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Biomechanical And Metabolic Changes Within Rabbit Articular Cartilage Following Treatment With Radiofrequency Energy

Christopher Larry Horstman

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BIOMECHANICAL AND METABOLIC CHANGES WITHIN RABBIT ARTICULAR

CARTILAGE FOLLOWING TREATMENT WITH RADIOFREQUENCY ENERGY

By

Christopher Larry Horstman

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Christopher Larry Horstman

Approved:

_____   _____
Ronald McLaughlin   Lora Ballweber
Associate Professor,   Graduate Coordinator,
Interim Head,   Veterinary Medical Science
Department of Clinical Sciences
(Director of Thesis)

_____   _____
Steven Elder   Robert Cooper
Assistant Professor in   Professor,
Agricultural and Biological Engineering Associate Dean for Academic Affairs
(Committee Member)   (Committee Member)

_____
Gregg Boring
Interim Dean of the College of
Veterinary Medicine

Name: Christopher Larry Horstman

Date of Degree: December 10, 2005

Institution: Mississippi State University

Major Field: Veterinary Medical Science

Major Professor: Dr. Ronald McLaughlin

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Candidate for Degree of Master of Science

The effects caused to articular cartilage by the remote use of arthroscopically-delivered RF energy to soft tissues in the joint are unknown. This investigation reported the short and long-term effects of bRF and mRF energy on the biomechanical properties and metabolic activity of articular cartilage. In addition, the effect of Cosequin® therapy was addressed. Thirty New Zealand white rabbits were randomly assigned to one of two treatment groups (Group 1 - placebo; Group 2 - Cosequin®). Histopathology, cell viability, GAG synthesis, and mechanical function of the articular cartilage were compared between groups. Data were analyzed using a mixed model ANOVA ($p=0.05$). Immediate chondrocyte death was created by both RF devices. This damage was noted to be superficial and did not lead to the progressive deterioration of the extracellular matrix or mechanical function of the articular cartilage. Cosequin® therapy was unable to demonstrate significant differences compared to the control group.

DEDICATION

I would like to dedicate this project to my family (Lori Engel, Larry Horstman, and Kelli, Kevin, and Tremayne Mungin) and friends for always being there when I needed them and always believing in my dreams.

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TABLE OF CONTENTS

CHAPTER

LIST OF FIGURES

CHAPTER I

INTRODUCTION

Heat has been used in surgery since first described in the fourth century BC by Hippocrates. Since that time dramatic advances have been made that allow greater control in applying heat to both cauterize and cut body tissues. The evolution of electrosurgery has moved from the use of laser energy to radiofrequency (RF) energy. Clinical and scientific reviews of the use of radiofrequency energy in arthroscopy began to appear in the literature in the late 1990's. Since then many researchers and clinicians have evaluated the basic science and clinical outcomes associated with the use of RF on human and animal joint structures. This article will review the use of RF energy during arthroscopic surgery; addressing the basic science, the types of radiofrequency energy available, and the effects of RF energy on supportive structures (e.g., joint capsule, ligaments, etc.) and articular cartilage within the joint.

BASIC SCIENCE

Electrocautery refers to a modality in which the tip of an instrument is heated with an electric current. This causes the affected tissues to be heated from the outside inward via thermal conduction. Electrocautery is inexact in its application and can lead to widespread tissue damage. Electrosurgery, on the other hand, refers to the passage of a current through the tissue, causing it to heat from the inside out. The tip of the device

does not increase in temperature (except by heat transfer from the tissue). Electrosurgery provides a more controlled treatment with less collateral tissue damage.

Electrical current can either be direct (flows in one direction) or alternating. When applied to living tissues, direct current will depolarize some of the cell membranes resulting in muscle and nerve excitation. When current duration exceeds the capacity of the cell to repolarize, the cell enters a refractory period and activity ceases. If this persists long enough, cell death can occur. However, cell viability can be maintained if the intermittent current pulses are applied in a manner that allows for the interval repolarization of some of the cellular membranes.[1] D'Arsonval published an experiment in the late 1800's in which he passed alternating current through the human body.[2] He found that current with a frequency less than 10,000 Hz (1Hz = 1 cycle per sec) caused undesirable neuromuscular stimulation. However, when the frequency was increased to more than 10,000 Hz muscular contractions were no longer present. The power company sends out an alternating current of 110 volts with a frequency of 60 Hz. To convert this current to a desired voltage and frequency, a step-up transformer increases the voltage and a steady-state oscillating circuit increases the frequency to over 10,000 Hz. Amplitude modulation (AM) radiowaves fall into the 55,000-160,000 Hz range of the electromagnetic spectrum. This is the same frequency at which electrosurgical current is used and, thus, electrosurgery is often referred to as radiosurgery.

Electrosurgical or radiosurgical currents can be altered into different waveforms that will perform a variety of functions. Coagulation is accomplished with a "damped" wave. The damped current starts at a given voltage and gradually decreases in amplitude

until it returns to zero. This leads to a brief moment where there is actually no current. This on/off cycle is referred to as intermittent current. Cutting is achieved with a "rectified" wave. This is also an intermittent current, but the amplitude does not decrease as in the "damped" wave. Filtering can be used to convert an intermittent current into a constant one. Pollack described four factors that influence the degree to which tissues are damaged during radiosurgery: the surface area of the treatment electrode, the time that the electrode is in contact with the tissue, the power setting, and the type of current (e.g., damped, rectified).[3] Taking all of these factors into consideration, the most precise cut with the minimum amount of peripheral tissue damage will result from the optimum combination of limited contact time, power setting, treatment electrode surface area, and a fully rectified and filtered current. Thermal energy for radiosurgery can be delivered arthroscopically by use of laser or RF energy probes.

Laser vs. RF Energy

Three laser systems have been used in arthroscopic surgery: carbon dioxide (CO_2), neodymium:yttrium-aluminum-garnet (Nd:YAG), and holmium:yttrium-aluminum-garnet (Ho:YAG) laser systems.[4] The CO_2 laser was first used in the early 1980s and required gaseous insufflation of the joint with CO_2. It lost its popularity because of the cumbersome delivery system, the potential for subcutaneous emphysema, and its propensity to cause CO_2 laser-induced synovitis. The use of Nd:YAG laser energy is limited as it creates considerable collateral tissue damage and fracturing of the probe tip permits uncontrolled escape of damaging laser energy. In 1987, the Ho:YAG laser was introduced as the first fiberoptic-delivered laser beam for arthroscopic

application in a water medium. However, the start-up cost of a Ho:YAG laser system exceeds $100,000. Its use has become limited since it is more expensive to use than traditional instrumentation and it has not been found to significantly improve postoperative performance in human clinical trials.

The use of RF energy with arthroscopy was first described in the late 1990's. Unlike laser systems which use direct photostimulation of cellular ions to produce heat, RF devices pass a high-frequency, alternating current from an electrical generator to the probe tip and into the tissue, leading to ionic agitation and eventual frictional heating within the tissue. This heat may be lost from the tissue by heat diffusion (conduction), lost in the irrigating solution (convection), or diverted into the circulating blood. In addition, the heat generated by the RF probe exponentially decreases as the distance (r) from the electrode tip increases, by a factor of $1/r^4$.[5] Heat generated within the tissue is also directly influenced by the RF current intensity (I), by a factor of I^2, and the time (T) that the RF energy is delivered to the tissue. Therefore the heat generated varies as I^2T.[5] Radiofrequency energy can either pass from the probe tip to a grounding pad (monopolar) or it can pass between two points within the same probe tip (bipolar).

Types of RF Used Arthroscopically

Monopolar: The Vulcan EAS generator (Oratec Interventions Inc, Menlo Park, California) is the only monopolar RF (mRF) unit available for arthroscopic usage. The current generated by this unit passes through the connection cable, through the probe (active electrode), through the patient to a return electrode (indifferent electrode or ground pad), and then back to a return in the RF unit. When RF energy is applied at

greater than 300 kHz, intracellular and extracellular ions have insufficient time to physically move from one side of the cell to the other.[6] This causes molecular friction. Heat is produced within the tissues as a result of this friction (resistive heating) because the tissue has a greater resistance than other parts of the circuit. This differs from laser energy, which produces heat through direct photostimulation of cellular ions. Thus, molecular friction is the source of the heat generated with RF energy and not the probe tip itself.[7]

The mRF device also has the added benefit of having probes with thermometric tips that can measure the temperature generated by the unit. The temperature is measured 50 times per second, and the voltage of the system is automatically adjusted to maintain the set temperature. In addition, both the temperature and the wattage can be adjusted to the ideal setting for the situation. As with all RF devices, there are a variety of different probe shapes, sizes, and functions for the necessary treatment.

Bipolar: There are two bipolar RF (bRF) devices available for use in arthroscopic surgery, ArthroCare 2000 (ArthroCare Corp, Sunnyvale, California) and Mitek VAPR (Mitek Products, Westwood, Massachusetts). Bipolar RF units produce heat within tissues similar to mRF devices. However, bRF devices place both electrodes (active and ground) within the probe tip and do not require RF energy to course through the patient to create thermal changes. In addition, the manufacturers of ArthroCare claim that their system may also be used for ablative procedures using a process they call coblation ("cold ablation"). Coblation replaces the thermally damaging vaporization and pyrolysis of standard electrosurgery with molecular disintegration via a cold ablative

process most closely resembling that of exclimer lasers.[8] This is achieved by employing an electrically conductive fluid (e.g., isotonic saline) in the physical gap between the electrode and tissue. Once a sufficiently high voltage difference has been created between the electrode and the tissue, the electrically active conducting fluid is converted into an ionized vapor layer, or plasma. As a result of the voltage gradient across the plasma layer, charged particles are accelerated towards the tissue. At sufficiently high voltage gradients, these particles gain adequate energy to cause dissociation of the molecular bonds within tissue structures.[8] This molecular dissociation produces volumetric removal of tissue. However, due to the short range of the accelerated particles within the plasma, this dissociative process is confined to the surface layer of the target tissue. In this way, coblation enables volumetric removal of the target tissue without producing extensive collateral damage.

The power setting on the bRF device can be adjusted as with the mRF device; however, the temperature and wattage cannot be independently set by the operator. The bRF device also does not contain a thermometric probe tip, and thus the voltage used is constant. As with the mRF system, there are a variety of sizes and shapes of RF probes available for arthroscopic use.

EFFECTS OF RF ON THE JOINT CAPSULE

Instability of the human glenohumeral joint is a challenging problem facing orthopedic surgeons worldwide. Shoulder instability is a growing concern in many veterinary patients with fore limb pain localized to the glenohumeral joint. Traditionally, this condition has been treated with medical management or arthrotomy with surgical

stabilization. In the mid-90s, Thabit presented a multi-institutional study involving the treatment of human unidirectional and multidirectional glenohumeral instability with arthroscopically delivered laser energy.[9] This treatment was shown to visibly shrink the joint capsular tissue, and the patients were clinically improved over the six months of the evaluation. Following this investigation, researchers began to investigate the mechanical, biochemical, and biologic changes associated with laser and RF energy use on joint capsular tissue.

Joint Capsular Shrinkage

The magnitude of joint capsular shrinkage caused by RF energy application has been extensively evaluated in cadaveric specimens. It was discovered that increasing power (watts)[10-13] or temperature[11,14-17] led to a significant increase in tissue shrinkage. The tissue shrinkage was immediate, and its magnitude correlated directly with the increases in power or temperature.

RF energy has been shown to cause significant tissue shrinkage when the temperature setting of the unit is greater than 65ºC. Although significantly greater tissue shrinkage is noted when the temperature increases, it appears that there is a maximum amount of tissue shrinkage that can be achieved by the application of RF energy. No added benefit was noted when temperatures greater than 75ºC were used to shrink joint capsular tissue.[11,16,17] Histologically, significantly greater tissue damage was seen when temperatures greater than 75ºC were compared to 65ºC. [11,16,17]

In addition to controlling the temperature of RF energy, the power or wattage can also be regulated with mRF. It was noted that a significant increase in tissue lesion area

and lesion depth in joint capsular tissue was created when the power of the RF energy used was increased.[10] When mRF energy was applied at 65ºC and 10 W of power, the depth of tissue penetration was 0.95 ± 1.16 mm. However, when mRF energy was applied at 65ºC and 30 W of power, the depth of tissue penetration increased to 4.75 ± 1.25 mm.

In 2000, Lu et al. evaluated the effect of different treatment delivery patterns on tissue shrinkage.[18] It was noted that when joint capsular tissue was treated with mRF energy at a temperature of 70ºC and a power setting of 15 W there was no significant difference in the amount of tissue shrinkage when a grid-like treatment pattern was compared to a paintbrush-like pattern (27% ± 8.7% and 29% ± 7.9%, respectively). The grid-like treatment pattern consisted of multiple, single, linear passes over the treatment area while the paintbrush-like pattern consisted of diffuse treatment of the entire area.

When Osmond et al. compared laser energy to mRF energy, it was noted that the mean shrinkage of joint capsular tissue was not significantly different between 10 and 15 W of laser energy and 75ºC of RF energy (42%, 41%, and 45% respectively).[11] It was also noted that laser energy applied at 5 W and RF energy applied at 55ºC were not significantly different than controls.

Joint capsular tissues were also placed in fluid baths at different temperatures for different lengths of time to assess the effect that temperature had on tissue shrinkage, independent of laser or RF energy. The effects of temperature alone on joint capsular tissue have also been analyzed. Tissues treated at or above 65ºC resulted in significant tissue shrinkage, with no differences noted between tissues placed in the 75º and 85ºC

fluid baths.[15,20]   Naseef et al. did find significant shrinkage of joint capsular tissue at 60ºC; however, this effect was not noted until the tissue had been in the water bath for over 5 minutes.[15]   All effects at or above 65ºC were noted to be immediate. [15,20]

It appears that the effects of laser and RF energy on joint capsular shrinkage are thermally induced. These effects are temperature and power sensitive. In addition, there is a maximum limit to the amount of tissue shrinkage achievable. This maximum amount of tissue shrinkage can be obtained by either a grid or paintbrush treatment pattern. In addition, to increase the depth of tissue penetration created by RF energy one can maintain a constant temperature setting while increasing the power (wattage) of the RF delivery system.

