or site of observation.

Light microscopic evaluation of H&E-stained samples of ligaments, tendons, and joint capsules obtained from treated and control limbs revealed no histologic changes associated with lavage of 5- and 10-minutes' duration in the treatment group and no changes in any time subset of the control group. In the limbs undergoing heat treatment for 15 minutes, moderate thermal injury of the surface of the joint capsule was observed, as indicated by loss of synovial cell lining; however, ligaments and tendons appeared unaffected by the 15-minute heat treatment. No lesions in the skin and subcutaneous tissues were observed in any of the limbs undergoing heat treatment, regardless of duration of treatment. Although the effect was not quantified, findings of the evaluation of confocal microscopy images indicated that some subchondral bone cell death occurred in the 15-minute treatment.

Discussion

Arthrodesis is typically performed in association with joint resection or at least with removal of cartilage.^{1,2} It is believed that articular cartilage may inhibit vascular invasion and ossification and that bony fusion is most successful when 2 cancellous bone surfaces are aligned, compressed, and stabilized to imitate fracture healing.^{1,2,6,7} After cartilage ablation in several species, joint surface preparation has been performed mechanically (through furrowing the subchondral bone with an osteotome) or chemically (through brushing the articular surfaces with carbolic acid, zinc chloride solution, or iodine tincture), all with varying reported successes.¹⁻¹⁴

Arthrodesis of the distal tarsal joints in horses has been attempted by use of a recently developed technique involving laser energy, and results have been favorable; the applied laser energy (applied at 25 to 30 W in continuous mode to provide 800 to 1,000 J/joint)

is believed to induce synovial fluid vaporization, secondary cartilage heating, and subsequent necrosis of the chondrocytes.^{5,15} Auxiliary thermal effects such as collagen denaturation (resulting in increased joint stability because of contraction of the joint capsule and intertarsal ligaments), increased friction (resulting in less joint motion), and decreased pain perception by nerves in the joint capsule and subchondral bone are believed to possibly contribute to the success rate of the technique^{5,15}; however, there are no scientific data available to support any of these possible thermal side effects.

Unlike laser and electrosurgical energies that are converted to heat as a result of tissue interaction, hydrothermal energy causes irreparable tissue damage by direct thermal transfer; compared with the effects of the former energies, the use of hydrothermal energy should achieve thermal equilibrium more rapidly and result in consistent chondrocyte death throughout the entire treated joint. With the advent of laser- and radiofrequency-chondroplasty for treatment of chondromalacia, the effects of heat on chondrocyte viability have been the subject of many investigations in humans and animals, although, understandably, the emphasis has been on optimizing thermal effects while minimizing cell death.^{16,17,19,21,22,31-44} Osteochondral sections obtained from human and bovine joints have been exposed to heat baths at temperatures of 37° to 100°C for 5 minutes; cell viability assessment via confocal microscopy revealed that the majority of chondrocytes were killed when exposed to temperatures of 50° to 65°C for 5 minutes.^{21,31,43} However, the osteochondral samples evaluated in those experiments were small (10 × 5 × 1 mm or 10 × 1.5 × 4 mm) and completely immersed in hot saline solution. During joint lavage with heated saline solution, heat conduction from the articular surface into the depth of the cartilage matrix has to occur and a thermal equilibrium throughout the entire cartilage matrix would have to be achieved to obtain thorough ablation.

The hydrothermal ablation used in the present study would appear to combine several different mechanisms of articular destruction in a minimally invasive technique. In addition to chondrocyte death, hydrothermal ablation should result in decreased synovial fluid production secondary to synovial cell ablation and may induce thermal capsulorrhaphy and possibly thermal denervation. Thermal shrinkage of collagen is a phenomenon commonly used in the treatment of ligamentous laxity.^{31,44-48} The predominant collagen in ligamentous tissues and joint capsules is type I collagen, which is comprised of 3 polypeptide chains arranged in a triple helical conformation. Proline and hydroxyproline, which compose up to 25% of the collagen molecule, form intramolecular cross-links, thereby imparting stability to the triple helix.^{31,44-48} When collagen is heated, the thermolabile intramolecular cross-links are broken. The disruption of these stabilizing hydrogen bonds releases the molecular strands, which collapse. This collapse, like the release of a tautly held spring, results in a new contracted state called the denatured or random coil conformation of the collagen fiber.^{31,44-48} The collagen shrinkage has been deter-

mined to be exponentially dependent on treatment temperature and linearly dependent on exposure time.^{31,44-48} A critical temperature range of 65° to 75°C for the thermal modification of dense collagenous tissues with a plateau at 85°C has been reported, and a temperature of 75° to 80°C has been recommended for thermal capsulorrhaphy.^{31,44-48}

