

# A long term study on the role of exogenous human recombinant basic fibroblast growth factor on the superficial digital flexor tendon healing in rabbits

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## Abstract

**Background:** This study was designed to investigate the effects of basic fibroblast growth factor on the remodeling phase of the tenotomized superficial digital flexor tendon in rabbits. **Methods:** Forty white New Zealand mature male rabbits were divided randomly into two equal groups of treated and control. After tenotomy and surgical repair, using modified Kessler technique and running pattern, the injured legs were casted for 14 days. Human recombinant basic fibroblast growth factor (bFGF) was injected subcutaneously over the lesion on days 3, 7 and 10 post injuries. The control animals received normal saline injection similarly. The weight of the animals, tendon diameter, radiographic and ultrasonographic evaluations was conducted at weekly intervals. The animals were euthanized 84 days post-injury and the tendons were evaluated at macroscopic, histopathologic and ultrastructural level and were also assessed for biomechanical and percentage dry weight parameters. **Results:** Treatment significantly reduced the diameter and increased the echogenicity and dry weight content of the injured tendons. Treatment also significantly enhanced the maturation of the tenoblasts, fibrillogenesis, the collagen fibrils' diameter, fibrillar density, stiffness, and ultimate and yield strength. **Conclusions:** Subcutaneous administration of human recombinant bFGF is effective in restoring the morphological and biomechanical properties of the injured SDFT in rabbits.

**Keywords:** Tendon Healing, Ultrastructure, Histopathology, Biomechanics, Basic Fibroblast Growth Factor (bFGF)

## Introduction

Tendons are dense connective tissues responsible for passively transferring forces generated by muscles to the opposite side of the joint, and support normal movements and the stability of the animal<sup>1,2</sup>. When subjected to high physiological loads, these tissues are commonly injured and fail to heal optimally due to their low cellularity and vascularity<sup>3</sup>. Tendon injuries produce substantial morbidity, and at present there are only a limited number of scientifically proven management modalities<sup>4</sup>. Tendons are at the highest risk for rupture if ten-

sion is applied quickly and obliquely, and the highest forces are seen during eccentric muscle contraction<sup>4</sup>.

Growth factors (GF) are short sequences of amino acids which usually transmit signals between cells and thereby modulate their activities<sup>5,6</sup>. They regulate cell activity by a number of mechanisms such as mitogenic activity, cell differentiation, cell migration and gene regulation, and play important roles in cell chemotaxis, proliferation, matrix synthesis, and differentiation. The use of growth factors to enhance tendon healing remains largely experimental and has been restricted to *in vitro* studies and animal models<sup>4,6</sup>.

The beneficial effects of bFGF on the proliferation and collagen production of the tenocytes<sup>6,9</sup>, differentiation of bone marrow stromal cells into osteoblasts<sup>10</sup>, local anabolic effects in bones<sup>11</sup>, angiogenesis<sup>12</sup>, regeneration of neurons in the central and peripheral nervous systems, and enhancing the healing processes of the corneal epithelium<sup>13</sup> have previously been reported.

Therefore, the present study was designed to investigate the effects of bFGF, as a mitogenic and maturation inducing agent, on the remodeling stage of healing of the SDFT of rabbits after

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84 days post tenotomy and surgical repair. This experiment was designed on the basis that bFGF can stimulate maturation of the tenoblasts, initiate earlier collagen formation and maturation, and result in an improved biomechanical performance of the injured treated tendon. These hypotheses were morphologically and biomechanically tested 84 days post-injury on a complete sectioned superficial digital flexor tendon in rabbits.

The proximal section of the SDFT has been selected as the tissue of choice by many investigators as an extra-synovial model because of its accessibility and easier exposure<sup>14-19</sup>. In addition, it simulates the hand flexor tendon of humans and SDFT injuries in horses<sup>20,21</sup>. Superficial digital flexor tenodinitis is a common career injury in sport horses, and when severe or with recurrence it can result in early retirement or destruction of the affected horse<sup>22</sup>.

## Material and methods

### *Experimental Design*

Forty skeletally matured male White New Zealand rabbits of 10 to 14 months of age, and 1.84±0.89 kg body weight were randomly divided into two equal injured treated and injured control groups.

The left SDFT of the rabbits of each group was designated as the injured tendon and the right (normal contra-lateral tendon) was designated as the index of normal tendon for each group. The rabbits were kept in individual standard rabbit cages and were maintained on standard rabbit diet with no limitation of access to food or water.

### *Injury induction*

The animals were anesthetized by intramuscular injection of 1 mg/kg Xylasin 2% as premedication and 60 mg/kg Ketamin HCl 10% for anesthesia. The left hind leg of each animal was designated as “experimental” and the skin over the common calcaneal tendon (CCT) was shaved and disinfected using normal surgical aseptic technique. After skin incision the SDFT was exposed and transversely tenotomized at the mid part between the gastrocnemius muscle and calcaneal tuberosity, and reconstructed with modified Kessler core suture and running pattern by the same absorbable polyfilament polygalactin 910, 4-0 and 6-0 sutures, respectively (Ethicon coated Vicryl, taper cut needle, J&J, USA). The paratenon was sutured using simple continuous pattern with the same material no 8-0, to complete the surgical repair of the injured area and prevent further adhesion formation by the invading tenoblasts. The skin over the lesion was closed with a simple continuous pattern, using non-absorbable silk material no 3-0 (Ethicon coated Silk, taper cut needle, J&J, USA). A cast (Dyna cast 5 CM, An-Yang, Korea Co., LTD) was then applied for two weeks at 70 degrees of extension. A rectangular window was created in the cast at the site of injury (plantar surface of the hind limb) to facilitate injection of the reagent around the lesion<sup>16</sup>.