Treatment Temperatures

Obrzut et al. noted that the recorded tissue temperatures, 0.3-mm below the probe, were 3.7º to 6.7ºC below the set temperatures of the RF device.[16]   Many authors have noted that tissue temperature decreases as the distance from the probe tip increases.[5,11,12,16]   Obrzut et al. also found that when RF energy was applied as high as 80ºC, the maximum temperature in joint capsular tissue 1.5-mm from the probe never exceeded 45ºC.[16]   Osmond et al. found mean tissue temperatures 0.3-0.5 mm below the probe to be between 55º and 65ºC when RF energy was applied at 65º-75ºC.[11]   Tissue temperatures 4.5 mm from the probe were not significantly different than controls when RF energy was applied.[16]

Shellock et al. noted that when the power setting of the bRF unit (Mitek) was increased, a subsequent increase in tissue temperature was found with 10, 16, and 20 W

of power leading to 50.0º-66.0º, 62.9º-70.7º, and 71.2º-76.8ºC, respectively.[12] The higher temperatures noted at each power setting were created by probes that had larger surface areas at the tip. The authors concluded that the surface area of the probe tip had a significant effect on tissue temperature, and larger probe tips generated higher tissue temperatures.

Recently, Lu et al. applied RF energy to joint capsular tissue in an artificial joint and recorded the fluid temperatures generated.[19] One mRF device (Oratec) and two bRF devices (ArthroCare and Mitek) were assessed in this investigation. All devices were used to deliver RF energy in both an intermittent and continuous fashion, with and without fluid flow. It was found that all fluid temperatures generated were below 40ºC when tissue was treated either intermittently (on for 10 seconds and off for 5 seconds for 5 treatments) or continuously (on for 3 minutes) under continuous fluid flow of 200ml/min. For intermittent treatment without flow, both bRF devices created significantly greater fluid temperatures than the mRF device. The ArthroCare (bRF) device was noted to create the highest tissue temperatures (46ºC at 55 seconds and 52ºC at 70 seconds). When continuous treatment was evaluated without flow, it was again noted, that bRF energy created greater fluid temperatures than mRF energy. The ArthroCare (bRF) device led to temperatures around 65ºC at 2 minutes and 80ºC at 3 minutes while the Mitek (bRF) device created temperatures around 52ºC at 3 minutes. In addition, fluid temperatures were still in excess of 75ºC 30 seconds after the ArthroCare (bRF) device was turned off. Overall, when RF energy was applied intermittently it led to lower temperatures than when applied continuously.

Tissue temperatures appear to be greatly affected by the amount of power used and the distance the tissue is located from the probe tip. An increase in power will generate higher tissue temperatures and as the distance from the probe tip increases the tissue temperature will decrease. In addition, when RF settings are equal (temperature, power, etc.), a greater surface area at the end of the probe tip will generate greater tissue temperatures. Finally, fluid temperatures can be affected by the manner in which RF energy is applied (continuous vs. intermittent) and whether or not there is continuous flow of fluids during RF energy application. It appears that intermittent treatment under continuous flow will generate the lowest joint fluid temperatures.

Microscopic Evaluation of Treated Tissues

As was stated previously, all effects created by RF energy in the joint capsule are visible instantaneously. These effects include significant tissue shrinkage that leads to a thickening of the joint capsular tissue. These changes are grossly evident for 30 to 60 days after RF application. After 60 days, however, the treated tissues grossly appear very similar to the control tissues.

Regardless of whether the joint capsule is treated with laser energy, RF energy, or the tissue is merely placed in a heated fluid bath, the resultant histological effects are similar. The following microscopic changes are evident with as little as 5 W of laser energy, 45ºC of RF energy, or 65ºC of heated fluid. The primary changes noted histologically include diffuse hyalinization and fusion of the collagen fibrils immediately after thermal treatment.[14,15,18,20-24] In addition, necrosis of fibroblasts is evident by pyknosis, karyorrhexis, and fragmentation of the nucleus. [14,15,18,20-24] Lopez et al. also

noted that as the temperature of RF energy increased the amount of collagen fusion and the percent area affected also increased (22.5% for 45ºC and 50.4% for 85ºC).[23] When transmission electron microscopy (TEM) was used to evaluate the ultrastructural changes immediately following joint capsular treatment, it was noted that the treated collagen began to increase in cross-section area, and the margins began to lose their distinct edges while maintaining a circular shape.[14,20,22,24-27] These fibrils also became more homogenous in size than those of the control tissues that had a variety of collagen fibril diameters. Additionally, the cross-sectional area of the collagen fibrils increased as the power/wattage of the energy device was elevated.[22,25] TEM also revealed that the treated tissue had a generalized decrease in cellularity, and the remaining intact fibroblasts contained pyknotic nuclei immediately after thermal treatment.[20,22,24,26] When CLM and a live/dead cell staining technique were used to evaluate RF treated tissues immediately after RF application, a clear demarcation was noted. No live cells were evident within the treated tissue, and the surrounding tissue was filled with live fibroblasts.[18]

Although many of the initial histological investigations involved cadaveric samples, the biological response following the application of laser or RF energy in the treated tissue was of great interest. Many studies noted that within the first week or two after thermal treatment an active capillary and fibroblastic response surrounded a distinct area of acellularity when the tissues were examined with light microscopy.[10,18,20,24,26] TEM revealed similar findings; however, the fibroblasts bordering the acellular region were large, filled with well developed endoplasmic reticulum (ER), and surrounded by finely secreted collagen fibrils.[20,24,26] When CLM was used for evaluation, Lu et al.

found only a few viable cells within the treated region during the first couple of weeks following RF application.[18]

Within one month after treatment, the inflammatory cells and active fibroblasts surrounding the treated tissue began to invade the acellular region and deposit collagen.[18,24,26,28] Ultrastructurally, TEM revealed increased cellularity within the treated tissues, and the cytoplasm of these fibroblasts contained more secretory vesicles than normal with a greater arrangement of rough ER, golgi apparatus, and mitochondria.[20,22,26] At six weeks following RF application, TEM revealed that the acellular region was completely incorporated with fibroblasts, and the large, swollen collagen fibrils were replaced by a homogenous array of small collagen fibrils.[24]

At two months following thermal treatment, histopathology revealed that the treated area had normal vascularity; however, the cellularity continued to be increased.[26,29] TEM revealed that the entire acellular region was covered with normal appearing collagen by 60 days after treatment.[26] When Lu et al. compared the biological response of joint capsular tissue treated with multiple linear passes (grid-like) to the diffuse treatment of a specific area (paintbrush-like), the grid-like treatment was entirely incorporated with viable cells (CLM) while the paintbrush-like treatment still had avascular areas six weeks following treatment.[18]

At three months after treatment, hyalinized collagen and necrotic cells were no longer evident histologically; however, the treated region still had increased cellularity.[18,21,24,26] Ultrastructurally, the collagen fibrils appeared normal, but there was still an increase in the number of active fibroblasts when compared to normal tissue.[24]

CLM revealed viable cells incorporating the entire treatment region by three months regardless if the tissue was treated in a grid-like or paintbrush-like manner.[18]

When tissues were evaluated at 6 months after thermal application, the fibroblasts appeared less active and the vascularity appeared to have decreased when compared to the tissues at three months.[24] There were, however, still a greater number of fibroblasts in the treated tissue than normal.[21,26] Fibroblasts and collagen also had a normal appearance when viewed with TEM at six months after thermal treatment, but the cross-sectional area of the collagen fibrils appeared to be uniformly smaller than controls.[26]

It appears that thermal heating of joint capsular tissue, whether with laser energy, RF energy, or a heated fluid bath, leads to the immediate damage of the collagen and fibroblasts in the treated area. Initially, the collagen fibrils swell and appear to fuse while the fibroblasts are severely damaged. Within a week, the treated tissue is surrounded by active fibroblasts. These fibroblasts begin to invade the acellular region approximately one month after treatment and lay down new collagen. Within 60 days the treated tissue begins to look more normal histologically, but continues to have an increase in cellularity as long as 6 months after treatment. Joint capsular tissue also appears to regain a more normal appearance sooner when it is treated in a grid-like pattern when compared to a paintbrush-like pattern.

Mechanical Changes Associated with Treated Tissues

The mechanical function of the treated joint capsular tissue became of utmost importance as changes in the joint capsule will dictate the overall function of the joint. When evaluating the mechanical function of the joint capsule, one must evaluate both its

structural and material properties. The relationship of these two tissue properties is important in determining whether the structural integrity and the intrinsic nature of the joint capsule can recover following thermal injury.

It was noted that when joint capsular tissue was treated with RF energy the tissue stiffness (resistance to load or stretching of the joint capsule) was initially decreased.[17,24] The decrease in joint capsular stiffness remained for the first two weeks after RF application, but returned by six weeks, and tissues evaluated at 6 and 12 weeks were not significantly different than controls.[24] When tissue was treated in a grid-like fashion, the tissue stiffness was significantly greater than tissue treated in a diffuse ("paintbrush") manner at six weeks after RF application; however, no other differences were noted at 0, 2, or 12 weeks in the study.[18]

The reported percent relaxation (which is a measure of how much an applied load relaxes the joint capsule with time when subjected to a constant deformation) of the joint capsular tissue has varied in published articles. Hecht et al. reported an initial increase in percent relaxation[24] while Lopez et al. reported no difference in percent relaxation between the control and treated tissues.[17] When calculating the percent relaxation, both studies subjected treated tissue to a similar stress-relaxation test where the tissue was stretched to a 10% strain.[17,24] Hecht et al. noted that the increase in percent relaxation of treated tissue was significant greater than control tissue at weeks 0 and 6, but the percent relaxation at weeks 2 and 12 was not.[24] In addition, Lu et al. noted that tissue treated in a paintbrush-like fashion had a significantly greater percent relaxation than tissue treated in

a grid-like manner immediately after RF application; however, no significant differences were noted at any other stage of the study.[18]

Tissue strength was not significantly different at any time when treated joint capsular tissue was compared to control tissue for 12 weeks after RF application; however, the tissue strength of treated tissue at 2 weeks after application was significantly less than that at 12 weeks.[24] In addition, Lu et al. noted that there was significantly less tissue strength at 0 and 2 weeks after RF application in tissue treated in a paintbrush-like pattern when compared to tissue treated in a grid-like manner; however, there were no significant differences between the two treatment patterns at 6 and 12 weeks.[18] No significant differences were noted between the treated and control tissues in any of the measured parameters at 12 weeks following RF application.[18,24]

Wallace et al. created stifle joint instability by moving the insertion of the medial collateral ligament (MCL) proximally and cranially on the tibia.[27] The MCL was then treated with RF energy to eliminate this created instability. The investigators then evaluated tissue creep, a time dependent increase in deformation, both cyclically (30 cycles at 5% ultimate stress of a normal MCL) and statically (following the 30 cycles the load was held constant for 20 minutes). It was noted that, immediately after RF energy application, the treated tissue had a cyclic creep strain that was three times that of normal MCL tissue and a static creep strain that was two times that of normal MCL tissue. In addition, low load stiffness was calculated from the ratio of load to deformation at the peak of the first cycle, and the treated tissue had a low load stiffness that was 25% that of a normal MCL.

Overall, treated tissues appear to lose tissue stiffness and may have a greater percent relaxation immediately after thermal treatment. Within six to 12 weeks, the mechanical properties of the treated tissues are regained to near normal strength. Tissue treated in a grid-like pattern regains mechanical function quicker than tissues treated in a paintbrush fashion. These mechanical changes correlate in time with the previously reported histological changes. These findings indicated that RF treated tissues may need to be immobilized for 6 to 12 weeks after surgery in order for the tissue to regain normal strength.

Biochemical Response of Treated Tissues

Hayashi et al. investigated the effects of laser treatment on collagen and the subsequent response of the treated collagen to trypsin digestion.[22] Trypsin is a proteolytic enzyme that will denature collagen fibrils only after the normal triple helix has been unfolded. Hayashi et al. noted that treated collagen not digested with trypsin and control tissues digested in trypsin had distinct α-bands of Type I collagen. However, when treated tissue was digested with trypsin there was a significant loss of these α-bands.[22] This indicates that laser treated collagen exposes cleavage sites for trypsin in the collagen molecule by unwinding the triple helix structure. This test may also be an indicator of the degree of collagen denaturation.

In a similar study, Hayashi et al. examined the molecular response of laser treated joint capsule for up to six months after the application of laser energy.[26] A significant decrease in the α-bands of Type I collagen were noted for the first 30 days after surgery. In addition, the trypsin susceptibility index (TSI) which was initially decreased began to

gradually return to near normal values.[26] This is indicative of the production of new native collagen. Hayashi et al. also noted an immediate decrease in collagen synthesis of the laser treated tissue immediately after application. This decrease was followed by a significant increase in collagen synthesis from days three to fourteen after treatment. From one to six months after laser treatment no significant differences were noted in treated and control tissue.[26] Hecht et al. is the only author to report the TSI of joint capsular tissue following RF application.[24] These results were similar to those following laser treatment where the TSI was significantly decreased for the first two weeks after RF treatment. However, the TSI subsequently increased until there was no significant difference between the TSI in the treated tissues at 6 and 12 weeks and the TSI of control tissues.

In general, it appears that changes in the joint capsule created by laser and RF energy are related to an unwinding of the collagen molecule. New collagen begins to be produced as early as three days after the insult. The amount of new, native collagen in the exposed area appears to be similar to pretreatment levels as early as one month post-treatment; however, from the previous data this collagen does not reach full strength until a later time.

EFFECTS OF RF ON ARTICULAR CARTILAGE

Many humans suffer from partial or full-thickness articular cartilage lesions that lead to significant pain and diminished function. Traditional treatment has focused on either treating the lesion with a mechanical shaving device or converting the partial-thickness lesion to a full-thickness lesion and allowing it to fill with fibrocartilage.

Newer treatment modalities have included the application of laser or RF energy to the damaged cartilage surface.[30-42]

Investigators have compared the changes that occur in the articular cartilage following the application of both types of RF energy (mRF vs. bRF).[35-38] CLM has been shown to be more sensitive than traditional light microscopy in assessing the acute changes in chondrocyte function following RF application.[33,35,36,40] Newer testing modalities recently appeared in the literature assessing the molecular changes that occur in RF treated articular cartilage.[42,43] To the author's knowledge, only one clinical trial has been published concerning the outcome following the use of RF energy to treat cartilage damage.[41]

Laser Energy

Many of the initial investigations related to the thermal treatment of articular cartilage involved electrocautery and laser energy. Rand et al. found that increasing the power of laser energy created an increase in the depth of the cartilage laceration; 25W = 11.4%, 82.5W = 57%.[30] Trauner et al. assessed the acute and chronic response of articular cartilage to Ho:YAG laser energy.[31] No evidence of tissue destruction was noted at exposures <50 J/cm2; however, at >80 J/cm2 there was consistent removal of cartilaginous tissue. Additionally, there was evidence of thermal damage 450-500μm below the articular surface and 250μm lateral to the treatment path within the articular cartilage. A linear relationship also was noted between the amount of energy used and the amount of tissue destruction. The chronic effects of laser application were assessed by artificially creating partial-thickness cartilage injuries that were either treated with

Ho:YAG laser energy or converted to a full-thickness lesion which was then treated with abrasion arthroplasty.[31] Samples were collected at 0, 2, 4, and 10 weeks after treatment. Histopathologic analysis revealed no healing of the laser treated defects. Chondrocyte necrosis extended greater than 900μm from the ablation craters caused by the laser application. In addition, chondrocyte cloning (partial-thickness) and granulation tissue (panus; full-thickness) was observed adjacent to the created lesions. These investigations demonstrated that although fibrillated cartilage could be smoothed with laser energy the heat produced lead to significant chondrocyte damage.

