Original article

The influence of radiofrequency ablation patterns on length, histological and mechanical properties of tendons

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Summary

The use of radiofrequency ablation for thermomodulation of connective tissues has gained acceptance with some surgeons. It is now mainly used for shoulder instability, and two techniques are commonly applied – ablation in a uniform pattern *(paintbrush)* and ablation in a linearly dispersed fashion *(grid)*. The use of these techniques for shrinkage of tendons or cruciate ligaments is not widely accepted but may be utilized in selected cases. We assessed the effects of thermomodulation via monopolar radiofrequency ablation using these two techniques on the histological and biomechanical properties of rabbit Achilles tendons.

16 paired rabbit achilles tendons were divided into two treatment groups. Using a Monopolar RF device, eight tendons were treated using the *paintbrush* technique, and eight using the *grid* technique. The tendons were shrunk to about 90% of their original length, and the paired tendons were used as control. Following thermomodulation, tendons were pulled to tear using the *Instron* 4502 (Instron, Mass.) device.

We found treated tendons were significantly less resistant to tear when compared to control; the average load to failure of the treatment group was 19.4% lower (p=0.05) than the control group values and the average tissue stiffness in the treatment group was 11.3% lower (p=0.051) than the control group. We found a tendency towards a lower resistance to pull in the tendon group treated using the *grid* technique. Histological analysis demonstrated areas of collagen denaturation correlated to areas of thermomodulation. A random point of failure was found along the tendons in the *paintbrush* group whereas the typical point of failure in the *grid* group was located at the treatment point or at its margins.

Our findings demonstrate that use of the *grid* technique in ablation of tendons creates typical failure points *(locus minoris resistenci)* which bring about failure and alter the biomechanical properties of the thermomodulated tendons. Thermomodulation of tendons may be used efficiently in selected cases but its detrimental effects to the biomechanical attributes of the tissue should be considered.

Key words: ablation, collagen, length, patterns, radiofrequency, tendon.

Introduction

Soft tissue injuries about joints are very common in the orthopedic practice, and can be found in all areas of population with no correlation to age, gender or type of activity. Soft tissues around the joint fulfill important functions of stabilization, movement creation and power transfer. The treatment for soft tissue joint injuries with instability may be conservative or surgical[1-3]. The surgical utilization of thermal ablation is mostly arthroscopic and is considered part of the surgeons' armamentarium of joint stabilizing procedures. The use of thermal energy for the shrinkage of soft tissue is accepted nowadays, mostly as an augmentation for capsular shift in the shoulder joint[3] and by some for wider indications. The use of this treatment method for the shrinkage of thicker tissues like tendons is less common. Literature considering this usage is scarce, and clinical studies present controversial (mostly disappointing) results. The two techniques most commonly used nowadays are the application of energy in separate lines leaving untreated areas in between them *(Grid)* or the application in one constant line treating a wide tissue area leaving no untreated tissue in between *(Paintbrush)*. The aim of this study was to evaluate the two RF thermo modulation techniques *(Grid and Paintbrush)* through evaluation of histological and biomechanical properties of affected tissue in a Rabbit in-vitro Achilles tendon model, supplying a basis for clinical application of RF thermal shrinkage in the clinical setting.

Molecular anatomy and biomechanics

Collagen is the key protein responsible for the mechanical properties of the musculoskeletal system. Soft tissues about the joint are mainly made of collagen type 1- a triple helix. The key factor differing between soft tissues – i.e. ligaments, joint capsule and tendons – is in the length of the collagen fibers, their number, arrangement and the number of inter-fibrous bonds. As a part of aging processes, the number of those bonds increases and the flexibility of the bonds decreases.