### *Treatment program*

Human recombinant bFGF (Vial, Rooyan Research Institute, Tehran, Iran) was diluted with normal saline, giving it the same

viscosity as the normal isotonic saline. Doses of 1200 ng/kg of this reagent were injected subcutaneously at the site of injury through the window cast on days 3, 7 and 10 post-injury according to the manufacturer’s recommendation, and with regards to the previous studies<sup>23-25</sup>. The manufacturer recommended that a dose of 1200 ng/kg was beneficial in tenocytes proliferation and tenoblasts infiltration on the *in vitro* and clinical situation (Rooyan Research Institute, Tehran, Iran).

In the control group, normal 0.9% saline was injected similarly on the site of injury at the same time and volume as the treated animals.

### *Pre euthanasia measurements*

The animals were weighed and the tendon and the covering skin diameter around the injury site and a comparable area of the uninjured contra-lateral tendon were blindly measured using a micrometer measurement device (SAMSUNG, Seocho-gu Seoul, Korea) before operation, and then at weekly intervals until the end of the experiment. Each measurement was taken three times to ensure that the repeatability of the measurements of the width was within 0.2 mm. From these, the average cross-sectional area of the tendon, together with the fascia and skin over it, was calculated by two blind observers<sup>14,16</sup>.

For the clinical investigations, two blind observers determined the swelling, lameness and locomotion activity of the rabbits in the cage. These criteria were checked, 3 times a day in 8 hour intervals. Based on the blind observer’s reports, the assessment was qualitative.

Lameness and comfortable/uncomfortable physical activities were defined as tarsal flexion degree of each animal, both in the cage and on the floor, the weight distribution of each animal on the hind limbs, both in the cage and on the floor, pain in palpation of the injured area, pain in complete extension of the hind paw and toe, and heel position of the injured leg<sup>26-28</sup>.

The radiographic and ultrasonographic observations were blindly evaluated by a veterinary radiologist at weekly intervals for 12 weeks.

The animals were sonographed at cross and longitudinal sections with a 12 MHz linear probe (Simense SLR-400 device, Berlin, Germany; Echowave 3.23 software). The cross sectional area, because of the narrow diameter of the rabbit’s SDFT, was not diagnostic, but the longitudinal sections were diagnostic, and as such were assessed for qualification<sup>16</sup>.

### *Ethics and euthanasia*

After administering sodium thiopental (50 mg/Kg) to induce coma, pancuronium (Pavulon Ink Co. USA) (1 mg/Kg) was delivered in order to stop breathing to perform a comfortable euthanasia.

The study was approved by the local ethics committee of our faculty, in accordance with the ethics standards of “Principles of Laboratory Animal Care”.

### *Sample collection*

The method has been described previously by the authors<sup>15-17</sup>, briefly; specimens from each of the injured and uninjured SDFT

of 10 animals of each group were collected for light and electron microscopic studies and percentage dry weight analysis. In the remaining 10 animals of each group, both injured and contra-lateral SDFT were carefully dissected from the surrounding tissues for biomechanical testing.

### Gross pathology

The left and right SDFT of all animals were carefully inspected and were then photographed. The pictures were transferred to Adobe Photoshop® CS-4 Program and qualitatively analyzed with computerized morphometric technique for determination of the hyperemia, pretendinous adhesion and tendon diameter. The method was blind and qualitative<sup>16</sup>.

### Histopathology

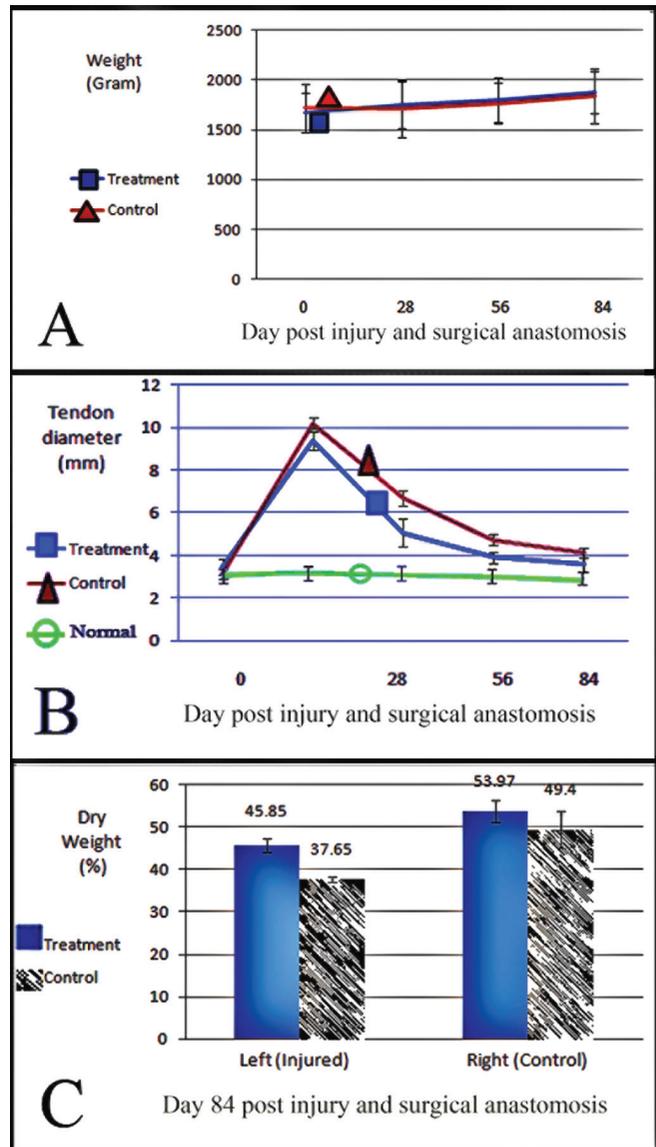
After fixation in 10% neutral buffered formalin, the tendon samples were washed, dehydrated, cleared, embedded in ester wax, sectioned at 4-5  $\mu\text{m}$ , stained with haematoxylin and eosin and examined by a light microscope (Olympus, Tokyo, Japan). The cell count and vascular populations of each section were estimated using an eye piece graticule. An average was then taken from five different microscopic fields for each cell type and blood vessel. Duplicate counts were carried out by double blind method. In addition, using a digital camera (Sony T-700, Tokyo, Japan), the pictures from each tissue section were transferred to a computer for morphometric analysis. The maturity of the tenoblasts and tenocytes together with the density of the collagen fibers and blood vessels on the normal and inverted photomicrographs were determined using Adobe Photoshop cs-3 10 final. The crimp pattern, tissue maturation, alignment and density, together with the types of degeneration and foreign body reactions in each sample, were qualitatively analyzed by the two blind pathologists<sup>16,29</sup>.