Device Settings

In 2000, Kaplan et al. investigated the acute effects caused in the articular cartilage by increasing the power setting/voltage of bRF.[34] Bipolar (ArthroCare) RF energy was applied to fresh human articular cartilage for three seconds at three separate power settings; 2 (133-147 volts), 4 (161-179 volts), and 6 (190-210 volts). Histologically, no significant changes were evident in the fibrillar component of the extracellular matrix or ground substance. In addition, the chondrocytes adjacent to each treated area appeared viable and similar to both control and areas remote to the treatment. The depth of cut created by the bRF probe gradually increased as the power setting increased (0.12 mm – setting 2; 0.23 mm – setting 4; 0.37 mm – setting 6) and settings 4 and 6 created scalloped excavation that was not present with setting 2. The authors concluded that RF energy was safe to apply to fibrillated cartilage since no discernible damage was created in the adjacent chondrocytes. In addition, the device was more

efficient when used in a non-contact manner. It was felt that the smooth surface created by RF application would help prevent further arthritis.

Lu et al. performed a similar project in which articular cartilage was treated with bRF (ArthroCare – settings 2, 4, and 6) until the fibrillated cartilage explants had a smooth surface.[36] When examined with standard H&E staining the chondrocytes appeared to have normal morphology; however, when the treated cartilage was stained with safranin-O the uptake was decreased within the superficial layer, and a clear demarcation was noted between the treated and untreated regions. In addition, CLM revealed that RF energy created immediate chondrocyte death, and some of the damage reached as deep as the subchondral bone (5 of 36 samples). It was also noted that as the power setting increased the area of chondrocyte death was wider and deeper.

Ryan et al. assessed the amount of proteoglycan synthesis and degradation in the articular cartilage of patellar explants from 11 horses following bRF treatment (Mitek – 20, 40, and 60 W).[42] In addition, cell viability and histopathology were evaluated. Chondromalacia was simulated on the patella, and the articular surface was treated with bRF in a "paintbrush" fashion until the surface was smooth. It was noted that treatment at all three settings significantly decreased proteoglycan synthesis, and the proteoglycan degradation increased with higher power setting. Additionally, treatment at 40 and 60 watts of power significantly decreased cell viability to 81% and 73% of normal tissue, respectively. All investigators concluded that increasing RF treatment energy levels created a dose-dependent increase in chondrocyte morbidity and mortality regardless of the testing modality.

Temperature

Thermal heating was the suspected cause of the chondrocyte damage observed in many of the RF investigations. In 2002, Edwards et al. used osteochondral sections from the femoropatellar joint of adult cattle to study thermal effects on articular cartilage.[38] Tip sensing fluoroptic thermocouples were placed at 200, 500, and 2,000 microns below the articular surface. Both bRF (ArthroCare, CoVac 50 – setting 2) and mRF (Oratec, TAC-C – setting 70ºC/30W) devices were tested with and without flow of saline at room temperature (22ºC). Temperature created by the bRF device was found to be consistently greater than the mRF device at all measured depths, regardless of fluid flow. Temperatures greater than 75ºC were noted at all depths during bRF application, and temperatures greater than 50ºC were noted at 200μm and 500μm below the surface during mRF treatment. Flow of saline did not affect the temperature of bRF at 200μm or 2,000μm below the surface, but did decrease the temperature at 500μm below the surface. When mRF was applied with fluid flow, greater temperatures were noted at 200μm and 500μm below the articular surface when compared to no fluid flow; however, this was not evident at 2,000μm. The authors concluded that the dissipation of heat created by the flow of irrigation fluids caused the temperature sensing probe in the mRF tip to increase the power level of the generator in order to maintain a constant temperature.[38] Cook et al. evaluated the effects of temperature culture media on chondrocytes and found that when the temperature was greater than 45ºC there was significantly less cell viability 24 hours after treatment.[43]

It appears that although temperatures decrease as the distance from the probe increases within the articular surface, most temperatures created by RF are much greater than the 45°C required to create severe chondrocyte damage. These elevated temperatures have been measured as far as 2 mm below the articular surface.

Testing Modalities

Many early studies of RF treatment used histological assessment and concluded that no damage occurred to the chondrocytes or the matrix within the articular cartilage.[32,34,39] However, newer testing methods and special staining techniques have challenged these conclusions.[33,35,40] CLM, along with a live/dead cell staining technique, has been use to assess chondrocyte viability even when cells appeared morphologically normal on histological examination.[33,36,38,40] The live/dead cell staining technique uses a combination of calcein and ethidium homodimer. The cell membranes of dead, damaged, or dying cells are penetrated by ethidium homodimer to stain the nuclei red. Living cells with intact plasma membranes and active cytoplasm metabolize calcein and show green fluorescence. All publications that have compared CLM to traditional hematoxylin and eosin (H&E) staining have found that significantly greater chondrocyte death/damage is detected with CLM immediately following RF application to the articular cartilage.[33,35,36,40] In addition, these authors have concluded that H&E may not be sensitive enough to detect functional from non-functional chondrocytes with normal histological morphology. Staining of the proteoglycan within the articular matrix with safranin-O, Masson trichrom, or alcian blue has also found distinct decreases in stain uptake within RF treated areas even when traditional light microscopy has indicated that

the chondrocytes have normal morphology.[33,36,38] Since chondrocytes are known to provide nutrition to the cartilage matrix, a decreased amount of proteoglycan staining likely indicates a decrease in the viability of chondrocytes despite normal morphology histologically. Therefore, when assessing acute damage of the articular cartilage, newer, more sensitive testing modalities are required to assess chondrocyte viability.

Monopolar vs. Bipolar RF Devices

Lu et al. used fresh bovine osteochondral sections to test the differences between bRF (Mitek, VAPR – setting V2:40 W; ArthroCare, 3.0mm/90º ArthroWand – setting 2) and mRF (Oratec, TAC-C – setting 55º/25 W).[37] Two separate treatment techniques were used in this investigation. First, a single pass at 1mm/sec was used with all devices to test the effects on the articular cartilage with and without contact between the probe and the surface. Second, an area (10 x 10 mm) was abraded with a specially designed tool and then treated in a "paintbrush" fashion to simulate clinical use. Scanning electron microscopy (SEM) revealed that all surfaces were smooth following RF treatment. However, when used in a contact manner, bRF resulted in significantly deeper penetration (ArthroCare 100%; Mitek 125%) of the articular surface and significantly more chondrocyte death (ArthroCare 128%; Mitek 100%) than the mRF (Oratec) device when evaluated with CLM. Bipolar RF (Mitek and ArthroCare) also penetrated the subchondral bone in nearly half (3 of 8) of the samples while mRF did not. When used in a non-contact fashion, visual smoothing was noted with bRF, but mRF had no effect. ArthroCare showed greater chondrocyte damage than Mitek with nearly all samples showing penetration to the level of the subchondral bone (7 of 8 vs. 3 of 8). When the

ArthroCare (bRF) device was used in a non-contact manner it led to 20% deeper and 25% wider damage than when used in a contact fashion. When the abraded surface was treated in a "paintbrush" manner, the bRF (ArthroCare 500%; Mitek 230%) treatment times were much shorter than the mRF. Although significant chondrocyte death was noted with all devices, bRF (ArthroCare 92%; Mitek 78%) penetrated the articular surface to a greater extent than mRF. Bipolar RF also penetrated a portion of the articular cartilage to the subchondral bone in all "paintbrush" treated samples where the mRF did not.

Edwards et al. conducted a similar study with human osteochondral explants obtained from patients undergoing total knee arthroplasty.[35] All grade 2 or 3 cartilage lesions were treated under arthroscopic visualization in a "paintbrush" fashion with either bRF (ArthroCare, CoVac 50 – setting 2; Mitek, 3.5mm VAPR – setting V2:40W) or mRF (Oratec, TAC-C – setting 70ºC/30W) energy until the articular surface was smooth. Bipolar RF (24 seconds) smoothed the surface faster than mRF (39 seconds), but lead to chondrocyte death at a significantly greater depth when evaluated with CLM (ArthroCare=2228 μm; Mitek=2819 μm; Oratec 737 μm). Bipolar RF also reached the subchondral bone in a significantly greater number of samples than the mRF (ArthroCare=6 of 10; Mitek=7 of 10; Oratec 0 of 10). Once again significant chondrocyte death was seen with all treatment devices.

When comparing the two types of RF energy it becomes evident that bRF energy results in greater treatment temperatures than mRF energy. Although this leads to decreased surgical time it also leads to significantly greater chondrocyte damage.

Despite the large discrepancy in the amount of damage created by the two devices, it is important to keep in mind that all devices created chondrocyte death when tested at the manufacture's recommended settings.

Chondromalacia Treatment

Turner et al. simulated chondromalacia in sheep and then subsequently managed the lesion arthroscopically with either a mechanical shaving device or bRF energy.[32] Animals were evaluated periodically for 6 months after surgery. Histologically, lesions treated with RF had 14% favorable responses and 36% favorable or neutral responses. The animals that received treatment with a mechanical shaving device had 0% favorable and 7% favorable or neutral responses. Additionally, chondrocyte damage to the subchondral bone was not evident, histologically, in the RF treated specimens. It was concluded that RF was superior to mechanical shaving.

Lu et al. performed a similar study in which they created a comparable lesion in mature sheep and provided either: no treatment or were treated. The treated lesions received mRF energy (Oratec, ORA-50 – setting 55ºC/15W) or were converted to a full-thickness lesion with curettage and microfracture.[33] Animals were evaluated periodically for 6 months after surgery. In addition to light microscopy all samples were evaluated with CLM and SEM. Cell viability (CLM) was largely unchanged in the control group at each time period. In contrast, the RF group had extensive chondrocyte death on day 0 that increased at week 2 and then remained constant throughout the remainder of the study. The area treated with curettage and microfracture had completely filled with fibrocartilage by week 24. Histologically, detrimental effects were noted in the

chondrocytes following RF treatment. The amount of chondrocyte damage and the lack of proteoglycan staining continued to increase throughout the study. SEM revealed that RF created a smooth surface that appeared to be melted at time 0 and continued to be smooth throughout the experiment. The authors concluded that the immediate chondrocyte death and progressive decrease in proteoglycan staining would eventually lead to complete loss of articular cartilage function despite the fact that the cartilage matrix was maintained, and the surface continued to be smooth throughout the experiment.

Gundel et al. compared the acute changes to the chondrocytes in the articular cartilage of adult sheep treated with bRF or mechanical shaving after the creation of a partial-thickness osteochondral lesion on the distal femoral condyle.[39] Treatment groups included: a rotary shaver; ArthroCare, CoVac 70; ArthroCare 2.5mm/90º; or no treatment. Samples were analyzed with light microscopy (H&E) for surface morphology, depth of tissue ablation, and amount of thermal damage. Lesions treated with RF showed no appreciable difference between the two treatment wands. Smooth concave defects with a variable amount of chondrocyte injury were noted adjacent to the defect, and the matrix appeared unaltered. The authors concluded that bRF had no immediate deleterious effects on cartilage when applied in this fashion.

Owens et al. treated 48 consecutive female patients with isolated patellar cartilage lesions who had failed a 6-month trial of conservative therapy.[41] A shaving device was used to mechanically debride the damaged cartilage, or bRF energy was use to smooth the fibrillated cartilage. Prior to surgery all patients had crepitus within the stifle. At the

2-year follow-up, 68% of the patients in the mechanical debridement group, compared to only 45% of the patients in the RF group, still had crepitus. No significant difference was noted between the two groups prior to surgery when a subjective scoring system for the evaluation of the femoropatellar joint was used; however, statistically greater improvement at both 1- and 2-year follow-ups was seen in patients that received RF treatment.

Clinically, it appears that treatment of chondromalacia with RF creates a smooth surface that is superior to the treatment with a mechanical shaving device as long as 2 years after surgery. However, severe, irreversible chondrocyte damage is created by the RF energy at the time of surgery. This leads to the depletion of the extracellular matrix that may eventually lead to the overall mechanical failure of the articular cartilage.

New Testing Modalities

Recently investigators have begun to assess the amount of proteoglycan synthesis and degradation in the articular cartilage following RF treatment. Ryan et al. assessed the amount of chondrocyte proteoglycan synthesis and degradation in the cartilage matrix of equine patellar articular cartilage that was treated with bRF energy (Mitek – setting 20, 40, and 60 W).[42] They found a significant decrease in proteoglycan synthesis following treatment at all three energy levels. In addition, proteoglycan degradation was significantly increased at 40 and 60 watts of power. However, this study only evaluated the acute effects of RF, and it is unclear as to whether this is an initial shock to the chondrocytes or if there are long-term detrimental effects.

Cook et al. assessed the glycosaminoglycan (GAG) and collagen content, type II collagen and matrix metalloproteinase (MMP)-13 immunoreactivity, and histopathology of cultured cartilage explants on days 0, 5, 10, and 20 following bRF treatment (Mitek 90W).[43] RF treated cartilage had reduced permeability and considerable histological damage. Most treated samples also had reduced collagen type-II staining and increased MMP-13 immunostaining. GAG content was largely unchanged throughout the study except for the samples treated in a diffuse "paintbrush" fashion at day 20. Chondrocyte viability was not significantly different between treatment groups.

CONCLUSIONS

Since the late 1990's RF energy has become very popular in human and veterinary arthroscopic surgery. RF energy is created at a similar frequency as AM radiowaves, and thus it is referred to as radiosurgery or electrosurgery. Monopolar and bipolar RF afford the surgeon a variety of options for the treatment of many orthopedic injuries. RF devices are much less expensive than laser energy devices and allow for greater control of collateral damage within the joint.

Application of RF energy leads to the immediate shrinkage of joint capsular tissue. This shrinkage is both temperature and power dependent with higher settings leading to greater tissue shrinkage. There does, however, appear to be a limit to the amount of tissue shrinkage attainable. Greater power settings also lead to higher tissue temperatures. However, tissue temperature decreases significantly as the distance from the probe tip increases. Histologically, it appears that there is immediate damage to the collagen and fibroblasts within the treated area. Thermal heating of the collagen

molecules leads to an unwinding of the normal triple helix and thus a loss of function. Within two months, however, the treated area looks more normal, histologically and ultrastructurally, as fibroblasts have filled the treated area with new collagen. Mechanically, an initial loss in tissue stiffness is restored 6 to 12 weeks after surgery. These mechanical changes coincide with the histological appearance of new collagen. Because of this initial loss in mechanical strength, treated joint capsular tissue should be protected for up to 12 weeks after application of RF energy.