In cats, dogs, and rats, thermal energy has also been proven to be an effective means of neurolysis in various areas of the body.⁴⁹⁻⁵² Irreversible nerve blocks have occurred at 45°C in the brain,⁵⁰ and radiofrequency treatment has been used to produce isotherms of 45°C for experimental induction of neural tissue lesions.⁴⁹ All sizes of both myelinated and unmyelinated nerve fibers are affected by thermal destruction after exposure to temperatures of 45° to 75°C for 2 minutes. An additional hypothesis for the mechanism of pain relief associated with heating that has been reported after thermal annuloplasty or capsulorrhaphy is that there are temperature-dependent biochemical alterations in inflammatory agents.⁴⁹⁻⁵²

The fact that hydrothermal ablation only causes chondrocyte death and the cellular debris and matrix remain within the joint cavity is a potential weakness of this minimally invasive technique. However, recently, successful percutaneous arthrodesis without debridement of the joint was reported in rheumatoid ankles of humans and healthy femoropatellar joints of rabbits.^{6,7} To obtain bony fusion with a percutaneous arthrodesis technique, the articular cartilage has to be absorbed quite quickly. In studies^{6,7} of the femoropatellar joints of rabbits, a combination of synovial fluid depletion and immobilization of the joint resulted in not only chondrocyte death but also extensive absorption of cartilage matrix within 7 weeks.

In horses, debilitating conditions, such as severe degenerative joint disease of the distal intertarsal, tarsometatarsal, and proximal interphalangeal joints are the most common indications for arthrodesis.^{1,2,4,5,9-14} Although these smaller joints have been more frequently arthrodesed, there are situations in which more challenging sites such as the metacarpophalangeal and metatarsophalangeal joints require arthrodesis. The development of methods of articular cartilage removal that do not require extensive surgical exposure or drilling, such as hydrothermal ablation, might make minimally invasive internal fixation techniques and arthrodesis of joints that are difficult to access by use of practical surgical approaches more feasible. The metacarpophalangeal and metatarsophalangeal joints each have a capacious joint capsule; this feature might make the achievement of a thermal equilibrium during hydrothermal ablation more difficult, but it allows accurate and secure needle placements without the need for imaging modalities to confirm their intra-articular positioning. In the present study, the variable cartilage thickness throughout the metacarpophalangeal and metatarsophalangeal joint components in horses was useful in the assessment of heat penetration through the articular cartilage.

Trypan blue dye exclusion has long been used as the standard method of determining cell viability.^{20,21,30,31,40,43,53,54} This nonfluorescent cytoplasmic dye

penetrates the plasma membrane of dead cells but is excluded from living cells with functional plasma membranes. When viewed with a light microscope, dead cells appear blue, whereas live cells remain translucent. The fluorescent dye system commonly used in confocal laser microscopic evaluations stains live and dead cells differently on the basis of 2 recognized parameters of cell viability—plasma membrane integrity and intracellular esterase activity. Live cells are distinguished by the presence of ubiquitous intracellular esterase activity, determined by the enzymatic conversion of the virtually nonfluorescent calcein-ace-toxymethylester to the green-fluorescent calcein. The second component dye is the red-fluorescent ethidium homodimer-1, which enters cells with damaged membranes and binds to nucleic acids.^{20,21,30,31,40,43,53,54} Trypan blue dye exclusion and the fluorescent dye system used in confocal microscopic evaluations yield nearly identical results when both methods of cell viability determination are used to assess chondrocytes isolated by enzymatic digestion.⁴⁰

Storage temperature of cartilage samples has an important effect on chondrocyte survival. A rapidly decreasing viability rate for chondrocytes (to as low as 44% within 48 hours) was associated with storage at room temperature (25°C); in contrast, the proportion of viable cells in samples stored at 4°C was > 80% after 48 hours.⁵⁴

In our study, the number of nonviable chondrocytes in the sham-operated control joints was surprisingly high and may be attributed in part to the time that elapsed between euthanasia of the horses and evaluation of chondrocyte viability and to the duration of storage of the collected specimens at room temperature (22°C) in the course of the experiment. The high number of nonviable chondrocytes in the sham-operated control joints was even more noticeable in the trypan blue dye exclusion test, as only the cartilage samples assessed with this method were enzymatically digested and therefore maintained at a temperature of 37°C for 36 hours; in contrast, confocal microscopic evaluations of chondrocyte viability were performed on intact osteochondral samples, which were kept on ice.