### Electron microscopy

The samples were fixed in cold 4% glutaraldehyde, dehydrated and embedded in Epon resin 97. Thin sections of 80-90 nanometer (nm) in diameter were cut and standard methods were employed for production of the transmission electron micrographs (Philips CM 10 transmission electron microscope, Eindhoven, Netherlands). Ultra-micrographs of different final magnifications (5,200-158,000) were taken for studying the collagen and elastic fibrillar morphology, differentiation, morphometri and density, inflammatory cell constituents and tenoblast maturity. The diameter of the collagen fibrils of five different fields of the same magnification for each tissue section was measured, their number counted, and their mean diameter calculated by a computerized morphometric technique using Adobe Photoshop CS4. In addition, the number of elastic fibers of each field was counted and their maturity was qualitatively evaluated by the blind pathologists<sup>15,16,29</sup>.

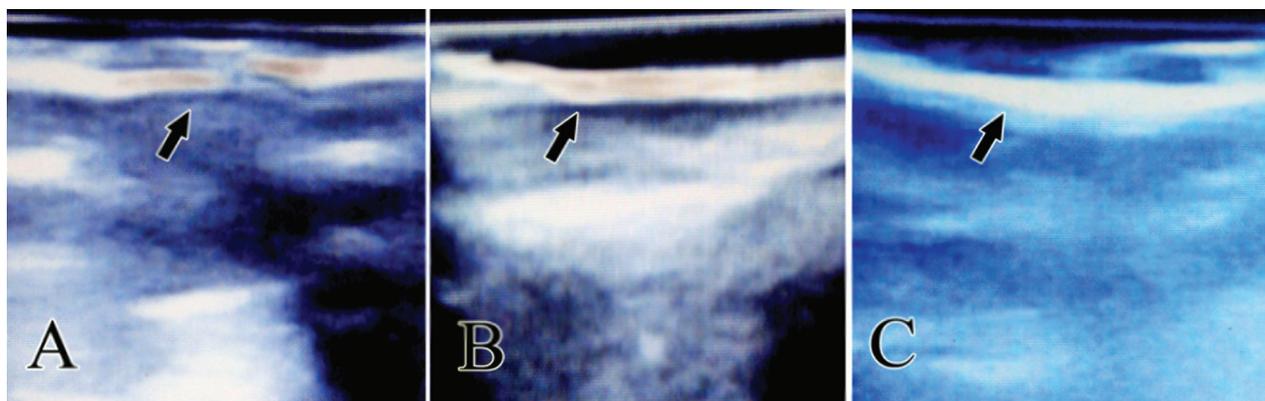
### Biomechanics

After application of standard preservation methods, the biomechanical tests were performed using a tensile testing ma-



**Figure 1.** A. The weight of the animals has no significant differences during the course of the experiments between the two groups. Note that there is a significant difference between the beginning and the end of the experiment in the weight of the injured treated animals. B: The tendon diameters of the injured treated tendons are significantly lower than those of the injured control ones from day 14 to day 28 post injury, however, there are some great differences between the diameters of the injured treated tendons with their normal contra-lateral tendons. C: The dry matter content of the injured treated tendons is significantly higher than in the injured control tendons, but they are still inferior to their normal contra-lateral tendons.

chine (Instron Tensile Testing Machine., London, England). The specimens were mounted between two metal clamps and were subjected to tensile deformation at a strain rate of 10 mm/min and the load deformation and stress-strain curves were recorded by a personal computer. The ultimate tensile strength, yield strength, ultimate strain, yield stain, stress and stiffness were determined<sup>17,30</sup>.



**Figure 2.** Ultrasonography: Diagnostic ultrasound in a longitudinal view at 12 MHz of the SDFT on day 28. A) Injured control tendon. It is not uniform and shows amputated view with hypo echoic, heterogeneous defective middle [arrowhead] (mag. x4). B) Injured treated tendon. Homogeneous highly echogenic fibers which is consistent with a near to normal, healthy and intact tendon [arrowhead] (mag. x4). C) Normal contralateral tendon. Homogeneous, highly echogenic fibers with no amputation view [arrowhead] (mag. x4).