Application of both bRF and mRF energy to the articular cartilage leads to significant and immediate chondrocyte damage. Increasing power leads to greater chondrocyte damage within the articular cartilage. Chondrocyte damage begins to occur at 45°C. When RF energy is used to directly treat the articular cartilage, all devices create temperatures greater than 45°C within the articular cartilage when used at the manufacture's recommended settings. Bipolar RF works at a higher power than mRF which leads to greater chondrocyte damage despite shorter surgical times. Clinically, it appears that patients who received RF treatment of their fibrillated cartilage respond more favorably than those that receive treatment with a mechanical shaving device. CLM and proteoglycan staining are more sensitive to functional differences within RF treated chondrocytes than traditional light microscopy. RF energy should be used with caution until further studies are conducted to devise an optimum temperature and power setting for arthroscopic use. If used, RF energy should be used at the lowest possible setting, for the shortest amount of time, to achieve the desired effects.

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CHAPTER II

CHANGES IN RABBIT ARTICULAR CARTILAGE FOLLOWING TREATMENT
WITH BIPOLAR OR MONOPOLAR RADIOFREQUENCY ENERGY AND WITH OR
WITHOUT COSEQUIN® THERAPY

INTRODUCTION

Radiofrequency (RF) energy is routinely used in conjunction with arthroscopy to
shrink exuberant joint capsular tissue, cauterize blood vessels, treat meniscal injuries,
smooth fibrillated cartilage, debride frayed cruciate ligaments, and to perform medial
meniscal release during the repair of cranial cruciate ligament deficient stifles.
Arthroscopically-assisted tibial plateau leveling osteotomy (TPLO) is gaining popularity
and becoming a well-recognized treatment for cranial cruciate disease in large breed
dogs. During arthroscopic surgery, the RF probe is often used in close proximity to the
articular cartilage and, in some cases, may incidentally contact the cartilage surface,
possibly creating damage or death to the chondrocytes and extracellular matrix.

Numerous research projects have documented the changes in the articular
cartilage resulting from the use of RF energy to treat cartilage defects.[1-13] Most of this
research, however, has focused on the acute effects of monopolar (mRF) or bipolar (bRF)
RF energy when applied directly to the cartilage surface (contact treatment).
Unfortunately, the results of these studies are conflicting and appear dependent on the
testing method used to detect cartilage injury. Kaplan et al. reported that cartilage treated

with bRF energy had no histological difference than non-treated cartilage when evaluated with a variety of staining techniques.[1] They concluded that RF energy was safe for use on articular cartilage. Studies in which articular cartilage was assessed with scanning electron microscopy (SEM) following the use of RF energy found that the surface was smooth and free from fibrillation or clefts when assessed up to 24 weeks following surgery.[2,3] However, Lu et al. and Edwards et al. reported that both mRF and bRF energy led to immediate chondrocyte death when the cartilage was evaluated with confocal laser microscopy (CLM) and a live/dead cell viability kit.[2,4] When evaluation was completed with CLM, chondrocyte death was observed as deep as the subchondral bone after direct application of RF energy.[2] In addition, bRF energy led to chondrocyte death 3-4 times deeper than mRF energy.[4] In another study, Lu et al. used both CLM and traditional light microscopic techniques (H&E and safranin- O) to compare articular cartilage treated with bRF energy.[5] No morphologic differences were observed in the chondrocyte nuclei stained with H&E in the RF treated cartilage. However, a clear demarcation between the treated and untreated regions was seen with safranin-O staining, and significant chondrocyte death was noted with CLM evaluation. Chondrocyte death was also noted to increase when more RF energy was applied. From this information it appears that the live/dead cell staining technique used with CLM may be superior to traditional light microscopy when assessing chondrocyte function immediately after application of RF energy.

To the author's knowledge, Lu et al. is the only group to date to evaluate the long-term effects of RF energy on articular cartilage.[3] A partial-thickness cartilage defect was

created on the trochlea of adult female sheep. The created defects were then treated with RF energy, converted to a full thickness lesion which was treated with curettage and microfracture, or left untreated. In the cartilage treated with RF energy, immediate chondrocyte death was noted with CLM. Furthermore, histological analysis found detrimental effects to the chondrocytes and proteoglycan concentration that progressed over the 24 weeks of the study. Though fibrillation or cartilage clefts were not seen in the RF treated tissues at 24 weeks, the authors concluded that the death of chondrocytes and the decrease in proteoglycan staining would inevitably lead to degradation and loss of cartilage function.

Recently, Cook et al. used *in vitro* techniques to study the cellular and biochemical changes in articular cartilage treated with RF energy.[6] It was noted that the glycosaminoglycan (GAG) content was largely unchanged, type-II collagen was significantly decreased, and matrix metalloproteinase (MMP)-13 was significantly increased in the treated tissue. In addition, significantly less GAG was released from diffusely treated cartilage when compared to focally treated cartilage. Chondrocyte cultures placed in tissue media greater than 45ºC were also found to have significantly less cell viability than controls.

Lu et al. recently assessed fluid temperatures created when RF energy was used to shrink joint capsular tissue in an artificially created joint.[14] They noted that fluid temperatures never exceeded 40ºC when tissue was treated under continuous flow at 200 mL/min. However, under no flow, treatment temperatures reached as high as 80ºC when RF energy was applied continuously for 3 minutes. However, the thermometry probe

used in this study was placed 1cm from the probe tip, and the joint was 25mL in size. It is known that tissue temperature decreases as the distance from the probe tip increases.[7,15-17] Most joints evaluated in veterinary surgery have structures that are much closer than 1cm from the probe tip; therefore, temperatures may reach much higher levels than those found in this study. These higher temperatures could potentially lead to chondrocyte damage *in vivo*.

Oral supplements containing glucosamine hydrochloride and chondroitin sulfate are reported to have a chondroprotective effect on cartilage and are commonly used to treat a variety of joint abnormalities in humans and animals.[18-28] Cosequin ® (Cosamin ®) is a commercially available brand of glucosamine hydrochloride and chondroitin sulfate that is often prescribed for use in dogs, horses, and humans. *In vitro* studies have supported the role of glucosamine as a modulator of cartilage metabolism that improves cartilage integrity through the stimulation of proteoglycan production.[18-20] Chondroitin sulfate, the most abundant GAG in the body, is especially important in articular (hyaline) cartilage as it aggregates with hyaluronic acid, other GAGs, and proteins to form proteoglycan macromolecules. These aggregate chains bind with collagen to form the characteristic resilient matrix of articular cartilage.[21] Studies have shown exogenous chondroitin sulfate can decrease interleukin-1 production, block complement activation, and competitively inhibit MMPs, thereby slowing degradation of cartilage and other joint tissue.[19,20] Other studies have shown, when Cosamin® DS was used in a rabbit stifle instability model, a significant decrease in the amount of moderate and severe cartilage lesions when treated rabbits were compared to those that did not receive treatment.[22,23] A

recent survey of small animal practitioners found that the average onset time to observed clinical efficacy following administration of glucosamine and chondroitin sulfate was approximately four weeks.[24] Although unproven, many investigators believe that the best results for chondromodulation are achieved in patients that do not have severe structural changes in the articular cartilage, and when viable chondrocytes are still present.[25,26] To date, the possible protective effects of glucosamine hydrochloride and chondroitin sulfate on articular cartilage have not been evaluated in patients undergoing arthroscopic surgery with RF treatment.

Currently, the short and long-term effects of mRF and bRF energy on the biomechanical properties and metabolic activity of the articular cartilage is controversial and requires further investigation. Additionally, the effect that RF energy has on articular cartilage when used in the joint to treat surrounding structures, without contacting the articular surface, has not been fully described. This study will document the effects on the biomechanical properties and metabolic activity of the articular cartilage following the remote use of mRF and bRF energy on the soft tissues within the stifle joint of rabbits. It will also evaluate the protective effects of Cosequin®, if any, on articular cartilage when RF energy is used during arthroscopic surgery.

MATERIALS AND METHODS

Experimental Animals

Thirty, New Zealand White rabbits were randomly assigned to one of two treatment groups. Group-1 consisted of 15 rabbits that received a normal rabbit diet

(placebo) for 4 weeks prior to surgery and throughout the study period. Group-2 consisted of 15 rabbits that received Cosequin® (2% by body weight) daily mixed into the food at processing for 4 weeks prior to surgery and throughout the study period. Each rabbit was subjected to arthroscopic stifle surgery using either mRF or bRF energy. This project was approved by the animal care and use committee at Mississippi State University.

Surgery

On the day of surgery, each rabbit was premedicated with ketamine (10 mg/kg body weight), midazolam (0.1 mg/kg body weight), and buprenorphine (0.015 mg/kg body weight), intramuscularly. An intravenous catheter was placed in the marginal ear vein prior to anesthetic induction, and Lactated Ringer's Solution (10 ml/kg/hr) was administered. Anesthesia was induced with mask induction and maintained with isoflurane in oxygen following endotracheal intubation. Both stifles were clipped and prepared for aseptic surgery. In one stifle joint (randomly selected), a 1.9 mm arthroscope with camera attached (Richard Wolf, 25°, 1.9mm Needle arthroscope with telescope, Medical Instruments Corp., Vernon Hills, Illinois) was inserted via a standard lateral portal and connected to a xenon light source (Richard Wolf, 180 W, Xenon Auto LP 5123 Light Source, Medical Instruments Corp., Vernon Hills, Illinois) and fluid pump. A medial instrument portal was then created. The infrapatellar fat pad was partially excised with a mechanical shaving device (Richard Wolf, Power Stick M3, Medical Instruments Corp., Vernon Hills, Illinois) to allow visualization of the distal femoral condyles. Then, under arthroscopic visualization, a mRF wand (Vulcan Micro

Ablator, 70ºC/40W, ORATEC Interventions, Inc., Menlo Park, California) was inserted through the instrument portal into the joint. The RF wand was then used for a total of 45 seconds in short (5-10 second) bursts to further remove the infrapatellar fat pad. A stop watch was used to record the total time the RF energy was applied. Taking care not to contact the articular surface, the wand was used in a "paintbrush-like" fashion. The instrumentation was removed, and the portals were closed with tissue glue. The same procedure was then performed on the contralateral stifle joint using a bRF wand (ArthroCare System 2000, Microblator 30 probe, ArthroCare Corp., Sunnyvale, California) on setting 2. Treatment devices were randomly assigned to each stifle (right vs. left) and each time (first vs. second). Total surgery time was recorded.

The rabbits were given meloxicam (0.5 mg/kg body weight, PO q 12 hr) during anesthetic recovery and, as needed, for the first 3 days postoperatively to control pain and inflammation. The rabbits were housed individually and allowed to exercise freely in their cages throughout the study period.

Sample Collection

Five rabbits from each group were humanely euthanatized on day 0 following surgery and at 4 and 14 weeks after surgery. The stifle joints were harvested bilaterally. Samples were also collected from unoperated hip joints and processed in the same manner to serve as controls for cell viability evaluation. A low-speed saw (Isomet Plus, Buehler Ltd., Lake Bluff, Illinois) was used to cut the distal femoral condyles into four equal osteochondral blocks. Phosphate-buffered saline (PBS) irrigation was used during cutting to prevent frictional heating of the samples. The four osteochondral blocks

collected from each joint were used for either histopathologic evaluation, analysis of cell viability (CLM), biomechanical testing, or evaluation of metabolic activity (GAG synthesis) within the treated cartilage.

Histopathology

The osteochondral blocks used for histopathologic evaluation were fixed in 10% neutral-buffered formalin, decalcified with Kristensen's solution, and sectioned prior to embedding in paraffin. Samples were then cut into 5μm thick sections and stained with hematoxylin and eosin to evaluate chondrocyte cytoplasmic and nuclear morphometry and shape. Adjacent sections were then stained with safranin-O to evaluate the proteoglycan within the extracellular matrix. Samples were evaluated with a modified Mankin scoring system to detect differences between the treatment groups relative to percent area of cartilage affected; necrosis of the superficial, intermediate, and deep cartilage layers; hyalinization of the matrix; and loss of safranin-O staining. (Figure 1) During evaluation, the area with the greatest damage was scored. Scores recorded for each criteria were then added together to create a total histological score. All samples were evaluated by two pathologists blinded to the treatment group from which the samples were collected. Mean scores from the two pathologists were used for analysis.

Cell Viability

The osteochondral blocks used to assess chondrocyte viability were placed in physiologic buffered saline (PBS) until testing with confocal laser microscopy. Testing was completed within four hours of the rabbits being euthanized. The osteochondral

<u>Modified Mankin Scoring System</u>

Percent area of cartilage affected
    0 = none
    1 = 10-30%
    2 = 30-60%
    3 = 60-100%
Necrosis of chondrocytes in superficial layer
    0 = absent
    1 = mild
    2 = moderate
    3 = severe
Necrosis of chondrocytes in intermediate layer
    0 = absent
    1 = mild
    2 = moderate
    3 = severe
Necrosis of chondrocytes in deep layer
    0 = absent
    1 = mild
    2 = moderate
    3 = severe
H & E (Hyalinization of extracellular matrix)
    0 = absent
    1 = mild
    2 = moderate
    3 = severe
Safranin-O (Loss of staining)
    0 = absent
    1 = mild
    2 = moderate
    3 = severe

Total Histopathologic Score
    0 (normal) – 18 (complete loss)

Figure 1 – Modified Mankin Scoring System

blocks were removed from the PBS solution and incubated with 1.0 mL of PBS containing 0.4 µL calcein (acetoxymethylester) per 13 µL ethidium homodimer (Live/Dead Viability/Cytotoxicity Kit [L-3224], Molecular Probes, Eugene, Oregon) for 30 minutes at room temperature. The ethidium homodimer penetrates the cell membranes of dead, damaged, or dying cells and stains the nuclei red. Living cells with intact plasma membranes and active cytoplasm metabolize the calcein and fluoresce green. All samples were examined with a confocal laser microscope (Leica Microsystems Inc., Exton, Pennsylvania) equipped with an argon laser and the necessary filter systems to assess the depth of chondrocyte death created by the RF energy. Measurements of the cartilage thickness and the depth of chondrocyte death were acquired in three separate sites within the samples to determine the percentage of cartilage damage. The mean of the three measurements was used for statistical analysis. All samples were evaluated without knowledge of the treatment each received. Samples collected from untreated hips (controls) were incubated and measured in the same manner.