Soft tissues have the ability to accept mechanical loads in order to function properly. The mechanical properties of the structure bone – ligament – bone can be presented on a load – elongation triple phase curve, which evaluates the tissue on one level[1]. The 1st phase is non-linear, presenting the preparation of the collagen fiber to requested resistance. The 2nd phase is a linear slope which defines the change in tissue length in correlation to the load applied on the tissue – the stretching of the collagen fibers. The slope reaches the peak point of elongation – its point of failure – where the 3rd phase begins – the ligament can not accept any further load. Like this curve, mechanical properties can be presented on a stress/strain curve, where stress is defined as a load per area and strain as a change in length unit (in percentage). The 2nd phase of this curve is called tangential modulus or the "Modulus of young". The higher the modulus is, the stiffer the tissue (e.g. more collagen per unit area and a larger fiber diameter)[1,2]. *In vivo*, the forces act on the ligaments through different planes, which increase the load and resistance required from the tissue. For example, a rotational knee injury that caused an ACL tear, forced the ligament through all phases of the modulus and the forces which caused the tear can be demonstrated by the direct load on the tissue *in vitro*. In shoulder dislocation the joint capsule reaches the point of failure (even if not completely torn) after which it can no longer fulfill its function.

Sources of thermal energy

The use of thermal energy for the treatment of joint instability was first described by Hippocrates, where he describes a hot probe placed in the axilla for the treatment of glenohumoral instability[3]. Nowadays, there are two main sources of thermal energy used for soft tissue shrinkage.

Radiofrequency (RF) is the older one, was first described at the end of the 19th century and is used in a wide variety of medical conditions[4]. RF is an electromagnetic energy produced by an alternating electrical current. RF can be produced when the current passes between two electrodes (BIPOLAR RF) or between the electrode and a "ground" pole which is the patients' body (MONOPOALR RF). Placing the probe against the tissue and then passing an alternating current, causes activation and movement of ions in the tissue, thereby producing heat that is generated from the friction between the ions – designated as frictional heating[4-7]. In Monopolar RF devices the probe does not create heat at all. It measures the heat created in the tissue using a thermocoupling device located at the tip of the probe. The data allows control of the energy levels and heat changes in the tissue, thereby causing the change in collagen structure without causing major injury[8-10], the heat produced by the tissue reflects the current level (I= current level), as I^2 and the time for the action (T=action time) and so the heat from the probe equals I^2T. Heat is lost by diffusion and fluid conduction. Therefore, the actual thermal influence on the tissue is the heat created by the probe subtracting the tissue heat loss. Another type of heat source is the Laser beam. The laser most commonly used for thermal shrinkage is the Holmium-Ytrium-Aluminum Garnet (Ho:YAG). This type of Laser device was first presented in 1987 and was the first to be passed through an optic fiber[11]. The laser beam concentrates light at a fixed wave length, usually in the infra-red area. The physical properties of the Laser beam allow its passing, spreading, breaking and absorption. The absorption of the beam heats the tissue[12,13]. The heat created in the tissue is the summation of the beam power, time of application and area of application. The distance from the Laser source and the color of the tissue influence the beam absorption as well[14]. The beam may be used in a stream or in a pulsed manner. The pulsed manner allows the tissue to cool down and thereby reduce the damage caused to the tissue. If the Laser beam passes the ablation threshold, the light energy will change to thermal energy and that will cause evaporation of tissue and water. In an *in vitro* research that compared the application of RF to Laser on the gleno-humeral joint, the results were similar[15]. The advantage of use of a laser beam is that one can treat the tissue in a precise manner and in a small and defined area[15]. The disadvantages of laser are its high cost and the need for special skills and instrumentation. The cost of RF devices is much lower and their use does not require special training or skills[12-15].