**Figure 3.** Gross pathology: A) Normal contra-lateral of the injured treated group shows smooth, yellowish and homogeny surface. B) Injured treated tendon. The color of the tendon is almost similar to the normal tendon. The diameter of the injured treated tendon is less than that of the injured control one. C) Injured control tendon. The color of the injured area is red to brown and hyperemic. Adhesion to surrounding tissue and high diameter is present at the site of injury. However, the diameter of the injured treated is less than that of the injured control one, but both are still larger than those of their normal contralateral tendons. D) Normal contra-lateral of the injured control group shows smooth, yellowish and homogeny surface.

#### Percentage dry weight

The samples were weighed immediately after euthanasia and were freeze-dried (Helosicc, Ink, Co. London, England) to a constant dry weight as previously described<sup>14,15,30</sup>.

#### Statistical analysis

After application of the normality distribution test, the injured tendons of the animals of each group were compared

with the normal contra-lateral tendons of the same group using paired sample *t-Test*. The right and left tendons of the injured treated animals were compared with the right and left tendons of the injured control animals, using the independent sample *t-Test*. Nonparametric tests were applied to check the results again. Statistics were performed using the computer software SPSS version 17 for windows (SPSS Inc., Chicago, IL, USA). Differences of  $p < 0.05$  were considered statistically significant.

Variable	Injured treated tendons	Injured control tendons	P value
Fibroblast	135.50+21.36	237.75+26.58	0.001
Fibrocyte	32.00+4.24	14.00+7.43	0.006
Macrophage	2.25+0.95	3.25+1.50	0.304
Lymphocyte	7.75+2.36	14.25+3.68	0.049
Neutrophil	negative	negative	
Mature fibroblast	31.25+1.75	15.50+3.33	0.021
Immature fibroblast	8.50+1.50	15+4.83	0.037
Vascularity	1.25+0.50	3.75+0.75	0.004
	<i>Normal contra-lateral tendons Of the injured treated group</i>	<i>Normal contra-lateral tendons of The injured control tendons</i>	
Fibroblast	0	33+49.65	0.232
Fibrocyte	28.25+7.5	12.03	0.436

*Number of fields for each tendon=5, P<0.05 was considered significant, independent sample t-test was used for comparison between left – left tendons of injured treated and injured control groups. Microscopic field magnification for cell count =x200 and for cellular maturation analysis=x800*

**Table 1.** Histopathologic cell differentiation analysis: comparison of the cell types of injured treated tendons with bFGF and injured control tendons, day 84 post injury and surgical anastomosis.

## Results

### Weight

No significant differences were found in the weight of both groups at all post surgical intervals. However, at the end of the experiments the weight of the injured treated rabbits were significantly greater than at the beginning of the experiment ( $P=0.049$ ) (Figure 1A).

### Tendon diameter

As is shown in Figure 1B, there were no significant differences between the tendon diameters of both groups before surgical operations ( $P=0.052$ ); however, treatment with human recombinant bFGF significantly decreased the diameter of the injured tendon compared to the injured control group from days 28 ( $P=0.005$ ) to 84 ( $P=0.001$ ) post-surgical operation. Compared to the injured control tendon, the diameter of the injured treated tendon showed a faster reduction so that it was comparable to the normal tendon at the end of the experiment, and with the injured treated tendon before injury induction ( $P=0.165$ ,  $P=0.324$ ).

### Clinical observations

Treatment reduced edema and lameness and the injured leg of the treated animals showed proper weight bearing capacity from day 21 onwards. From day 28 post injury until the end of the experiment, the weight bearing capacity and locomotors activity of these animals was comfortable and comparable to the normal animals. However, most of the injured control animals showed lameness and uncomfortable physical activity up to day 40 post injury.

### Radiography

No lesion such as soft tissue swelling and calcification, osteoarthritis and bone fracture with the presence of abnormal radiolucency and radio-opacity was observed in either group.

### Ultrasonography

Cross sectional ultrasonography was not diagnostic with regard to the diameter of the SDFT of rabbits. At longitudinal sections, the treatment increased echogenicity and homogeneity, while decreasing the tendon diameter. No amputated view was observed in the injured treated tendons compared to those of the injured control one (Figure 2).