The data obtained from the confocal microscopic and digestion assays in the present study were continuous, and an ANOVA is a more commonly used method of statistical analysis for this type of data; however, these data could not be normalized to perform an ANOVA because of the large number of zero values (0% chondrocyte viability). Therefore, the data were analyzed categorically with each site being categorized as having viable cells or having no viable cells. A Cox proportional hazard model was used to analyze the data because this type of analysis could take into consideration the categorical nature of the data as well as the random variable of horse. There was no association between sample site and the percentage of viable cells; therefore, the percentage of sites with viable cells was used as the final outcome variable. The limitations of analyses that include categorical rather than continuous data are a loss of statistical power and an increase in the chance of a type II statistical error (failing to detect a statistical difference when one exists); however, there was a significant difference between the groups over time.

In our study, confocal microscopic examination of articular cartilage samples after lavage of the metacarpophalangeal and metatarsophalangeal joints of horses with heated saline solution (80°C) for 5 minutes revealed viable chondrocytes, especially in the deeper layers of the articular cartilage; lavage periods of 10 and 15 minutes resulted in death of almost all the cartilage cells. Although the 15-minute lavage with heated saline solution resulted in the greatest amount of cell death, the signs of subchondral bone necrosis and periarticular soft tissue injury associated with that treatment combination (ie, duration of treatment and temperature of the lavage solution) do not permit its recommendation for clinical use. It appears that adequate thermal ablation of cartilage in the metacarpophalangeal and metatarsophalangeal joints of horses is likely to be achieved via a 10-minute lavage with saline solution heated to 80°C; this duration of treatment should not be exceeded if unnecessary bone and soft tissue damage is to be avoided.

Burn injuries result from an increase in tissue temperature that exceeds a threshold value for a period of time.⁵⁵ Although low-grade thermal injury to the articular and periarticular soft tissues might result in beneficial side effects such as capsulorrhaphy, thermal denervation, and inactivation of inflammatory agents, exceeding the threshold temperature can result in detrimental consequences, such as deactivation of enzyme systems, protein denaturation, or microvascular stasis because of platelet aggregation.^{31,44-52,55} The clinical manifestations of the morphologic, ultrastructural, and biochemical changes associated with temperatures in excess of the threshold value range from inflammation with hyperemia and edema formation to more severe complications, such as rupture of the periarticular ligamentous structures secondary to thermal modification of their collagenous components or soft tissue necrosis.^{31,44-48} Because the threshold for thermal damage is a first-order rate process that is dependent on exposure time and temperature, thermal injuries of a standard severity can be produced by progressively decreasing temperatures while the thermal insult period is logarithmically increased.^{22,55,56} Therefore, further evaluation of the in vivo effects of various time-temperature treatment combinations for use in hydrothermal ablation (such as those investigated in our study) might aid in limiting undesirable adverse effects.

Because cartilage is an avascular structure, it is limited in its heat transfer capacity, compared with that of other vascular tissues; however, the environment within cartilage should allow heat to be held in this tissue with relatively little fluctuation during thermal treatments.^{22,31,55,56} In vivo, structures adjacent to the cartilage may be additionally protected from thermal injury by the vascular circulation, which is known to help dissipate heat.^{29,55} Several studies^{23-29,55} have been performed to investigate the safety of hydrothermal endometrial ablation in sheep and women in vivo; solutions were heated to 60° to 90°C, and duration of treatment was 10 to 17 minutes, but the measured uterine serosal temperatures were 33.1° to 42°C (maximum measured temperature, 42°C).