Molecular and biomechanical effect

Depending on the thermal energy applied to the tissue, the quartiary structure of collagen is broken followed by unwinding of the triple helix[16]. This causes a disruption of fiber and histological structure. The tissue shortens significantly and looses its primary elastic properties. In studies it was shown that even at 55° Celsius there is a change in collagen structure and shortening of fibers. The amount of shortening increases as temperature increases, it was shown that at 65^{o} Celsius collagen shortens to 50 % of its original length[17], while when temperature reaches over 80^{o} Celsius collagen looses its structure and function altogether[18]. In one study, performed on sheep joint capsules, it was found that rising ablation temperature correlated with minor changes in collagen structure to complete loss of normal structure and eventual tissue necrosis[19]. Yet another study found there was no difference in shrinkage level once the temperature rises above 75^{o} [4]. In an *in vivo* study where rabbit patello-femoral joint capsule was treated by Ho:YAG Laser it was shown that the reaction to tissue injury was to create new collagen tissue but the general tissue structure remained short. 7 days following damage, a large number of fibroblasts were demonstrated in the area of hyaline and collagen deposition with no other cells. 30 days following damage, fibrosis was evident with a loose connective tissue matrix, with minimal collagen deposition (20). Another study examined the response of shoulder joint capsule 180 days following Laser capsular shrinkage[21]. The immediate response was the same as described before. 30 days following shrinkage there were fibroblast infiltration and tissue capillaries. 60 days following shrinkage collagen fibrils were uniformly small but the tissue was otherwise normal. 90 and 180 days post shrinkage a normal tissue structure was found, which was shorter than initially. Biochemical evaluation using "tripsin sensitivity index" showed that the laser ablation caused collagen denaturation which returned to its initial properties at healing completion. The index was low at 7 days following ablation and returned to normal 180 days later. The higher the energy absorbed by the tissue, the greater was the damage caused to the collagen fibers. The energy distribution during application is of utmost importance, influencing collagen denaturation and thereby its biomechanical properties[4]. Too high an energy may cause mesenchymal damage and interrupt the normal healing process, and possible differentiation and migration of Tendon-Derived stem cells[22]. Hayashi et al.[23] treated rabbit patellofemoral joint capsules by Ho:YAG laser using 5,10 and 15 watts per square centimeter. The capsules were shrunk to 9 to 38% of original capsule length. It was shown that in tissue where 10 and 15 watts were applied, there was a significant increase in tensile stiffness compared to untreated tissue. It was also found that the load required for returning the applied tissue to its original length was significantly larger in these groups. Vangsenss et al.[18] examined the resistance of human patella tendons to Ho:YAG Laser. After shrinkage of 10% of tissue length, a 70% decrease in tissue tensile strength was found. But when the beam was applied to one area in the tendon, thus causing severe burn, failure of the tissue was at that point i.e. the *"locus minoris resistenci"*. Thus, since tissue should be shortened and remain at least partially functional and later regain full functionality, an acceptable range of temperatures used for shrinking collagen tissue has been derived. This range is between 60° and 70° Celsius according to different studies[4,15,17-19,24]. Considering tissue length, most studies show shrinkage of about 10% of original length[18,19]. The type of tissue – i.e. tendon ligament or capsule – is important as well. It was also shown that shrinkage percentage decreases as the tissue area exposed to thermal energy decreases[18] and this changes according to heat application technique as well. The two techniques most commonly used nowadays are the application in separate lines leaving untreated areas in between them (*Grid*) or the application in one constant line treating a wide tissue area leaving no untreated tissue in between (*Paintbrush*)[5, 18]. This study was aimed at assessing the effects of utilization of these two techniques on tendon biomechanical and histological properties.

Clinical use of thermo-modulation

Thermal soft tissue shrinkage is acceptable nowadays as treatment of loose tissue like the shoulder joint capsule. This technique is used as augmentation in arthroscopic surgery. It seems that appropriate use of thermal energy for gleno-humeral capsule shrinkage allows joint stability with no changes in the tissue functional properties[25]. Effectiveness of this technique in ligaments and tendon has not been thoroughly studied. Recently published case reports reveal an ambivalent to pessimistic picture. In one case, an ACL reconstruction (hamstrings) in a 16 year old female athlete was shrunk after it had been found to be lose using RF. Three months later the reconstruction was found to be in a state of auto digestion[26]. Carter et al.[27] used monopolar RF for shrinkage of ACL reconstructions (of different types) in patients who demonstrated symptomatic instability of the injured knee. 11 of 18 patients treated with RF shrinkage had gone through complete reconstruction 4 months following shrinkage. 7 went back to normal activity at an average of 20 months following shrinkage. Indelli et al.[28] used monopolar RF in 28 patients for shrinkage of bone patellar-tendon-bone ACL reconstruction, followed by at least 2 years of follow-up. 96% of the patients went back to sports activities similar to those before injury. One of their conclusions is that a strict rehabilitation protocol and patient's compliance are as important as the form of application. No technique of shrinkage (Grid vs. Paintbrush) is uniformly accepted.