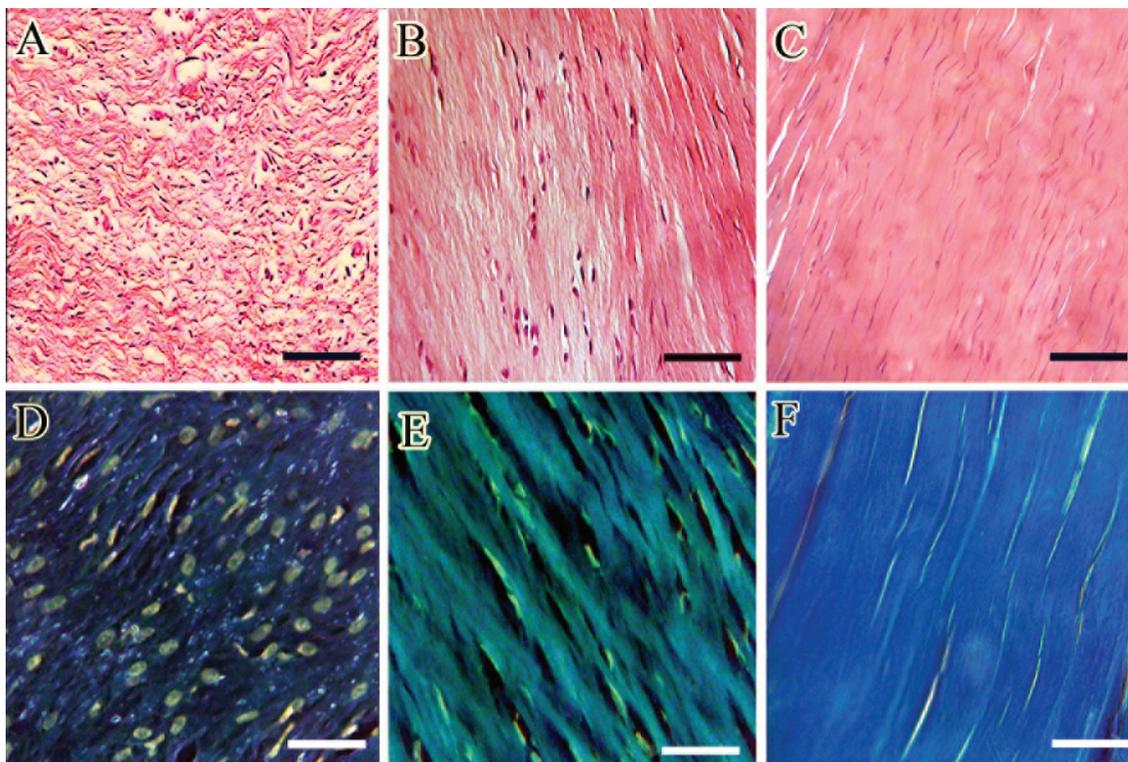
### Gross pathology

The injured tendons of all the control animals were thicker and hyperemic, and additionally showed severe adhesions to the surrounding peritendinous fascia. Treatment reduced the diameter of the injured tendon. The injured treated tendons had a shiny glistening appearance similar to those of the normal contra-lateral tendons. Except for the injured lesions of three animals that showed loose adhesion to the surrounding fascias, the tendons of the other remaining rabbits showed no peritendinous adhesions (Figure 3).

### Histopathology

Both injured treated and injured control tendons showed no areas of necrosis, acute inflammation and calcification. The lesions of the injured control or treated animals showed hypercellularity with proliferation of tenoblasts and mild infiltration of macrophages, lymphocytes and plasma cells (Figure 4). However, compared to those of the injured control tendons, treatment significantly reduced the swelling and cellularity of the lesions (Table 1). In addition, treatment enhanced the maturity of the tenoblasts so that number of the tenocytes and mature tenoblasts of the injured treated tendons were significantly higher than those of the injured control tendons ( $P=0.006$ ,  $P=0.021$ ) respectively.

Although the newly regenerated fibrous connective tissue showed a haphazard orientation with no crimp pattern in the lesions

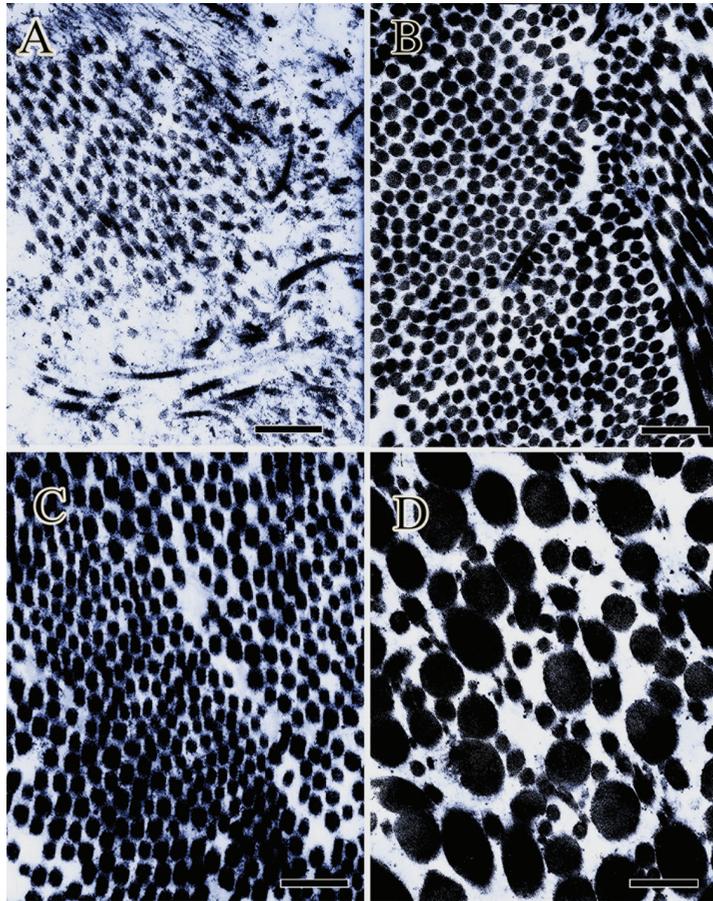


**Figure 4.** Histopathologic findings: A, D) Injured control, day 84 post injury. The lesion is organized. Most of the tenoblasts are still immature with no alignment. The vessels and perivascular edema are characteristic in the histopathologic field. The collagen appearance around the vascular appearance is not oriented in normal anatomical of the tendon direction. Most of the fibroblasts are immature and show a haphazard pattern of organization. B, E) Injured treated tendon, day 84 post injury. The tissue is more organized and shows a better alignment and maturation than the injured control ones. The tenoblasts are more mature and show a more advanced organization. The collagen density is much higher than the injured control tendons. The cigar and longitudinal shaped tenocytes with low cytoplasm and dense nuclei are characteristic as a mature tenocyte. C, F) The normal contra-lateral tendon: the collagen density is high and there are a limited number of tenocytes with no vascular or edema appearance. The collagen fibers are oriented in one direction, exactly on the normal anatomical directional line (Scale bar for Figs. A, B, C=75  $\mu$ m and for D, E and F=15  $\mu$ m).