Biomechanical Testing

The osteochondral segments used for mechanical testing were wrapped in a saline moistened paper towel and stored at -70ºC until the time of testing. On the day of testing, the osteochondral segment was thawed at room temperature and mounted to the custom testing jig with cyanoacrylate glue. The cartilage and underlying subchondral bone underwent compressive indentation testing on a stepper motor-driven micromechanical testing machine (Mach-1, Biosyntech, Montreal, Canada) stationed in a 37ºC incubator.

Prior to testing, the samples were submerged in 37ºC saline and allowed to equilibrate for 10 minutes. Samples were subjected to a 5-segment stress relaxation test. Each segment was composed of a 30µm ramp indentation at 15µm/second. This position was maintained until the slope of the force verses time curve fell below 1 gram/minute. The force at this point was used to calculate equilibrium stress, and the test immediately proceeded to the next segment. Thus the total indentation was 150µm, achieved in a stepwise fashion with stress relaxation occurring at each step. Equilibrium stress was plotted against applied strain, and the slope of this curve was taken as the equilibrium compressive modulus. The equilibrium compressive modulus was recorded at three separate, random locations within each sample. All indentions were made perpendicular to the cartilage surface. All samples were tested by the same individual who was blinded to the diet and device used.

After indentation testing was completed, osteochondral bone blocks were decalcified and sliced (perpendicular to the surface) through the testing sights which were previously marked with a tissue dye. Cartilage slices were digitally photographed next to a ruler with 1-mm gradations. Imaging software (Scion Image, Scion Corp., Frederick, Maryland) was used to calculate the thickness of the cartilage. Measurements were performed manually using a line tool, with the scale set using the ruler marks. Five separate measurements were taken at each testing location and averaged to acquire the cartilage thickness at each site. These cartilage thickness measurements were used in the calculation of strain (displacement divided by cartilage thickness).

Metabolic Activity

A stock solution of culture media was made by placing 50mL of adult bovine serum into a 500mL vial of Ham's nutrient mixture. One milliliter of the culture media was placed into each well of a 24-well plate. The cartilage from the final osteochondral block was removed from the subchondral bone with a scalpel blade and place individually in the wells for transportation prior to digestion. GAG synthesis testing was performed as previously described.[29] Briefly, the cartilage samples were incubated for 4 hours at 37°C in a medium containing 20 µCi/mL ^{35}S aqueous sulfate. Radioactive medium was removed and replaced with 500µL detergent lysis buffer. DNA was quantified from an aliquot of the lysis buffer using the Hoescht dye procedure (DNA-QF, Sigma-Aldrich, St. Louis, Missouri) using a Vicam Series 4 fluorometer with calf thymus DNA as a standard. Residual lysis buffer was removed and replaced with 1% weight/volume papain in 50 mM sodium acetate (pH = 6). Samples were incubated overnight at 60°C, and the macromolecular ^{35}S was separated from unincorporated label using sephadex desalting columns (PD-10 columns, Amersham Biosciences, Pittsburg, Pennsylvania). Four milliliters of scintillation fluid was added to each elution fraction, and radioactivity was determined by liquid scintillation counting (Beckman Coulter Scintillation Counter, GMI, Inc., Albertville, Minnesota). Macromolecular incorporation was taken as the area under the first peak of the elution curve. Incorporation, in disintegrations per minute (DPM), was normalized to DNA content. All samples were tested without knowledge of the diet or device used.

Statistical Analysis

The effects of Cosequin, type of RF probe, and time after surgery on measures of cartilage health were analyzed using a mixed model analysis of variance (ANOVA) for a repeated measures design with one within-subject factor and two between-subjects factors in a factorial arrangement. The within-subject factor was the type of RF probe (mRF vs. bRF), and the between-subjects factors were the drug regimen (Cosequin vs. placebo) and the time after surgery (0, 4, and 14 weeks). The ANOVA was performed using the SAS procedure MIXED.[30] If significant effects were found, means were separated using the least significant difference test. The clinical importance of statistically significant differences were assessed using confidence intervals.[31] The percentage of chondrocyte death (0-25, 26-50, 51-75, >75%) was further divided into four subgroups and analyzed using Fisher's exact test. All calculations were performed using the SAS System for Windows, Version 9.1 (SAS Institute Inc., Cary, North Carolina); all statistical tests used the 0.05 level of significance.

RESULTS

Rabbits weighed an average of 3.7 kg (range, 3.2 – 4.1 kg) in group-1 (normal diet) and 3.7 kg (3.2 – 4.3 kg) in group-2 (Cosequin® therapy). The average total surgery time for rabbits in group-1 was 30.4 minutes (range, 15 – 81 min.) and 31.3 minutes (14 – 82 min.) in group-2. The total time that the probe was activated within the joint was 45.6 seconds (range, 44.6 – 46.4 sec.) in group-1 and 45.5 seconds (44.8 – 46.12 sec.) in group-2. No significant differences were noted between group-1 and

group-2 with respect to the weight of the rabbits (p = 0.86), total surgery time (p = 0.47), or the total time the RF probe was used in each joint (p = 0.49).

Histopathology

Percent Area of Cartilage Affected: (Figure 2) A significant interaction was found between diet (group) and device (type of RF; p = 0.02). There was significantly less cartilage affected in group 2 compared to group 1 when bRF energy was used. There was no significant difference between diet groups when mRF energy was used.

A significant interaction was also found between time (0, 4, and 14 weeks) and device (p = 0.03). In joints treated with bRF, the percent area of the cartilage affected was significantly greater at weeks 4 and 14 than at week 0, irregardless of diet. The percent area affected was not significantly different between weeks 4 and 14. In joints treated with mRF, the percent area of cartilage affected was significantly greater at 14 weeks than at weeks 0 and 4, irregardless of diet. There was no significant difference between weeks 0 and 4. At week 14, the percent area of cartilage affected was significantly less in group-2 rabbits treated with bRF than in group-1 rabbits treated with bRF or group-2 rabbits treated with mRF (p< 0.05).
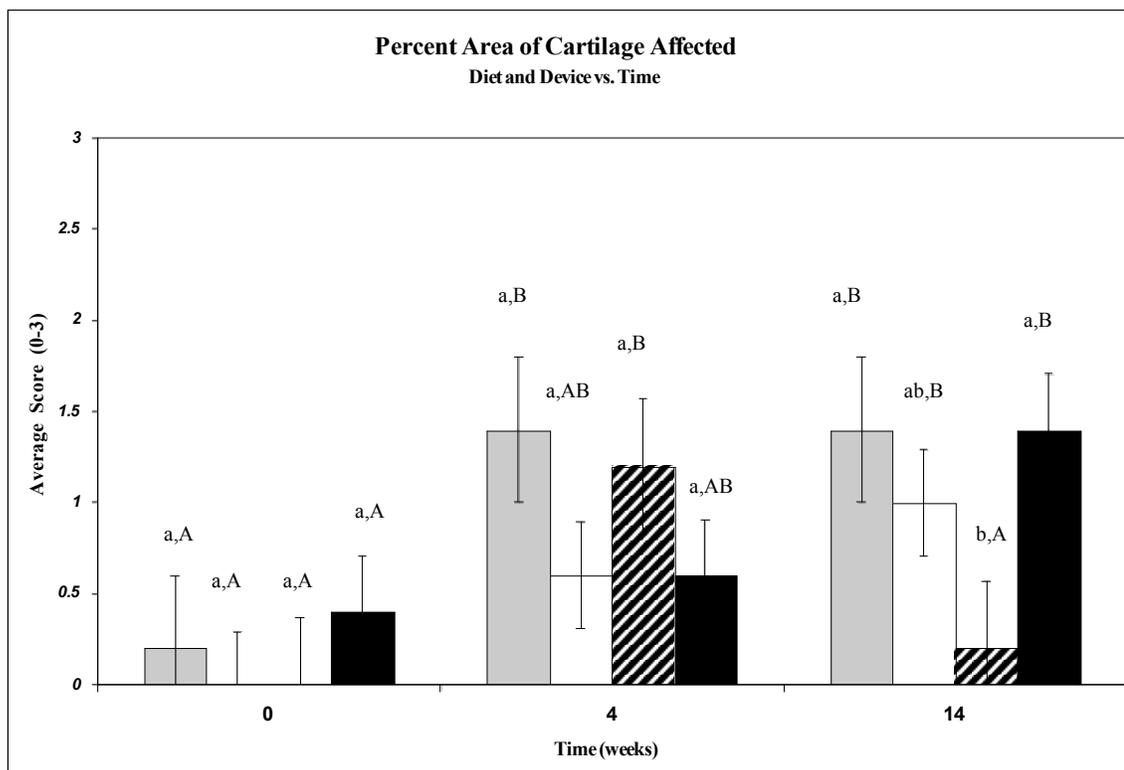
Figure 2 – Percent Area of Cartilage Affected
    Each diet and device is graphed over time.
    Group-1, bRF=grey; Group-1, mRF=white;
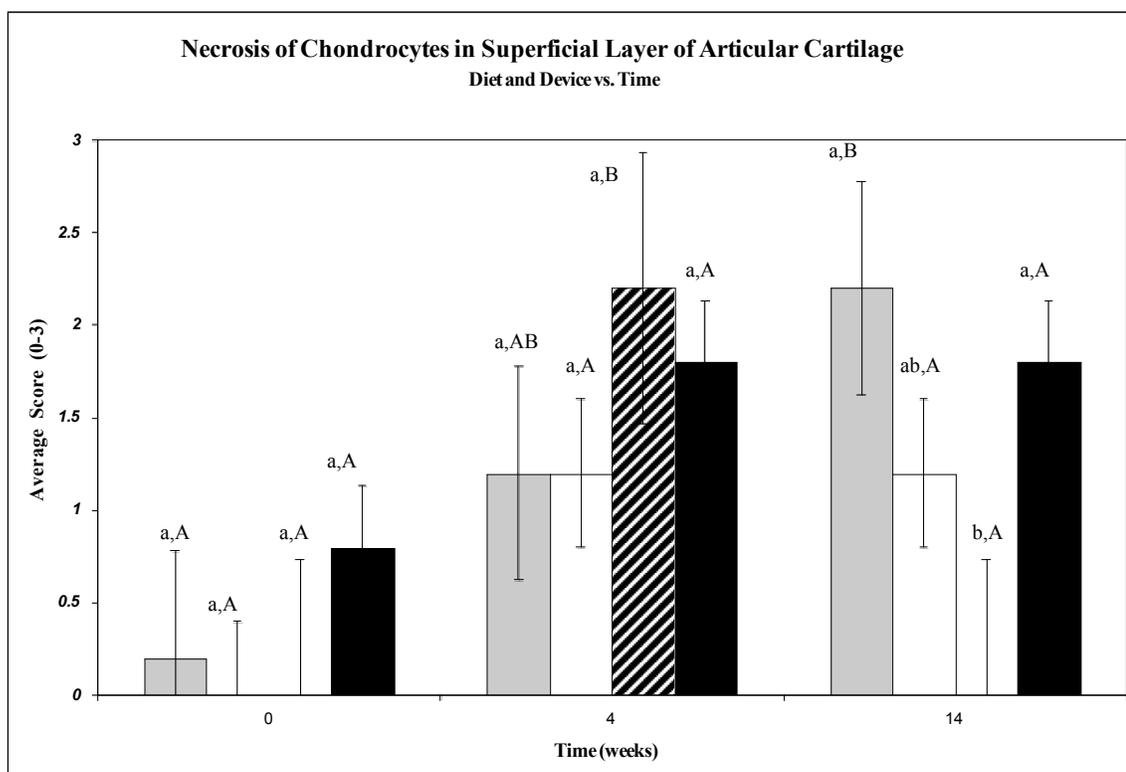   Group-2, bRF=striped; Group-2, mRF=black.
    Within each time point, means followed by the same lowercase letter are not significantly different at p=0.05. Within each treatment group, the same capital letters are not significantly different at p=0.05.

Necrosis of Chondrocytes in Superficial Layer of Articular Cartilage: A significant three-way interaction was found with diet, time, and device (p = 0.046). (Figure 3) Rabbits in group-1 treated with bRF had significantly more necrosis in the superficial layer of the articular cartilage at week 14 than at week 0 (p<0.05).

There was no significant difference between week 0 and week 4 or between week 4 and week 14. Rabbits in groups-2 treated with bRF had significantly more necrosis in the superficial layer at week 4 than at weeks 0 and 14 (p<0.05). There was no significant

difference between weeks 0 and 14. When mRF was used, no significant differences were noted in chondrocyte necrosis of the superficial cartilage layer in either group at any time period. At week 14, chondrocyte necrosis in the superficial cartilage layer was significantly less in group-2 rabbits treated with bRF than in group-1 rabbits treated with bRF or group-2 rabbits treated with mRF (p< 0.05).

Figure 3 – Necrosis of Chondrocytes in Superficial Layer of Articular Cartilage
Each diet and device is graphed over time.
Group-1, bRF=grey, Group-1, mRF=white;
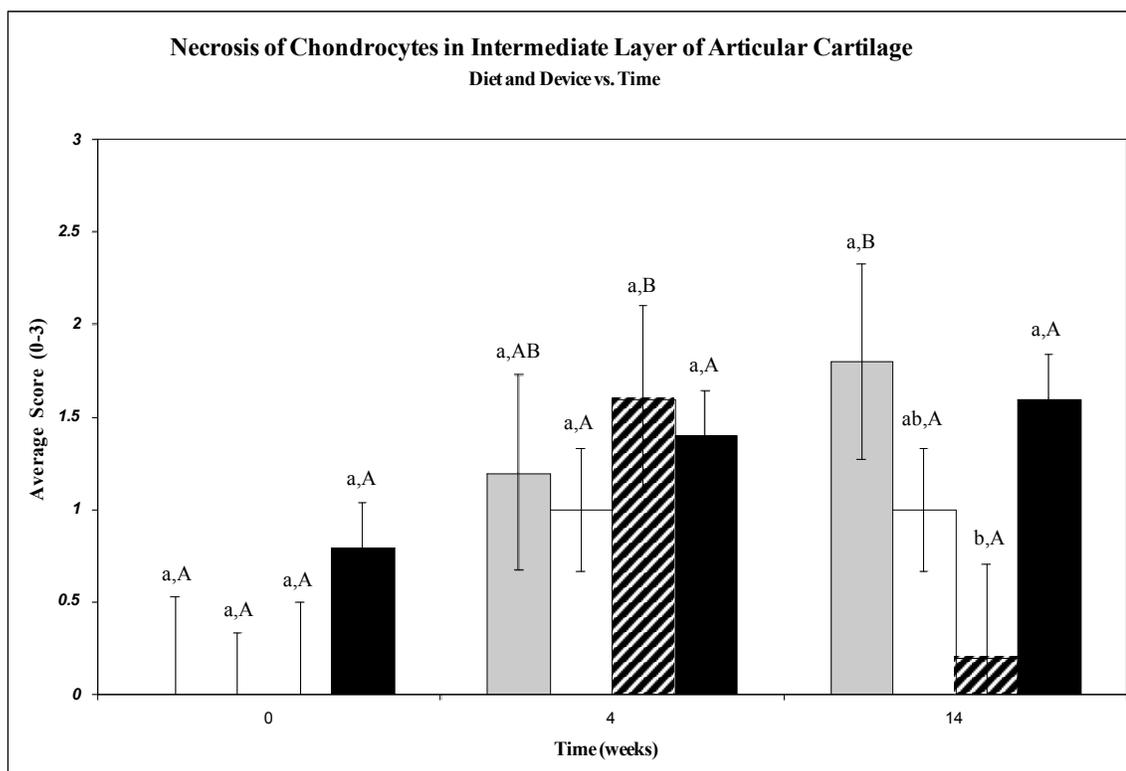Group-2, bRF=striped; Group-2, mRF=black.
Within each time point, means followed by the same lowercase letter are not significantly different at p=0.05. Within each treatment group, the same capital letters are not significantly different at p=0.05.