Materials and methods

16 white adult rabbits, with equal age and weight were sacrificed using an acceptable protocol for animal experiments and with animal committee authorization. 16 paired Achilles tendons were harvested. The paired tendons were divided into two groups containing 8 paired tendons each. Thermal modulation was performed in a saline bath using a Monopolar RF device (VAPR Radiofrequency system, DePuy Orthopaedics, Warsaw, Indiana, USA). In each group one of the paired tendons was treated with Monopolar RF and the other used as control. In group A, the thermo-modulation was performed along the tendon, implementing the Paintbrush technique in an effective time of 1 minute per tendon. In group B, thermomodulation was performed in separate lines parallel to tendon width implementing the Grid technique in an effective time of 1 minute per tendon. The power of the device was 5 kilovolts. The length of treated tendons was measured before and after thermal treatment. Tendons were bathed in a 37° saline bath. Up to 2 hours from thermal treatment, the tendons were mounted on a 10 Kilo-Newton load-cell device (Instron 4502, Canton, Mass.) via clamping jigs (Fig. 1). The tendons were connected to the jigs with no resistance or stretch, anchor points were frozen using liquid nitrogen, and the tendons were pulled to tear at a 500 mm/min speed while load and deformation were acquired automatically (series IX, Instron, Canton, Mass.). Data concerning force at tear and stiffness characterizing the Young's modulus were extracted. Tendons were sent to histological examination following mechanical testing. A simple t-test was applied to assess significance of results. Statistical significance was set at p=0.05. The specimens were processed and stained with Hematoxylin & Eosin stain. The histologic preparations were assessed for failure location and local tissue denaturation.

Results

As a result of thermal modulation, denaturation marks (color changes, shortening of the tendon, appearance of a rigid clear area) appeared in all treated tendons. Treated tendons demonstrated an average shortening of 9.13% (8.76% in the Paintbrush group and 9.50% in the Grid group). In general, the stiffness (Young's modulus) was lower in treated tendons compared to control. The average load at failure in the treated group was 19.4% lower compared to the control group (p=0.05). The load at failure of the treated tendons was lower than the control group; the average tissue stiffness in the treated group was 11.3% lower than that of control (p=0.05). (Tab. 1) This was also found true when comparing each treated tendon to its control. Treated tendons from group A (*Grid*) demonstrated a lower resistance to stretching than treated tendons from group B (*Paintbrush*). Average load at failure in group A (*Grid*) was 19.4% lower than in group B (*Paintbrush*) (p=0.092), and the average tissue stiffness was 14.1% lower (p=0.088). In treated tendons of group A (*Grid*), failure occurred consistently at the transition point between the treated and untreated areas. In treated tendons of group B (*Paintbrush*), the point of failure was found at an incidental point along the tendon. In the control group there was no specific point of failure either. Histological examination of the treated tendons, using Hematoxylin & Eosin stain demonstrated areas of denaturation and defects to the normal collagen structure in the areas of thermal manipulation. In the treated tendons of group A (*Grid*), there were areas of denaturation and loss of normal structure lying aside areas of normal collagen structure. In the treated tendons of group B (*Paintbrush*), there was a large consistent area of denaturation and destruction of normal structure of long collagen fibers.

Mean tissue strength (N/mm^2)	**Mean load to failure (N)**	**Treatment groups**
8.35	446.75	**Paintrush (n=8)**
7.18	360.00	**Grid (n=8)**
8.75	500.56	**Control (n=16)**

Table 1. Mean tissue strength and load at failure.