Variable	Injured treated tendons	Injured control tendons	P Value
<i>Number of collagen fibrils at different ranges</i>			
33-64 (nm)	95.50+48.34	378+51.55	0.001
65-102 (nm)	169.25+34.44	88+25.88	0.009
Total	264.75+55.66	466+67.14	0.004
Collagen Density (Fibrils/area) (%)	97.64+1.35	84.56+3.16	0.001
<i>Collagen fibril diameter at different range</i>			
33-64(nm)	56.40+5.53	46.79+4.37	0.035
65-102 (nm)	93.59+5.65	70.50+4.91	0.001
Total	80.80+7.95	51.12+4.11	0.001
Number of Elastic fibers	3.25+0.25	0.075+0.75	0.049

*Number of fields for each tendon=5, (P<0.05 was considered significant), analysis magnification =39000, independent sample t-test was used for comparison between left and left tendons between injured treated and injured control groups.*

**Table 2.** Ultrastructural morphometric analysis of the number of the collagen fibrils and fibrillar diameter at different ranges (nm), collagen fibrils density (%) and number of elastic fibers-comparison between injured treated with bFGF and injured control



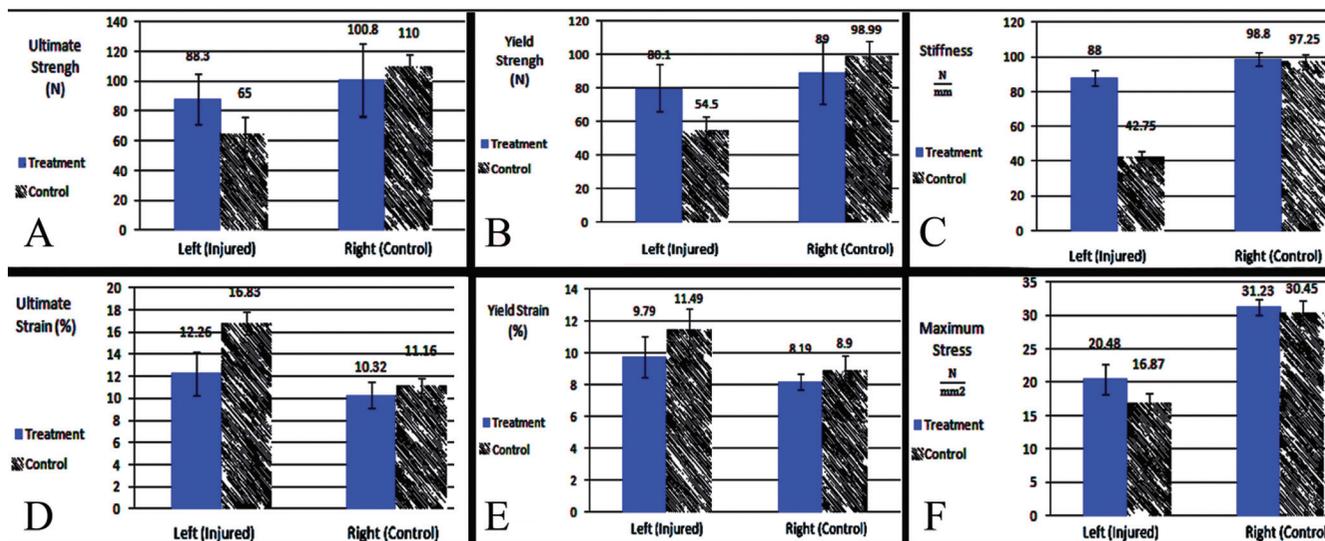
**Figure 5.** Ultrastructural findings: A): Injured control tendon. The newly regenerated small diameter collagen fibrils are unimodally distributed. B, C): Injured treated tendon. The newly regenerated collagen fibrils are numerous with bimodal distribution pattern. They become more mature and aligned in the normal anatomical orientation. D) Normal contralateral tendon with multimodal distribution of collagen fibrils (scale bar=360 nm).

of the injured control tendons, a parallel alignment with crimp formation along the longitudinal axis of the injured treated tendons was seen. The density of the collagen fibers of the treated lesions was comparable to the normal tendons. Compared to those of the injured control tendons, there were significantly fewer blood vessels with a larger caliber in the tendon proper of the injured treated tendons ( $P=0.004$ ). In addition, the blood vessels of the injured control tendons were surrounded by perivascular edema. The paratenon of the injured treated tendons were properly aligned along the longitudinal axis of the tendon mass and showed no abnormalities, however, the paratenon of the injured control tendons were thick and contained numerous irregularly orientated small blood vessels and tenoblast. Also while remnants of the suture material were still present in the control lesions, no suture material was seen in the treated lesions and the suture material of the injured treated lesions resorbed faster than the injured control ones.