However, prior to considering the clinical use of hydrothermal energy to achieve chondrocyte necrosis as an adjunctive measure for arthrodesis in horses, the

effects of this technique in live animals would have to be determined. It may be possible to minimize soft tissue thermal injury in vivo by simultaneous or sequential cooling of the surface of the treated limb in an ice bath. In vivo evaluation of this technique would also allow evaluation of external cooling as well as more direct assessment of the effects of thermal injury on the periarticular structures. In our opinion, hydrothermal cartilage ablation appears to provide a means with which to achieve consistent, uniform chondrocyte death throughout the articular surface of a treated joint via a minimally invasive, low-cost approach. The decreased soft tissue damage (compared with that associated with conventional ablation techniques) and the possible additional benefit of thermal capsulorrhaphy and neurolysis achieved via hydrothermal ablation may reduce morbidity rates associated with arthrodesis and contribute to the success of such procedures in horses.

- a. Masterflex, PharMed precision tubing (HV-06485-16), Cole-Parmer Instrument Co, Vernon Hills, Ill.
- b. Physitemp IT-14, Physitemp Instruments Inc, Clifton, NJ.
- c. BAT-12 microprobe thermometer, Physitemp Instruments Inc, Clifton, NJ.
- d. PT300 pressure transducer, Grass-telefactor, West Warwick, RI.
- e. 6600 signal conditioners-transducer amplifier, Gould Instrument Systems, Valley View, Ohio.
- f. Dataq DI-205 16-channel signal interface termination panel, Windaq Software, DATAQ Instruments Inc, Akron, Ohio.
- g. Live/dead viability/cytotoxicity kit, Molecular Probes L-3224, Eugene, Ore.
- h. Confocal laser microscope, MRC-1000 Bio-Rad, Hemel Hempstead, Herts, UK.
- i. Scion Image Beta 4.02 Win, Scion Corp, Frederick, Md.
- j. UTHSCSA ImageTool 3.0, UTHSCSA Dental Diagnostic Science, San Antonio, Tex.
- k. SAS software, SAS Institute Inc, Cary, NC.

References

1. Bramlage LR. An initial report on a surgical technique for arthrodesis of the metacarpophalangeal joint in the horse. *Proc Am Assoc Equine Pract* 1982;27:257-261.
2. Zamos DT, Honnas CM. Principles and applications of arthrodesis in horses. *Compend Contin Educ Pract Vet* 1993;15:1533-1541.
3. Bohanon TC, Schneider RK, Weisbrode SE. Fusion of the distal intertarsal and tarsometatarsal joints in the horse using intra-articular sodium monoiodoacetate. *Equine Vet J* 1991;23:289-295.
4. Caron JP, Fretz PB, Bailey JV, et al. Proximal interphalangeal arthrodesis in the horse. A retrospective study and a modified screw technique. *Vet Surg* 1990;19:196-202.
5. Hague B, Guccione A. Laser facilitated arthrodesis of the distal tarsal joints. *Clin Tech Equine Pract* 2002;1:32-35.
6. Lauge-Pedersen H, Aspenberg P. Arthrodesis by percutaneous fixation: patellofemoral arthrodesis in rabbits without debridement of the joint. *Acta Orthop Scand* 2002;73:186-189.
7. Lauge-Pedersen H. Percutaneous arthrodesis. *Acta Orthop Scand Suppl* 2003;74:1, 1-30.
8. Miller RA, Firoozbakhsh K, Veitch AJ. A biomechanical evaluation of internal fixation for ankle arthrodesis comparing two methods of joint surface preparation. *Orthopedics* 2000;23:457-460.
9. Penraat JH, Allen AL, Fretz PB, et al. An evaluation of chemical arthrodesis of the proximal interphalangeal joint in the horse by using monoiodoacetate. *Can J Vet Res* 2000;64:212-221.
10. Schaer TP, Bramlage LR, Embertson RM, et al. Proximal interphalangeal arthrodesis in 22 horses. *Equine Vet J* 2001;33:360-365.
11. Stanger P, Lauk H, Plocki K. Treatment of spavin by arthrodesis of the distal tarsal joint: longterm results. *Pferdeheilkunde* 1994;10:75-79.