Figure 1. At right - Tendon in the process of pull to tear, while freezing the anchor points by spraying liquid Nitrogen. At left - the tendon after completion of the process.

Discussion

Thermal modulation of soft tissue alters normal collagen structure and, as a result, the tissue will shrink. Our results show that thermal modulation decreases tendon resistance to stretch when compared to control, and that thermal modulation using the Paintbrush technique causes less damage to tendon resistance than thermal modulation using the Grid technique. The reasoning behind using the Paintbrush technique is that collagen modulation will be done uniformly; this will allow for initiation of rehabilitation following surgery with a lessened chance of failure and in hope that the healing process will be uniform as well. The reasoning behind using the Grid technique is that, theoretically, leaving healthy areas adjacent to treated areas will allow better tendon healing, since the tendon is a living tissue carrying self-healing abilities [29,30]. During the healing process, fibroblasts migrate from the peritendon and tendon sheath to the damaged area. Those fibroblasts will produce collagen that will help the healing tissue regain its structure and return to near normal strength when healing is completed. On-the-other-hand, this technique creates possible points of failure in the vicinity of the treated areas, which may fail during the tendon healing period, especially when challenged by a rehabilitation process.

In our study we utilized RF energy on an *in vitro* rabbit Achilles tendon model and did not utilize laser energy. This was done as the goal of the study was to evaluate the mechanical effects of the different energy implementation techniques, i.e. grid as opposed to paintbrush. We believe that use of laser has merit as an alternative source of thermal energy and hence it is described in the introduction.

Our study has several limitations to it, most notably the fact it is an *in vitro* study may be rendered a downside where performing an adjunctive assessment on an *in vivo* model may elucidate behavior of the tissues in a larger span of conditions and add to our understanding of the impact of use of the grid and paintbrush modes of thermal energy ablation. This study is also limited by the use of tendons and not joint capsule or ligaments in that although the molecular composition is similar the fiber orientation and arrangement may be different in these tissues. We decided to use the Achilles tendon model due to the rotational orientation of the fibers within the tendon itself and the uniformity and ease of performing the biomechanical testing on this tissue.

Few studies evaluated the effects of thermal modulation on the biomechanical properties of soft tissue. Lu et al.[5] compared the effect of thermal shrinkage on joint capsule of sheep, using Grid and Paintbrush techniques. They evaluated the level of shrinkage and the healing abilities after modulation. Comparing the shrinkage level, no statistical difference was found, as in our study. Comparing tissue healing abilities, it was found that at the end point of the study (12 weeks of treatment) tissue strength was similar, but the joint capsules that were treated by the Grid technique, reached peak strength 6 weeks before the joint capsules treated by the Paintbrush technique. Lu et al.[5] claim that treatment using the Grid technique in gaps of 5mm between treated lines decreases the amount of damaged tissue and allows better contact between living and necrotic tissue. Accordingly, this contact enables more effective fibroblast passage and better replacement of necrotic tissue by new tissue. This study, however, did not evaluate the influence of implementation of these techniques on mechanical properties.

Clinically, the Paintbrush technique is much more commonly used. In this technique, the surgeon passes the RF probe in a uniformal manner along the treated tissue leaving no untreated areas. Indelli et al.[28], published their experience with 28 patients who suffered from clinical instability following ACL reconstruction. All patients were treated using monopolar shrinkage of their reconstructed ligament in accordance with the Paintbrush technique leaving only 4mm gaps at the ends of the graft. At 2 years, 96% of patients went back to the activity level as prior to the injury, and knee stability was normal or near normal. They emphasized the importance of immediate rehabilitation treatment after thermal shrinkage as well. In their study, the treated knee was protected by a knee brace with non weight bearing for the first 6 weeks, with passive mobilization and ROM training beginning the second week after the thermomodulation procedure. This protocol is based on an in vivo study, showing that after thermal shrinkage using RF energy, tissue regains its mechanical strength 6-8 weeks following manipulation[31]. Other studies involving thermal ACL reconstruction shrinkage did not emphasize the rehabilitation protocol and presented ambivalent results[25, 26]. We believe our results may shed some light on these results in showing that treated tissue fails at smaller loads and, thus, when thermal energy is used as a surgical tool strict rehabilitation protocols are called for and should be adhered to.