#### Ultrastructure

Treatment enhanced the orientations and alignment of the collagen fibrils towards the normal longitudinal direction of the ten-

don. Compared to the injured control tendons, treatment enhanced the collagen fibrils differentiation and maturation so that the diameter and density of the collagen fibrils of the injured treated tendons were significantly higher than those of the injured control tendons ( $P=0.001$ ,  $P=0.001$ ) respectively (Figure 5). The collagen fibrils in the injured control tendons were of small-sized fibrils in the range of 33 to 64 nm and showed a unimodal distribution pattern. However, the collagen fibrils in the lesions of the injured treated tendons showed a bimodal pattern and, in addition to the small sized collagen fibrils of 33 to 64 nm, larger collagen fibrils in the range of 65 to 102 nm were also present in these lesions. While the collagen fibrils in the range of 33 to 64 nm in diameter were significantly fewer in the lesions of the treated tendons compared to those of the injured control tendons ( $P=0.001$ ), larger diameter collagen fibrils in the range of 65 to 102 nm were only present in the lesions of the treated tendons, so that at this stage the total mean collagen fibrils diameter of the injured treated tendons were also significantly greater than those of the injured control tendons ( $P=0.009$ ) (Table 2). Treatment also significantly increased the number and maturation of the elastic fibers ( $P=0.049$ ) (Figure 5).



### Day 84 post injury and surgical anastomosis

**Figure 6.** Ultimate strength (A), yield strength (B), stiffness (C) and maximum stress (F) of the injured treated tendons are significantly greater and the ultimate strain (D) and yield strain (E) are significantly lower than those of the injured control tendons.

#### Biomechanical properties

Treatment strongly improved the biomechanical properties of the injured treated tendons and the ultimate strength ( $P=0.034$ ), yield strength ( $P=0.006$ ), ultimate strain ( $P=0.002$ ) and stiffness ( $P=0.001$ ) of the injured treated tendons showed significant improvement compared to those of the injured control tendons. However, there were no significant differences between the yield strain ( $P=0.069$ ) and ultimate stress ( $P=0.094$ ) of the injured treated with the injured control tendons. On the other hand, except for ultimate strength ( $P=0.058$ ) and ultimate strain ( $P=0.107$ ), at this stage, these parameters were still inferior to their normal contra-lateral tendons. In addition, there were no significant differences between the biomechanical properties of the normal contra-lateral tendons of both groups (Figure 6).

#### Dry matter

Treatment significantly increased the dry matter content of the injured treated tendons compared to those of the injured control ones ( $P=0.001$ ). However, at this stage the dry weight contents of the treated lesions were still significantly inferior to those of their normal contra-lateral tendons ( $P=0.005$ ). The contra-lateral tendons of both groups had no significant differences in their percentage dry weight content ( $p=0.125$ ) (Figure 1C).

#### Discussion

The findings of the present experiment clearly showed that repeated administration of human recombinant bFGF could enhance the structural and biomechanical properties of the ex-

perimentally tenotomized SDFT in rabbits. Many factors such as peritendinous adhesion, delayed inflammation, immaturity and lack of proper organization of the collagen fibers and many other factors interfere with the healing process and influence the biomechanical performance of the healing tendon<sup>4</sup>. The results of the present study showed that injured SDFT of rabbits are at a high risk of peritendinous adhesion formation. Adhesion formation poses a major clinical problem, and if it is not prevented, the tendon is unable to move in its location and is not able to transfer the load between muscle to bone, resulting in enhanced alignment and maturation of the tenoblasts and collagen fibers<sup>1,4</sup>. Treatment with bFGF possibly relieved acute inflammation and post-surgical edema and additionally inhibited peritendinous adhesion formation and resulted in painless movement. It has been shown that bFGF inhibited acute inflammation and pain by reducing the prostaglandine E2 concentration, nitric oxide activity and scavenging oxygen free radicals<sup>13</sup>. These results explain why bFGF conserved viscoelasticity of the tendon and facilitated earlier locomotion activity of the injured treated animals compared to those of the injured control ones.

There are limited data regarding the anti-adhesive effects of bFGF on tendon healing. The findings of the present study were in accordance with those of Sha et al. (2004), who showed that bFGF diminished further degradation of Achilles tendon in rat, enhanced intrinsic healing of the tendon and inhibited adhesion formation in the extra synovial flexor tendon<sup>31</sup>. However, the result of the present study is not in agreement with that of Sheng et al. (2007), who demonstrated that exogenous bFGF stimulated adhesion formation in a

leghorn chicken model after eight weeks post injury<sup>32</sup>. The difference between the results of the present study with those of Sheng et al. (2007) may be due to the differences in the animal models, the dose of this growth factor, the time and mode of administration, and also the tendon model under experiment such as intra or extra synovial tendon<sup>32</sup>. It has been stated that the root and timing of the administration of bFGF is very important on the results of the healing process, and if administered after the first week post injury, results in an improvement of the biomechanical properties and structural performance of the injured tendon<sup>1,33-36</sup>. The administration time in the present study was on days 3, 7 and 10 post injuries, while in the previous study, the time of administration was at the time of injury induction.

The Influence of bFGF on the structural organization of the tendon including improved tissue alignment and crimp formation, enhanced cell maturation, increased collagen fibrils differentiation and maturation with decreased peritendinous adhesion are possibly the most significant effects of bFGF on the tendons healing. Enhanced percentage dry weight and collagen density are the consequences of these structural changes.

Therefore, due to the enhanced hierarchical organization, improved biomechanical parameters seen in the treated animals of the present study are expected. The tensile strength of the tendons is co-related to the total collagen content, type of collagen, diameter and unimodal or multimodal distribution pattern of the collagen fibrils, quality of the cross links of the collagen fibrils, and quantity and quality of the non-collagenous material of the ground substance<sup>1,4,14,15,17,29,30</sup>. Enlargement of the collagen fibrils may occur through deposition of more collagen on the existing fibrils or could be due to aggregation of a number of the newly regenerated small-sized collagen fibrils<sup>1,4,14,29</sup>.