12. Watt BC, Edwards RB 3rd, Markel MD, et al. Arthrodesis of the equine proximal interphalangeal joint: a biomechanical comparison of three 4.5-mm and two 5.5-mm cortical screws. *Vet Surg* 2001;30:287–294.
13. Watt BC, Edwards RB 3rd, Markel MD, et al. Arthrodesis of the equine proximal interphalangeal joint: a biomechanical comparison of two 7-hole 3.5-mm broad and two 5-hole 4.5-mm narrow dynamic compression plates. *Vet Surg* 2002;31:85–93.
14. Wyn-Jones G, May SA. Surgical arthrodesis for the treatment of osteoarthritis of the proximal intertarsal, distal intertarsal and tarsometatarsal joints in 30 horses: a comparison of four different techniques. *Equine Vet J* 1986;18:59–64.
15. Baxter G, Dechant J, Southwood L. Treatment of horses with distal tarsal osteoarthritis. *Compend Contin Educ Pract Vet* 2003;25:148–155.
16. Jones N, Sviridov A, Sobol E. A prospective randomized study of laser reshaping of cartilage in vivo. *Lasers Med Sci* 2001;16:284–290.
17. Sviridov A, Sobol E, Jones N. The effect of holmium laser radiation on stress, temperature, and structure of cartilage. *Lasers Med Sci* 1998;13:73–77.
18. Grogan S, Aklin B, Frenz M. In vitro model for the study of necrosis and apoptosis in native cartilage. *J Pathol* 2002;198:5–13.
19. Kaplan LD, Chu CR, Bradley JP, et al. Recovery of chondrocyte metabolic activity after thermal exposure. *Am J Sports Med* 2003;31:392–398.
20. Mainil-Varlet P, Monin D, Weiler C, et al. Quantification of laser-induced cartilage injury by confocal microscopy in an ex vivo model. *J Bone Joint Surg Am* 2001;83A:566–571.
21. Zuger BJ, Ott B, Mainil-Varlet P, et al. Laser solder welding of articular cartilage: tensile strength and chondrocyte viability. *Lasers Surg Med* 2001;28:427–434.
22. Diaz SH, Nelson JS, Wong BJ. Rate process analysis of thermal damage in cartilage. *Phys Med Biol* 2003;48:19–29.
23. Baggish M, Paraiso M, Breznock EM, et al. A computer-controlled, continuously circulating, hot irrigating system for endometrial ablation. *Am J Obstet Gynecol* 1995;173:1842–1848.
24. Bustos-Lopez HH, Baggish M, Valle RF. Assessment of the safety of intrauterine instillation of heated saline for endometrial ablation. *Fertil Steril* 1998;69:155–160.
25. Goldrath MH, Barrionuevo M, Husain M. Endometrial ablation by hysteroscopic instillation of hot saline solution. *J Am Assoc Gynecol Laparosc* 1997;4:235–240.
26. McGurgan P, O'Donovan P. Endometrial ablation. *Curr Opin Obstet Gynecol* 2003;15:327–332.
27. Perlitz Y, Rahav D, Ben-Ami M. Endometrial ablation using hysteroscopic instillation of hot saline solution into the uterus. *Eur J Obstet Gynecol Reprod Biol* 2001;99:90–92.
28. Romer T, Muller J, Foth D. Hydrothermal ablation. A new simple method for coagulating endometrium in patients with therapy-resistant recurring hypermenorrhea. *Contrib Gynecol Obstet* 2000;20:154–160.
29. Weisberg M, Goldrath M, Berman J, et al. Hysteroscopic endometrial ablation using free heated saline for the treatment of menorrhagia. *J Am Assoc Gynecol Laparosc* 2000;7:311–316.
30. Allison PD. *Survival analysis using the SAS system: a practical guide*. Cary, NC: SAS Institute Inc, 1995;158.
31. Edwards RB 3rd, Lu Y, Markel MD. The basic science of thermally assisted chondroplasty. *Clin Sports Med* 2002;21:619–647, viii.
32. Hojo T, Fujioka M, Otsuka G. Effect of heat stimulation on viability and proteoglycan metabolism of cultured chondrocytes: preliminary report. *J Orthop Sci* 2003;8:396–399.
33. Karamzadeh AM, Rasouli A, Tanenbaum BS, et al. Laser-mediated cartilage reshaping with feedback-controlled cryogen spray cooling: biophysical properties and viability. *Lasers Surg Med* 2001;28:1–10.