**Necrosis of Chondrocytes in Intermediate Layer of Articular Cartilage:** (Figure 4) A significant difference was found in the amount of necrosis in the intermediate cartilage layer over time (p=0.004), regardless of diet and device. Chondrocyte necrosis was significantly greater at weeks 4 and 14 than at week 0. Necrosis was not significantly different between week 4 and week 14. When bRF was used in group-1 rabbits, necrosis in the intermediate zone was significantly greater at week 14 than at week 0 (p<0.05); however, there were no differences between week 0 and week 4 or between week 4 and week 14. When bRF was used in group-2 rabbits, necrosis in the intermediate zone was significantly greater at week 4 than at weeks 0 and 14 (p<0.05). When mRF was used, no significant differences were noted in chondrocyte necrosis of the intermediate cartilage layer in either group at any time period. The only significant difference found in the amount of necrosis present within the intermediate layer of cartilage between mRF and bRF energy was noted at week 14 when significantly less chondrocyte necrosis was found in group-2 rabbits treated with bRF than in group-1 rabbits treated with bRF or group-2 rabbits treated with mRF (p< 0.05).

Necrosis of Chondrocytes in Deep Layer of Articular Cartilage: A significant interaction was found in the amount of chondrocyte necrosis in the deep layer of the articular cartilage between diet and device (p = 0.04). (Figure 5) Necrosis was significantly greater at week 14 in group-1 rabbits treated with bRF when compared to week 0 (p<0.05); however, there were no difference between weeks 0 and 4 or between weeks 4 and 14. At week 14, chondrocyte damage in the deep layer was significantly

greater in the group-1 rabbits treated with bRF than group-1 rabbits treated with mRF and

group-2 rabbits treated with bRF (p<0.05).

Figure 4 – Necrosis of Chondrocytes in Intermediate Layer of Articular Cartilage
Each diet and device is graphed over time.
Group-1, bRF=grey; Group-1, mRF=white;
Group-2, bRF=striped; Group-2, mRF=black.
Within each time point, means followed by the same lowercase letter are not
significantly different at p=0.05. Within each treatment group, the same
capital letters are not significantly different at p=0.05.
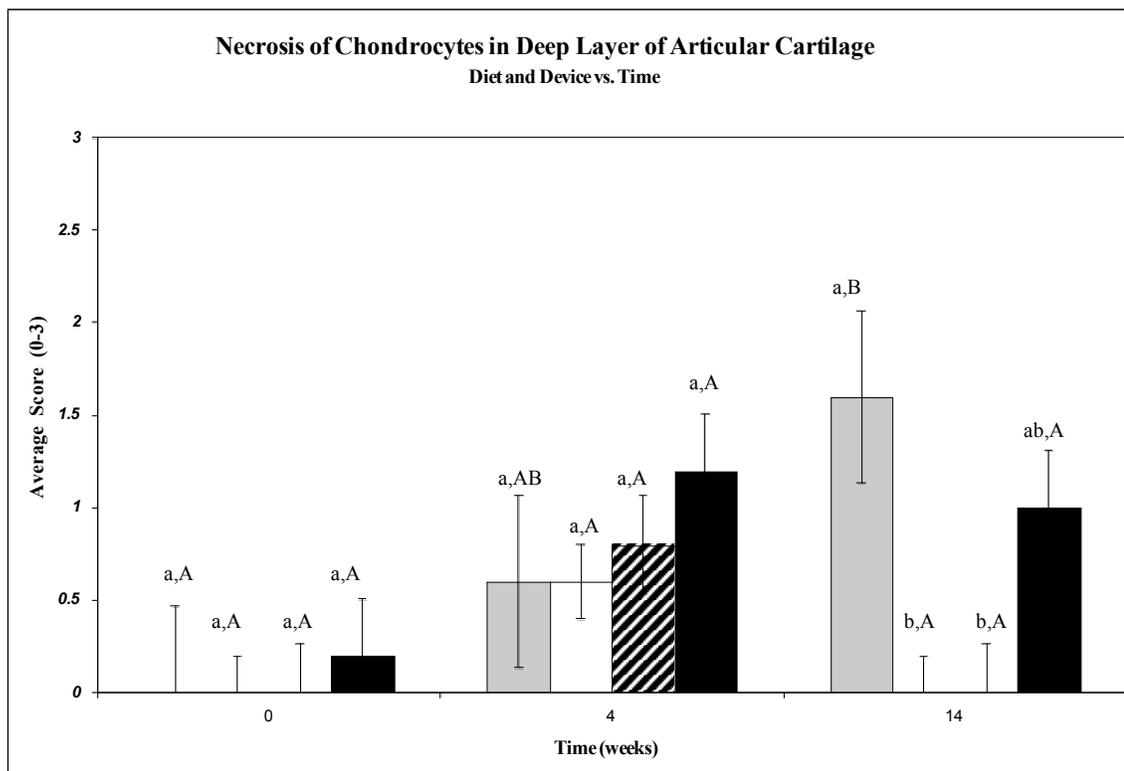
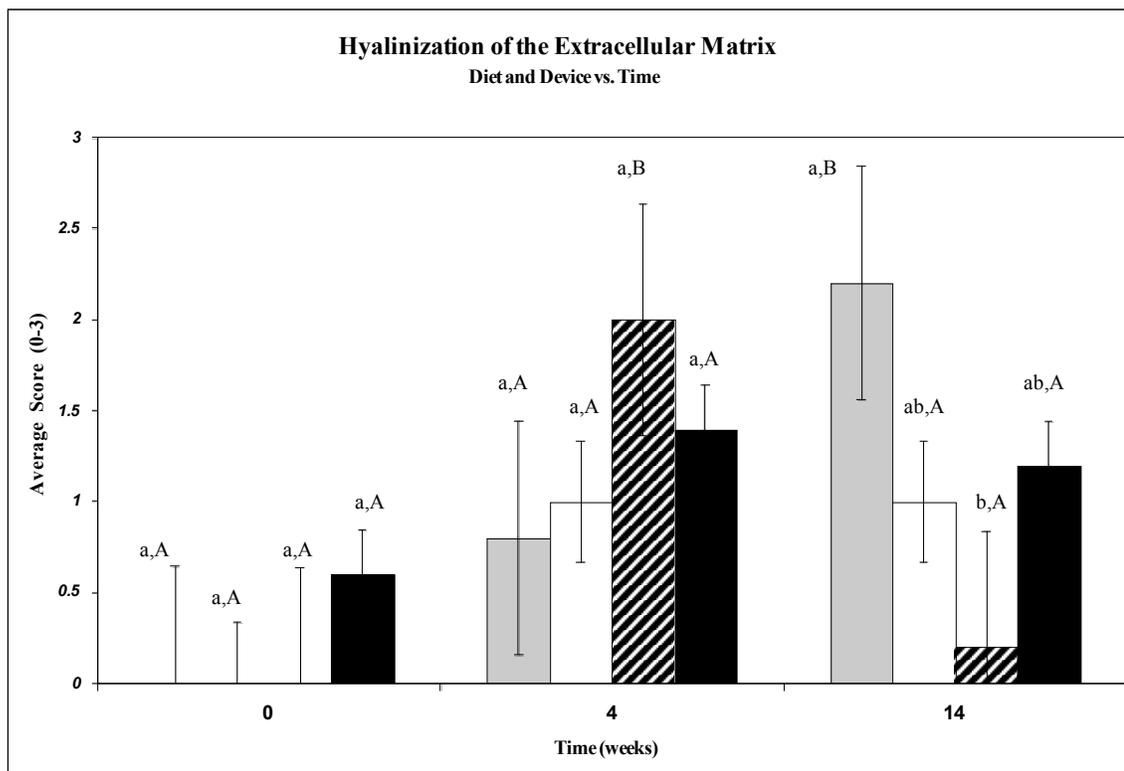**Figure 5 –** Necrosis of Chondrocytes in Deep Layer of Articular Cartilage
Each diet and device is graphed over time.
Group-1, bRF=grey; Group-1, mRF=white;
Group-2, bRF=striped; Group-2, mRF=black.
Within each time point, means followed by the same lowercase letter are not
significantly different at p=0.05. Within each treatment group, the same
capital letters are not significantly different at p=0.05.

Hyalinization of Extracellular Matrix: A significant interaction was noted between diet and time (p = 0.02). (Figure 6) In group-1, there was significantly more hyalinization, which correlates with more proteoglycan destruction, at weeks 4 and 14 than at week 0, but there was no difference between weeks 4 and 14. In group-2, there was significantly greater hyalinization of the matrix at week 4 than at weeks 0 and 14; however, weeks 0 and 14 were not significantly different from one another.

Figure 6 – Hyalinization of the Extracellular Matrix
Each diet and device is graphed over time.
Group-1, bRF=grey; Group-1, mRF=white;
Group-2, bRF=striped; Group-2, mRF=black.
Within each time point, means followed by the same lowercase letter are not
significantly different at p=0.05. Within each treatment group, the same
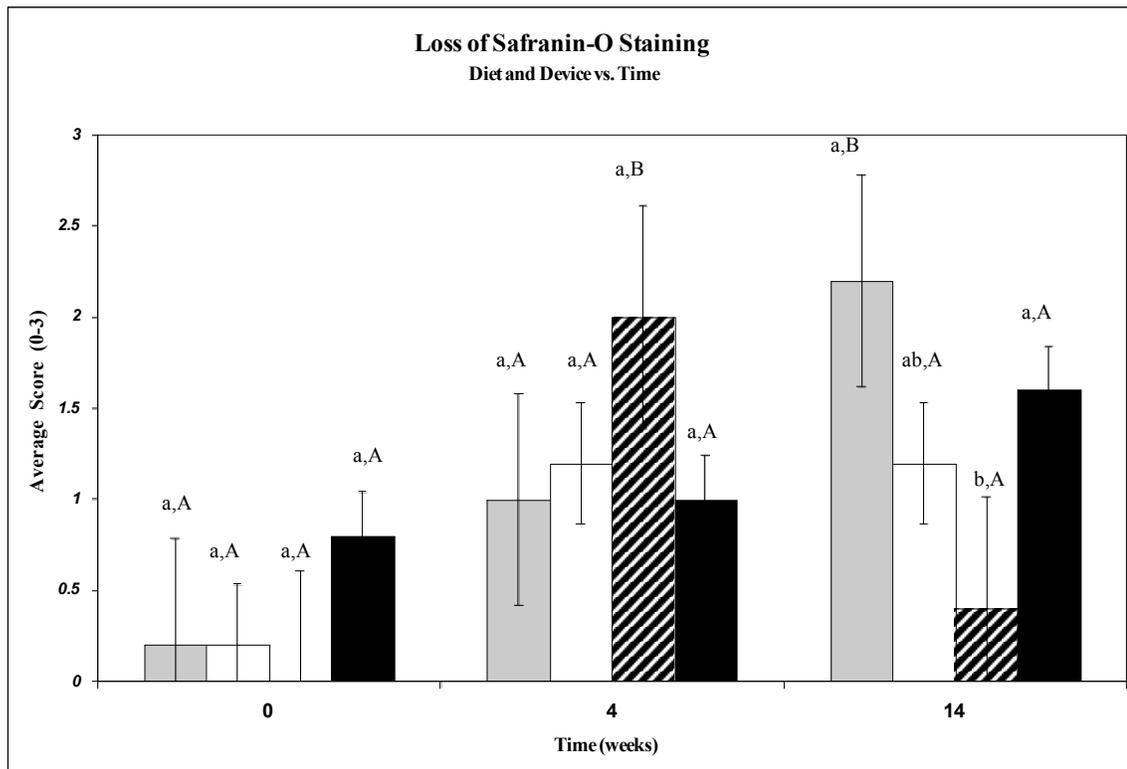capital letters are not significantly different at p=0.05.

When bRF was used in group-1 rabbits, significantly greater hyalinization was present at week 14 than at weeks 0 and 4 (p<0.05). When bRF was used in group-2 rabbits, significantly greater hyalinization was present at week 4 than at weeks 0 and 14 (p<0.05). When mRF was used, no significant differences were noted in matrix hyalinization in either group at any time period. There was no significant difference in hyalinization caused by mRF and bRF at any time period. Hyalinization of the cartilage

at week 14 was significantly greater in group-1 rabbits treated with bRF when compared to group-2 rabbits treated with bRF (p<0.05).

Loss of Safranin-O Staining: A significant three-way interaction was found between diet, time, and device (p=0.02). (Figure 7) Group-1 rabbits treated with bRF had significantly less stain uptake at week 14 when compared to weeks 0 and 4 (p<0.05). Group-2 rabbits treated with bRF had significantly less stain uptake at week 4 when compared to weeks 0 and 14 (p<0.05). Group-2 rabbits treated with bRF had significantly more staining at week 14 than group-1 rabbits treated with mRF and group-2 rabbits treated with mRF (p<0.05).

Total Histological Score: When all scores were combined to create a total histological score, there was a significant difference over time, regardless of diet or device, (p = 0.0004). (Figure 8) Total histological scores were significantly greater at weeks 4 and 14 when compared to week 0. There was also a significant interaction between diet and device (p = 0.046).