Our findings do not coincide with those of Lu et al.[5], however, who found no significant differences between tissue that was treated by either the Grid or Paintbrush techniques at the 0 and 2 week intervals from treatment. In our study we found that using the Grid technique weakened the tissue such that the load to failure in the Grid group (A) was 19.4% lower than in the Paintbrush group (B) (p=0.092). Although we did not evaluate the healing process *in vivo*, we assume that treatment using the grid technique will require strict rehabilitation, while remembering treated tissue is most vulnerable during the first few weeks after treatment.

In conclusion, the findings of our study show that thermal modulation causes changes in the mechanical and histological properties of rabbit Achilles tendons. Our findings show that utilizing the Grid technique causes a point of failure along the treated tendons and thus they are less resistant to stretch. Our findings demonstrate the need for a conservative rehabilitation protocol for patients intending to go through thermal RF shrinkage and to regard patient willingness and ability to adhere to conservative rehabilitation as one of the inclusion criteria for such treatment.

References

1. Woo SL, Debski RE, Withrow JD, Janaushek MA. Biomechanics of knee ligaments. Am J Sports Med 1999; 27: 533-543.
2. Woo SL, An KN, Arnocky SP, Wayne JS, Fithian DC, Meyers BS. Anatomy, biology, and biomechanics of tendon, ligaments and meniscus. In: Simon SL, ed. Orthopaedic basic science. Chicago: American Academy of Orthopaedic Surgeons, 1994; 45-87.
3. Rockwood CA Jr, Wirth MA, Subluxations and dislocations about the glenohumeral joint. In: Rockwood CA Jr, Green DP, Bucholtz, RW, Heckman JD, eds. Rockwood and Green's fractures in adults. Ed 4. Lippincott - Raven, 1996: 1194.
4. Lopez MJ, Hayashi K, Vanderby R, Jr, Thabit G 3rd, Fanton GS, Markel MD. Effects of Monopolar radiofreqency Energy on ovine joint capsular mechanical properties. Clin Orthop 2000(374): 286-297.
5. Lu Y, Hayashi K, Edwards RB 3rd, Fanton GS, Thabit G 3rd, Markel MD. The Effect of Monopolar Radiofrequency Treatment Pattern on Joint Capsular Healing. Am J Sports Med 2000; 28(5) 711-719.
6. Organ LW. Electrophysiological Principles of Radiofrequency Lesion Making Appl Neurophysiol 1996; 39: 69-76.
7. Van Haesendonck C, Sinnaeve A, Willems R, Vandenbulcke F, Stroobandt R. Biophyssical and electrical aspects of Radiofrequency catheter ablation. Acta Cardiol 1995; 50: 105-115.
8. Nath S, Dimarco JP, Haines DE. Basic aspects of Radiofrequency Catheter ablation. J Cardiovasc Electrophysiol 1994; 5: 863-876.
9. Cosman ER, Nashold BS, Bedenbaugh P: Stereotactic radioferquency lesion making. Appl Neurophysiol 46: 160-166, 1983.
10. Fanton GS, Khan AM. Monopolar Radiofrequency energy for arthroscopic treatment of shoulder instability in the athlete. Orthop Clin North Am 32: 511-523, 2001.
11. Thabit G III. Therapeutic Heat: A historical perspective. Oper Tech Sports Med1998; 6: 118-119.
12. Achuaer BM. Lasers in plastic surgery: current practice. Plast Reconstr Surg 1997: 1442-1450.
13. Sherk HH. The use of lasers in orthopaedic procedures. J Bone Joint Surg Am 1993; 75A: 768-776.
14. Anderson RR, Parrish JA. Selective photothermolysis: precise microsurgery by selective absorption of pulse radiation. Science 1983; 220: 524-527.
15. Osmond C, Hecht P, Hayashi K, et al. Comparative effects of laser and Radiofrequency Energy on joint capsule. Clin Orthop 2000(375): 286-294.
16. Hayashi K, Peters DM, Thabit G 3rd et al. The Mechanism of Joint Capsule Thermal Modification in an In Vitro Sheep Model. Clin. Orhtop. 2000(370): 236-249.
17. Naseef GS 3rd, Foster TE, Trauner K, Solhpour S, Anderson RR, Zarins B. The thermal properties of bovine joint capsule. The basic science of laser – and Radiofrequency induced capsular Shrinkage. Am J Sports Med 1997; 25(5) 670-674.
18. Vangsness CT Jr., Mitchell W 3rd, Nimni M, Erlich M, Saadat V, Schmotzer H. Collagen Shortening. An Experimental Approach With Heat. Clin. Orthop.1997(337): 267-271.
19. Lopez MJ, Hayashi K, Fanton GS, Thabit G 3rd, Markel MD. The effect of radiofreqeuncy energy on the ultrastructure of joint capsular collagen. Arthroscopy 1998; 14(5): 495-501.
20. Hayashi K, Thabit G 3rd, Bogdanske JJ, Cooly AJ, Orwin JF, Markel MD. Effect of nonablative laser energy on the joint capsule: An in vivo rabbit study using holmium:YAG laser. Lasers Surg Med 1997; 20: 164-171.
21. Hayashi K, Hecht P, Thabit G 3rd et al. The Biological Response to Laser thermal modification in an In Vivo Sheep Model, Clin Orthop 2000(373) 265-276.
22. de Mos M, Koevoet WJ, Jahr H et al. Intrinsic differentiation potential of adolescent human tendon tissue: an in vitro cell differentiation study. BMC Musculoskelet Disord 2007, 23: 8-16.
23. Hayashi K, Thabit G 3rd , Bogdanske JJ, Theilke RJ. The effect of nonablative laser on joint capsular properties: An