34. Keefe MWMD, Rasouli AMD, Telenkov SAP, et al. Radiofrequency cartilage reshaping: efficacy, biophysical measurements, and tissue viability. *Arch Facial Plast Surg* 2003;5:46–52.
35. Khan AM, Dillingham MF. Electrothermal chondroplasty—monopolar. *Clin Sports Med* 2002;21:663–674.
36. Lee EW, Paulos LE, Warren RF. Complications of thermal energy in knee surgery—Part II. *Clin Sports Med* 2002;21:753–763.
37. Lu Y, Edwards RB 3rd, Kalscheur VL, et al. Effect of bipolar radiofrequency energy on human articular cartilage. Comparison of confocal laser microscopy and light microscopy. *Arthroscopy* 2001;17:117–123.
38. Lu Y, Edwards RB 3rd, Nho S, et al. Lavage solution temperature influences depth of chondrocyte death and surface contouring during thermal chondroplasty with temperature controlled monopolar radiofrequency energy. *Am J Sports Med* 2002;30:667–673.
39. Lu Y, Edwards RB 3rd, Nho S, et al. Thermal chondroplasty with bipolar and monopolar radiofrequency energy: effect of treatment time on chondrocyte death and surface contouring. *Arthroscopy* 2002;18:779–788.
40. Rasouli A, Sun C, Basu R. Quantitative assessment of chondrocyte viability after laser mediated reshaping: a novel application of flow cytometry. *Lasers Surg Med* 2003;32:3–9.
41. Shellock FG, Shields CL Jr. Radiofrequency energy-induced heating of bovine articular cartilage using a bipolar radiofrequency electrode. *Am J Sports Med* 2000;28:720–724.
42. Shellock FG. Radiofrequency energy-induced heating of bovine articular cartilage: evaluation of a new temperature-controlled, bipolar radiofrequency system used at different settings. *J Knee Surg* 2002;15:90–96.
43. Edwards RB 3rd, Lu Y, Markel MD. Radiofrequency energy-induced heating of bovine articular cartilage using a bipolar radiofrequency electrode. *Am J Sport Med* 2001;29:263–266.
44. Arnoczky SP, Aksan A. Thermal modification of connective tissues: basic science considerations and clinical implications. *Instr Course Lect* 2001;50:3–11.
45. Hayashi K, Hecht P, Thabit G III, et al. The biologic response to laser thermal modification in an in vivo sheep model. *Clin Orthop* 2000;373:265–276.
46. Hayashi K, Markel MD. Thermal capsulorrhaphy treatment of shoulder instability: basic science. *Clin Orthop* 2001;390:59–72.
47. Moran K, Anderson P, Hutcheson J, et al. Thermally induced shrinkage of joint capsule. *Clin Orthop* 2000;381:248–255.
48. Wallace AL, Hollinshead RM, Frank CB. The scientific basis of thermal capsular shrinkage. *J Shoulder Elbow Surg* 2000;9:354–360.
49. Cosman E, Nashold B, Ovelman-Levitt J. Theoretical aspects of radiofrequency lesions in the dorsal root entry zone. *Neurosurgery* 1984;15:945–950.
50. Letcher FS, Goldring S. The effect of radiofrequency current and heat on peripheral nerve action potential in the cat. *J Neurosurg* 1968;29:42–47.
51. Smith HP, McWhorter JM, Challa VR. Radiofrequency neurolysis in a clinical model. Neuropathological correlation. *J Neurosurg* 1981;55:246–253.
52. Saal JA, Saal JS. Intradiscal electrothermal therapy for the treatment of chronic discogenic low back pain. *Clin Sports Med* 2002;21:167–187.
53. Rendal-Vazquez ME, Maneiro-Pampin E, Rodriguez-Cabarcos M, et al. Effect of cryopreservation on human articular chondrocyte viability, proliferation, and collagen expression. *Cryobiology* 2001;42:2–10.
54. Malicev E, Wozniak G, Velikonja N. Determination of optimal transport conditions for biopsies of human articular cartilage as well as for suspensions of cultured chondrocytes used in autologous transplantation. *Eur J Physiol* 2001;442:171–173.
55. Jiang SC, Ma N, Li HJ, et al. Effects of thermal properties and geometrical dimensions on skin burn injuries. *Burns* 2002;28:713–717.
56. Baldwin SA, Pelman A, Bert JL. A heat transfer model of thermal balloon endometrial ablation. *Ann Biomed Eng* 2001;29:1009–1018.