Group-1 rabbits treated with bRF had a significantly greater total histological score at week 14 than week 0 (p<0.05), but week 4 was not different than the other two time points. Group-2 rabbits treated with bRF had significantly higher total histological scores at week 4 than weeks 0 or 14 (p<0.05); however, week 0 was not significantly different from week 14. Group-2 rabbits treated with bRF had a significantly lower total histological score at week 14 than group-1 rabbits treated with mRF and group-2 rabbits treated with mRF (p<0.05).
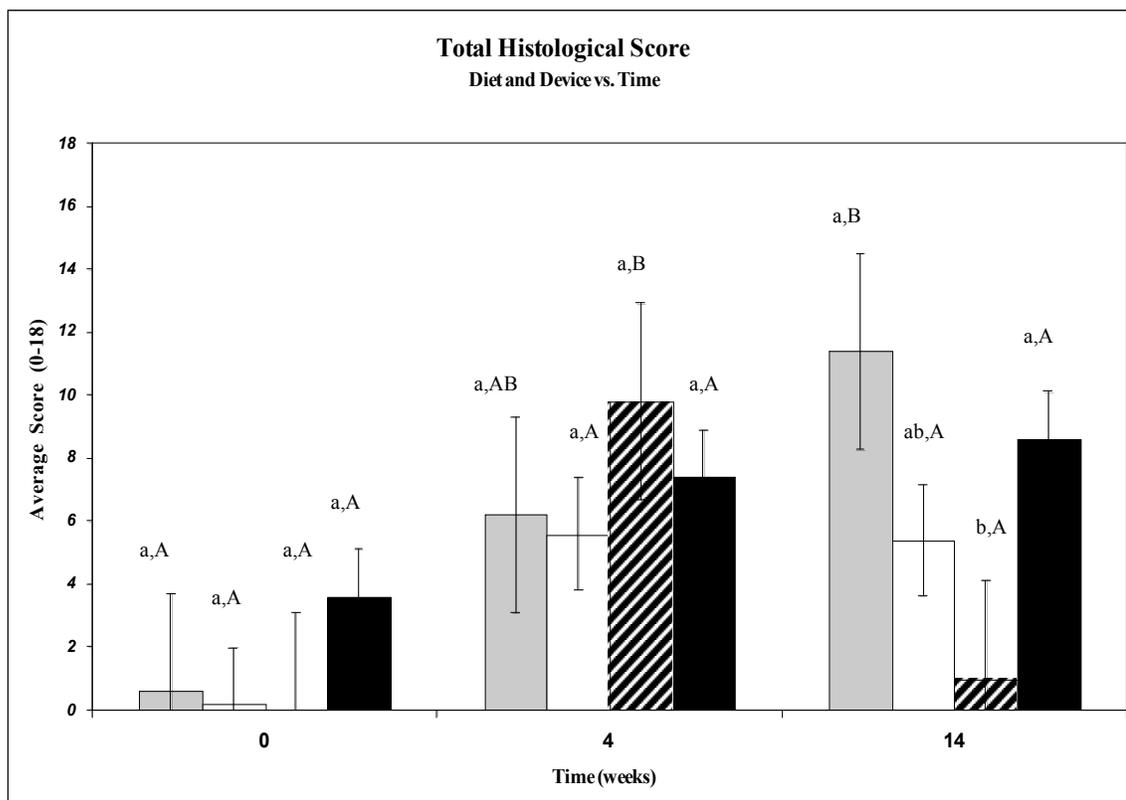
Figure 7 – Loss of Safranin-O Staining
Each diet and device is graphed over time.
Group-1, bRF=grey; Group-1, mRF=white;
Group-2, bRF=striped; Group-2, mRF=black.
Within each time point, means followed by the same lowercase letter are not significantly different at p=0.05. Within each treatment group, the same capital letters are not significantly different at p=0.05.

Figure 8 – Total Histological Score
Each diet and device is graphed over time.
Group-1, bRF=grey; Group-1, mRF=white;
Group-2, bRF=striped; Group-2, mRF=black.
Within each time point, means followed by the same lowercase letter are not significantly different at p=0.05. Within each treatment group, the same capital letters are not significantly different at p=0.05.

Cell Viability

The thickness of the cartilage samples evaluated at each time period was not significantly different between the type of RF energy used or the type of diet fed. Depth of chondrocyte death was significantly greater in treated stifle joints than in control hip joints (p=0.01). The average depth of chondrocyte death (±SE) was 17.58μm (±23.38μm) for the control samples, 94.52μm (±15.61μm) for bRF samples, and 102.72μm

(±15.61µm) for mRF samples, regardless of time or diet. There was no statistically significant difference in the depth of chondrocyte death caused by mRF and bRF. There was no statistically significant difference in the depth of chondrocyte death between group-1 and group-2 at any time period.

The percentage of chondrocyte death (relative to total cartilage thickness) was significantly greater in treated stifle joints than in control hip joints (p=0.007). The average percent of chondrocyte death (±SE) was 4.05% (±3.94%) for the control samples, 17.93% (±2.63%) for the bRF samples, and 18.88% (±2.63%) for the mRF samples. There was no statistically significant difference in percentage of chondrocyte death caused by mRF and bRF. (Figure 9) There was no statistically significant difference in the percentage of chondrocyte death between group-1 and group-2 at any time period. The percentage of chondrocyte damage was further broken down into four subgroups (0-25, 26-50, 51-75, and >75%) and analyzed with Fischer's exact test. No significant differences were noted with respect to diet, time, or device. Forty-nine of the 56 samples available for testing had chondrocyte damage between 0 and 25% of the cartilage thickness. Four samples were unable to be analyzed due to sample collection error (two rabbits from each diet and device at time 0).

Cell Viability
Diet and Device vs. Time

Figure 9 – Cell Viability
　　　　Each diet and device is graphed over time.
　　　　Group-1, bRF=grey; Group-1, mRF=white;
　　　　Group-2, bRF=striped; Group-2, mRF=black.
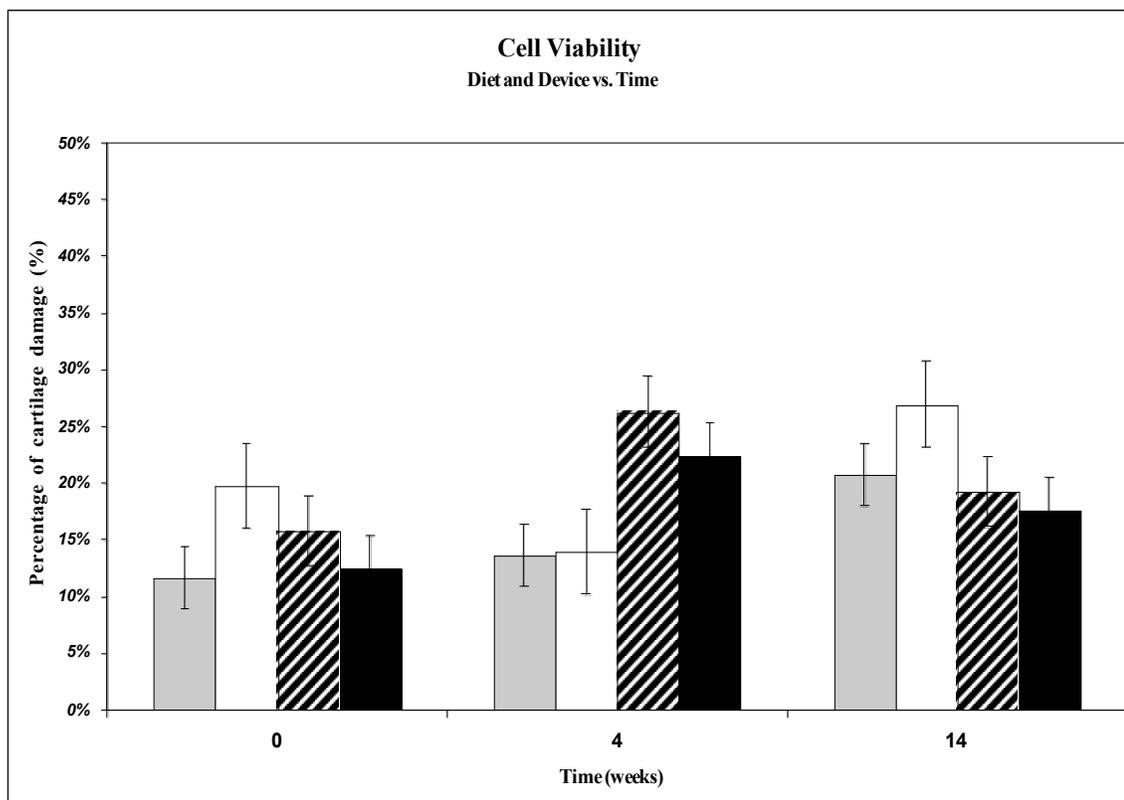　　　　No significant differences were noted with respect to diet, time, or device.

Biomechanical Testing

There was a significant difference found in equilibrium compressive modulus over time when all samples were combined (p = 0.014). (Figure 10) The compressive modulus at week 4 was significantly less than week 14, but neither time was different from week 0. There was no difference between group-1 and group-2 at any time period. There was not difference between cartilage treated with mRF and cartilage treated with bRF energy at any time period.

Figure 10 – Biomechanical Testing
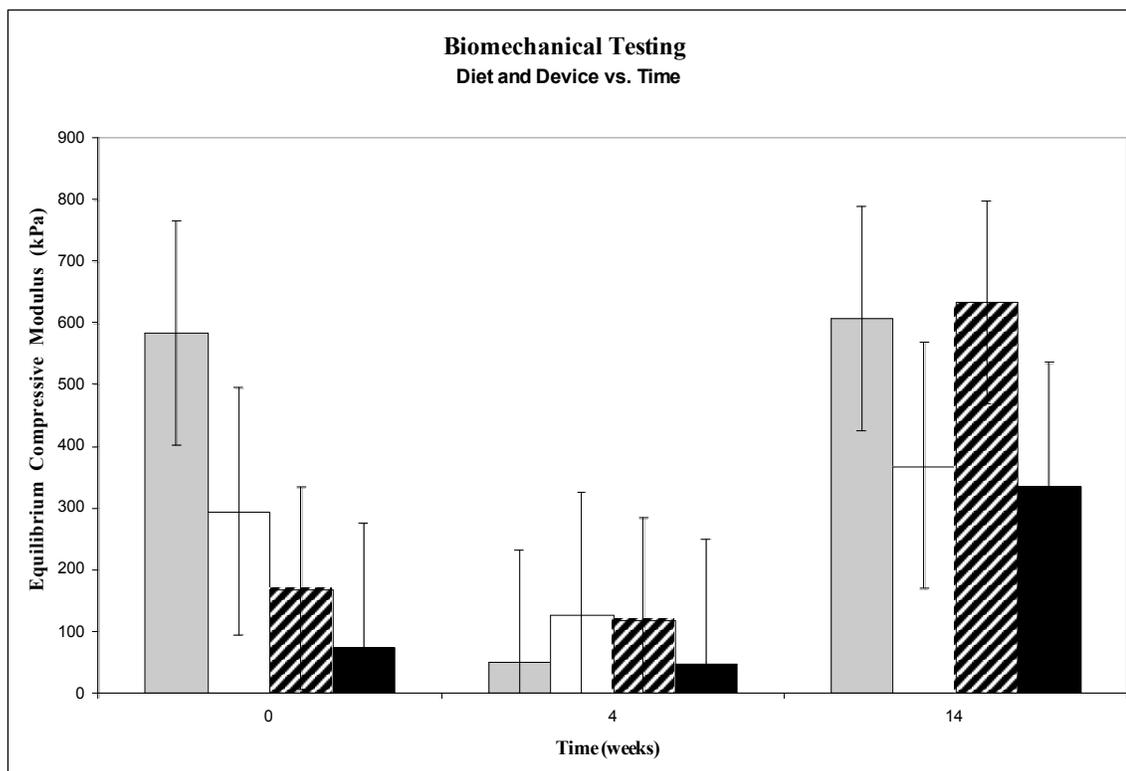   Each diet and device is graphed over time.
        Group-1, bRF=grey; Group-1, mRF=white;
        Group-2, bRF=striped; Group-2, mRF=black.
        No significant differences were noted with respect to diet or device.
        Values at 4 weeks were significantly less than 14 week; however, no
        significant differences between weeks 4 and 14 when compared to week 0.

Metabolic Activity

There were no significant interactions between diet, device, or time when the amount of GAG synthesis was evaluated. (Figure 11) GAG synthesis was significantly greater in group-1 rabbits treated with bRF at week 4 when compared to week 14 ($p<0.05$); however, neither time was significantly different than time 0. At 4 weeks, GAG synthesis was significantly greater in the group-1 rabbits treated with bRF than in group-1 rabbits treated with mRF and group-2 rabbits treated with bRF ($p<0.05$).
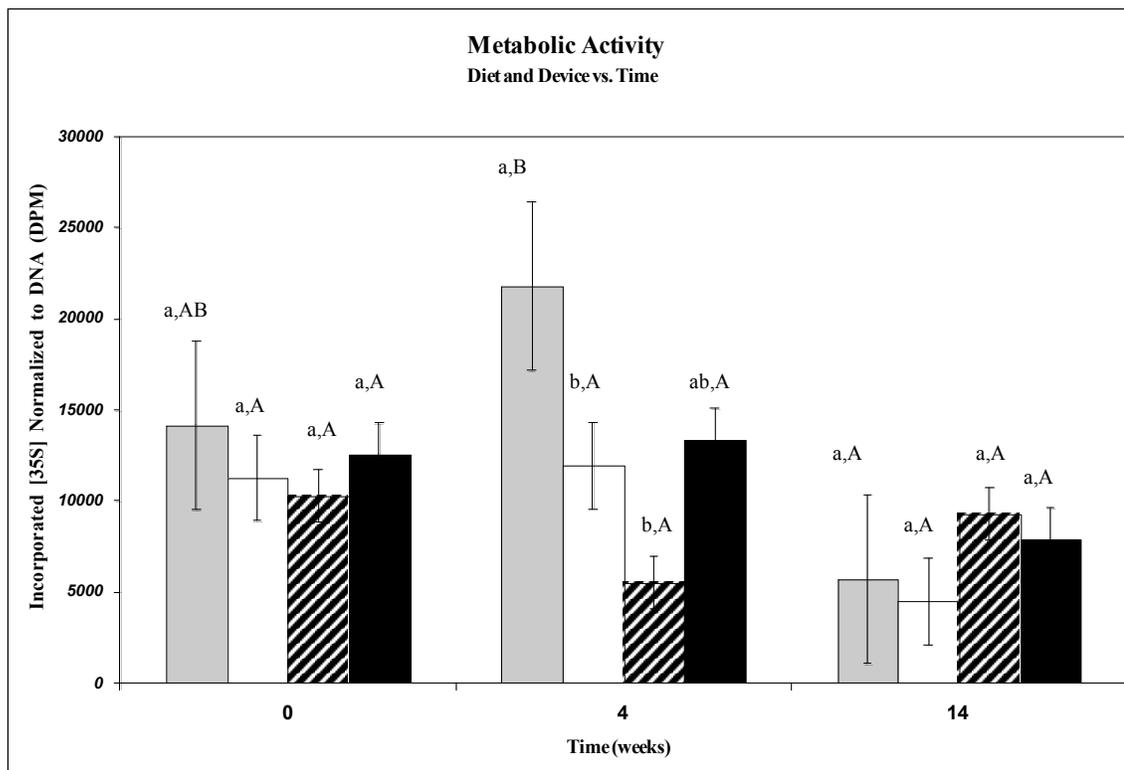
Figure 11 – Metabolic Activity
Each Diet and Device is graphed over time.
Group-1, bRF=grey; Group-1, mRF=white;
Group-2, bRF=striped; Group-2, mRF=black.
Within each time point, means followed by the same lowercase letter are not significantly different at p=0.05. Within each treatment group, the same capital letters are not significantly different at p=0.05.