in vitro mechanical study using a rabbit model. Am J Sports Med 1995; 23: 482-487.

24. Obrzut SL, Hecht P, Hayashi K, Fanton GS, Thabit G 3rd, Markel MD. The effect of radiofrequency Energy on the length and temperature properties of the glenohumoral joint capsule. Arthroscopy 1998; 14(4): 395-400.
25. Medvecky MJ, Ong BC, Rokito AS, Sherman OH. Thermal Capsular Shrinkage: Basic science and clinical applications. Arthroscopy July 2001: Vol 17(6); 624-635. Review.
26. Sekya JK, Golladay GJ, Wojtys EJ. Autodigestion of a Hamstring Anterior Cruciate Ligament Autograft, Following Thermal Shrinkage. JBJS Am Oct. 2000, NO.10, Vol. 82-A: 1454-1457.
27. Carter TR, Bailie DS, Edinger S. Radiofrequency Electrothermal Shrinkage of Anterior Cruciate Ligament. AM J Sports Med 2002; 30(2): 221-226.
28. Indelli PF, Dillingham MF, Fanton GS, Schurman DJ. Monopolar thermal treatment of symptomatic anteriot Cruciate ligament instability. Clin Orthop Relat Res 2003 (407): 139-147.
29. Oliva F, Via AG, Maffulli N. Role of Growth factors in rotator cuff healing. Sports Med Arthrosc 2011 Sep; 19(3): 218-226. Review.
30. Maffulli N, Benazzo F. Basic sciences of tendons. Sports Medicine and Arthroscopy Review 2000; 8:1-5.
31. Hecht P, Hayashi K, Lu Y, et al. Monopolar radiofrequency energy effects on joint capsular tissue: Potential treatment for joint instability: An in vivo mechanical, morphological, and biomechanical study using an ovine model. Am J Sports Med 1999 (27): 761-771.