DISCUSSION

Arthroscopic surgery is commonly used by human orthopedic surgeons, and its use is growing rapidly in veterinary surgery. Many investigators have reported the direct effects of RF energy on the articular cartilage[1-13] and the joint capsular tissue,[14-17,32-42] independently. However, to the author's knowledge, the current literature does not contain any documentation of the changes that occur in the articular cartilage following

the remote treatment of the soft tissues in the joint with RF energy. Lu et al. recently reported the fluid temperatures created in the joint during capsular shrinkage with RF energy,[14] and Cook et al. reported that cartilage explants incubated in culture media greater than 45ºC lead to chondrocyte death;[6] however, the biological response of chondrocytes subjected to indirect RF energy is still unknown. This investigation simulated clinical use of both bRF and mRF energy, at tissue ablative settings, to describe the changes in the articular cartilage following RF treatment of the soft tissues within the joint.

In the study reported here, confocal laser microscopy (CLM) identified significant chondrocyte death in the treated cartilage samples immediately after surgery. Chondrocyte death was evident in the samples from the treated stifle joints; however, the control samples, collected from untreated hip joints, were free of chondrocyte injury. This indicates that the cell death in this study was a result of RF treatment and not a result of the sampling technique used. This chondrocyte death was evident even though the RF energy was applied to the soft tissues in the joint and not directly to the cartilage. This is consistent with other reports in which significant loss of chondrocyte viability was noted immediately after direct application of RF energy to the cartilage.[2-6,12]

Radiofrequency-associated chondrocyte death occurred primarily in the superficial portion of the articular cartilage (outer 25% of the total cartilage thickness) in this investigation. This was true in the majority (49 of 56) of the samples evaluated, and this damage remained consistent over the 14 weeks of the study. Other studies evaluating the effects of RF energy following direct cartilage treatment have found full thickness

cartilage injury to the level of the subchondral bone.[2,4,5] The more superficial injury noted in this study is likely because the RF energy was used within the joint but was not applied directly to the cartilage surface. Another factor may have been the duration of RF treatment. In this study, RF energy was applied in 5 to 10 seconds bursts for a total of 45 seconds, under continuous lavage with lactated Ringer's solution to maintain adequate joint distention. This is consistent with the clinical use of RF energy to address synovial, meniscal, or joint capsular disease. Time has been shown to be directly proportional to the heat generated by RF energy, and thus longer application of RF would be expected to cause chondrocyte death to greater depths.[14,16,32] In addition, continuous flow of fluid has previously been shown to reduce temperatures within the joint, and this could further minimize the degree of cartilage injury.[7,14] It is important to note that the depth of chondrocyte death and the percentage of dead chondrocytes did not increase after the initial RF treatment (week 0). This indicates that the injury sustained was immediate, and no residual effects were present to cause continued cell death following the discontinuation of RF treatment.

Despite the death of chondrocytes in the superficial portions of the cartilage, only minor changes were detected in the extracellular matrix over the 14 weeks of the study. Matrix hyalinization and loss of safranin-O staining were both increased at weeks 4 and 14 (when compared to week 0); however, there was not a difference between weeks 4 and 14 suggesting that the initial decrease in proteoglycan content was not progressive. In another study, Lu et al. evaluated articular cartilage subjected to RF energy and noted that, though cell viability remained constant, safranin-O staining of the proteoglycans

within the matrix continued to decrease throughout the 24 weeks of the study.[3] The authors concluded that the extracellular matrix would likely continue this depletion until the mechanical function of the cartilage was eventually lost. In general, however, no significant difference in the amount of GAG synthesized by the treated cartilage was found in this study between the samples obtained 4 and 14 weeks after surgery. In addition, the equilibrium compressive modulus of the RF treated cartilage at weeks 4 and 14 was not significantly less than week 0, indicating that the cartilage did not deteriorate mechanically. These finding suggest that, despite the injury caused by the RF energy to the superficial layer of the articular cartilage, the remaining chondrocytes were able to maintain GAG synthesis and mechanical function over the 14 weeks of the study. This may be attributable, in part, to the fact that the injury was superficial, and approximately 75% of the chondrocytes in the articular cartilage remained viable throughout the study. In addition, more time may be required for significant matrix depletion to occur. An evaluation of longer duration is warranted to address the issue of matrix health following RF treatment. The rabbits used in this study were also confined to a cage throughout the investigation. Studies evaluating the effects of exercise on RF treated cartilage would help determine if the injury noted in this investigation is more detrimental than was described.

This study also found that the effects created in the articular cartilage by mRF and bRF energy were similar when used to treat the soft tissue in the joint. This finding differs from others who have reported that bRF leads to the production of significantly higher tissue temperatures and significantly greater chondrocyte destruction.[2,4,7] The fact

that both devices caused similar injury to the cartilage may be because both were set at the level recommended for tissue ablation (e.g., treatment of damaged meniscus) and may have created similar treatment temperatures within the joint. Temperatures greater than 45ºC have previously been reported to damage articular chondrocytes.[5,6] In the study reported here, the temperature within the joint fluid or articular surface was not recorded during RF application. It would have been difficult to ensure that the distance from RF probe to the thermometer was identical throughout the RF application, that the thermometer was accurately placed in a consistent manner within the small stifle joints, and that the thermometer was not dislodged during the arthroscopy procedure. However, previous investigations have documented the tissue and fluid temperatures expected by direct application of RF energy at various settings.[3,7,14-17] Lu et al. found that when RF energy was used in an artificial joint to shrink joint capsular tissue either continuously for three minute or intermittently (on for 10 seconds and off for 5 seconds for 5 treatments) the fluid temperature never exceeded 40ºC, as long as there was continuous fluid flow (200mL/min).[14] However, the temperature recording device used in that study was placed 1cm from the tip of the RF probe. A previous investigation documented that temperature exponentially decreased as the distance (r) from the RF probe tip increased $(1/r^4)$.[42] In addition, the temperature created by the RF probe was directly proportional to the intensity (I) and the time (T) that the RF probe was in use, (I^2T).[42] Thus, higher temperatures have been measured closer to the probe tip.[7,15-17] In the study reported here, because of the small size of the rabbit stifle joints, all tissue structures were less that 1cm from the RF probe tip. In addition, the artificial joint used in the study by Lu et al. held

25mL of fluid; significantly more than the rabbit stifle. In order to minimize tissue temperature, yet accurately simulate the clinical use of RF energy to debride a damaged meniscus or shrink a stretched joint capsule, RF treatment was applied intermittently (5-10 second bursts) for a limited time (total of 45 seconds) under continuous fluid flow. The RF devices in this investigation were used at settings very similar to those employed by Lu et al., (Oratec, 75ºC/40 W; ArthroCare, setting 2).[14] However, with the small size of the joints in our study, it is possible that fluid temperatures created would have exceeded 45ºC and led to the changes noted in the articular chondrocytes.

Cartilage injury in rabbits fed Cosequin® before and after RF treatment was similar to that seen in rabbits given a regular diet throughout this study. The fact that supplementation with Cosequin was not shown to have a protective effect, or to encourage cartilage repair in the 14 weeks of the study, may be attributable to the severity and type of cartilage injury caused by the RF treatment. As demonstrated using CLM, RF treatment caused immediate chondrocyte death, particularly in the superficial portion of the cartilage, which persisted throughout the study. The proposed mechanism of chondromodulation by glucosamine and chondroitin sulfate (Cosequin®) requires viable chondrocytes.[25,26] Therefore, the dead chondrocytes in this study would not be expected to benefit from Cosequin® therapy. In addition, the amount of GAG synthesized by the chondrocytes in this investigation did not significantly decrease at weeks 4 and 14 (compared to week 0) despite the death of the superficial chondrocytes. It is important to note that serum or synovial glucosamine and chondroitin sulfate levels were not measured in this study. Thus, we cannot confirm that rabbits fed a diet containing 2%

Cosequin®, by body weight, actually had levels greater than those of control rabbits. The dose used in this investigation was based on a previous study in which moderate and severe cartilage damage was significantly decreased in rabbits with stifle instability fed 2% Cosamin®.[22,23] Interestingly, significant improvement in many of the histological parameters (percent area of cartilage affected, necrosis of the superficial and intermediate layers of articular cartilage, hyalinization of the extracellular matrix, safranin-o staining, and total histological score) were noted at week 14 in rabbits fed 2% Cosequin®. However, these findings were not corroborated with GAG synthesis data, CLM findings, or mechanical properties of the articular cartilage.

In this study, histologic evaluation of cartilage immediately after surgery (week 0) failed to identify the chondrocyte injury detected by CLM. This finding is similar to that of others who reported little or no change in articular cartilage immediately after the application of RF energy.[1,8,11] It is possible that the inability to detect significant histological changes in the chondrocytes immediately after RF application is because morphologic changes are not yet present, or that routine histological evaluation is limited in its ability to assess chondrocyte function immediately after this injury occurs. Though histological evaluation did not detect significant cartilage change the day of surgery, CLM analysis confirmed that chondrocyte death occurred immediately after RF treatment. This is consistent with the findings of other investigators who demonstrated that the use of CLM, with a live/dead cell staining technique, is more sensitive to acute chondrocyte death than traditional histopathology following thermal energy application.[3,5,44]

In summary, both bRF and mRF, when applied remotely to the soft tissues in the rabbit stifle joint, led to chondrocyte death, particularly in the superficial 25% of the articular surface. The remaining chondrocytes within the articular cartilage appeared to maintain the extracellular matrix which provided ample mechanical strength to the cartilage throughout the 14 weeks of this investigation. In addition, similar chondrocyte damage was noted between RF devices when used at tissue ablation settings. These results suggest that RF energy, even when applied remotely to the soft tissues within the joint during arthroscopic surgery, is detrimental to chondrocyte health, and its used should be limited.

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CHAPTER III

SUMMARY, IMPLICATIONS, AND CONCLUSIONS

Human and veterinary orthopedic surgeons have made a shift to be more minimally invasive over the past few decades. The arthroscopic use of radiofrequency (RF) energy has minimized the need for arthrotomy with open surgical manipulation of many conditions within the joint. Surgeons have been able to shrink exuberant joint capsular tissue, treat various meniscal injuries, and smooth fibrillated cartilage with both bipolar and monopolar RF. However, RF energy has been shown to damage collagen molecules and fibroblasts in joint capsular tissue and cause chondrocyte death in articular cartilage. Although joint capsular tissue has the ability to repair itself articular cartilage does not. Numerous investigations have documented the changes following the direct treatment of joint capsular tissue and articular cartilage with RF energy; however, to the author's knowledge, this is the first study to document the changes in the articular cartilage following remote treatment of the soft tissues in the joint. This is the first investigation to report the mechanical function of RF treated cartilage and the response of RF treated cartilage to Cosequin® therapy.

This study was able to document immediate chondrocyte death in the superficial region (outer 25%) of the articular cartilage following the remote application of both bRF

and mRF energy at tissue ablative settings. Confocal laser microscopy, with a live/dead cell staining technique, was able to verify this immediate chondrocyte death when traditional histopathology revealed normal chondrocyte morphology. This is consistent with the findings of other investigators. A recent investigation reported a progressive loss of proteoglycan staining in the cartilage extracellular matrix for 24 weeks following direct application of RF energy. The authors concluded that this loss would continue until there was complete mechanical failure of the articular cartilage. The study reported here was unable to document progressive loss of chondrocyte function or proteoglycan staining in the treated articular cartilage for 14 weeks after remote application of RF energy. Safranin-O staining of the proteoglycans in the extracellular matrix, glycosaminoglycan (GAG) synthesis by the articular chondrocytes, chondrocyte viability (CLM), and mechanical function of the articular cartilage were not significantly different between weeks 4 and 14 in this investigation. This documents that, even though the superficial layer of chondrocytes were dead, the chondrocytes in the deeper layers were able to maintain the extracellular matrix and mechanical function of the treated articular cartilage.

Other investigations have documented that bRF energy leads to significantly greater treatment temperatures and chondrocyte destruction than mRF energy. This study was unable to find consistently significant differences between the two treatment devices. This is most likely because both devices were set at tissue ablative settings, used intermittently, and evaluated under continuous fluid flow in an attempt to create similar treatment temperatures.

Rabbits fed 2% Cosequin®, by body weight, were not found to have significant improvement in chondrocyte viability, proteoglycan staining, or GAG synthesis over rabbits fed a normal diet. This is most likely because of the severity of the damage created to the chondrocytes given that Cosequin® has previously been shown to only affect chondromodulation in live cells.

One drawback of this investigation is that we did not measure the fluid or tissue temperatures created within the rabbit stifles during RF treatment. A recent investigation documented fluid temperatures 1cm from the RF probe tip in an artificially created joint that was able to hold 25mL of fluid. It has previously been shown that the temperature created by RF energy exponentially decreases as the distance from the probe tip increases. It has also been shown that fluid temperatures greater than 45°C will bring about chondrocyte death. Thus, because of the small size of the rabbit stifles, fluid temperatures created during this investigation may have contributed to the chondrocyte damage seen.

Additional investigation of fluid and articular cartilage temperatures created by remote RF application is warranted. The rabbit joints used in this investigation would be similar in size to human wrist, ankle, or phalangeal joints. Studies investigating larger joints would help document the changes created in the articular cartilage during RF treatment of joint capsular and meniscal tissue. Different sized RF probes tested at a variety of settings and various distances would be necessary to document the optimum treatment required to eliminate chondrocyte death during remote application of RF energy. Finally, the length of this investigation may not have been long enough to

document depletion of the extracellular matrix or mechanical function of the articular cartilage. Therefore, a study of greater length is indicated.

In conclusion, remote application of both bRF and mRF energy in rabbit stifles at tissue ablative settings, under continuous fluid flow, in an intermittent fashion causes immediate damage to the superficial (outer 25%) chondrocytes of the articular cartilage. This immediate damage was found to be non-progressive and the metabolic activity and mechanical function of the articular cartilage in this study was maintained throughout the entire 14 weeks. Cosequin® was unable to create significant differences in treated articular cartilage function when compared to controls.