The findings of the present study are in agreement with those of Hamada et al. (2006) who showed that, after placement of the nylon monofilament coated with bFGF in the injured area of the flexor tendon of rabbits, the treated animals showed significantly enhanced ultimate strength compared to those of the control, after 21 days post injury<sup>37</sup>. Tang et al. (2008) also found that injured SDFT treated with bFGF showed a higher tensile strength at 2, 4, and 8 weeks post injury<sup>38</sup>. On the other hand, it has also been reported that bFGF affected the initial events of tendon healing on the cell proliferation and type III collagen expression, but had no significant effect on the ultimate stress of the injured tendon during the first two weeks post injury in a rat patellar model<sup>24</sup>. Additionally it has been demonstrated that bFGF accelerated the cell-proliferation phase of tendon healing, but it failed to produce improvements in either the mechanical or functional properties of the injured area on day 21 post injury in a canine intrasynovial flexor tendon study<sup>23</sup>. The differences in the ultimate stress in the present study with those of Chan et al. (2000) and Thomopoulos et al. (2010) were probably due to the duration of the study and the stage of healing<sup>23,24</sup>. Low biomechanical performance of the injured tendons, two and three weeks post injury, in the above experiments is expected because the collagen fibrils of the injured area at this stage are mostly imma-

ture and of small-sized, unimodally distributed, and randomly organized type III collagen fibrils<sup>4,14,17,29,30</sup>. At the earlier stages of tendon healing, the non-collagenous constituents of the matrix such as glycosaminoglycan and proteoglycan constituents, together with water content and cellular elements are still high, while the fibrous connective tissue that is responsible in load bearing capacities is still low<sup>16,17</sup>. After a significant period of time post injury in the present study, maturation of these initially newly regenerated, small-sized, unorganized collagen fibrils was promoted by bFGF administration so that at 84 days post injury, the collagen fibrils differentiated to a mixed population of longitudinally oriented small and medium sized collagen fibrils. The mechanical strength of the healing tendon therefore increased with time, and the longitudinally oriented collagen fibrils showed more normal functional properties than the disoriented small-sized, unimodal collagen fibrils present in the lesions of the injured control tendons.

It is indicated that after approximately six weeks post tendon injury, the remodeling phase commences, with decreased cellularity and reduced collagen and glycosaminoglycan synthesis. The cells start to become mature and the collagen content and the collagen fibrils diameter increases<sup>4,15</sup>. The effects of bFGF on proliferation of the tenoblasts and type III collagen expression can explain why, after twelve weeks post injury, the injured treated tendons had an optimum biomechanical performance.

Possibly the selected dosage and time of administration of bFGF are other potential reasons for the enhanced structural and physical performance of the injured treated tendons and three application of 1200 ng (totally 3600 ng/kg) of this drug on days 3, 7 and 10 post injuries seems effective in the restoration of the structural hierarchy and physical activity of the injured tendons. A correlation between the dose and the efficiency of the bFGF has been shown<sup>39</sup>.

Based on the present findings, by designing suitable times of administration and dosage and leaving enough time for structural and functional development, it is possible that bFGF might be one of the drugs of choice in improving the structural organization and biomechanical performance of the injured tendons. These beneficial effects are possibly related to the potential capacity of bFGF to inhibit peritendinous adhesion formation, shorten the inflammatory processes, enhance cellular maturation, improve collagen deposition and facilitate collagen fibrils aggregation and differentiation. Further biochemical and molecular studies are needed to elucidate other aspects of the mechanism of action of this reagent on the structural and functional performance of tendon injuries.

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## References

1. Kannus P. Structure of the tendon connective tissue. *Scand J Med Sci Sports* 2000;10:312-20.
2. Ross WS, Dyson SJ. Diagnosis and management of lameness in the horse. Philadelphia: Saunders; 2003.
3. Strauss EJ, Ishak C, Jazrawi L, Sherman O, Rosen J. Operative treatment of acute Achilles tendon ruptures: an institutional review of clinical outcomes. *Injury* 2007; 38:832-8.
4. Sharma P, Maffulli N. Tendon Injury and Tendinopathy: Healing and Repair. *J Bone Joint Surg (Am)* 2005; 87:187-202.
5. Chan BP, Chan KM, Maffulli N, Webb S, Lee KK. Effect of basic fibroblast growth factor. An *in vitro* study of tendon healing. *Clin Orthop Related Res* 1997;342:239-47.
6. McGeachie J, Tennant M. Growth factors and their implications for clinicians: A brief review. *Aust Dent J* 1997;42:375-80.
7. Takahashi S, Nakajima M, Kobayashi M, et al. Effect of recombinant basic fibroblast growth factor (bFGF) on fibroblast-like cells from human rotator cuff tendon. *Tohoku J Exp Med* 2002;198:207-14.
8. Thomopoulos S, Harwood FL, Silva MJ, Amiel D, Gelberman RH. Effect of several growth factors on canine flexor tendon fibroblast proliferation and collagen synthesis *in vitro*. *J Hand Surg [Am]* 2005;30:441-7.
9. Zakine G, Ermisch C, Loaille B, et al. Comparison of two ways of administration of growth factors (bFGF and RGTA) to improve latissimus dorsi muscle flap vascularization and trophicity. *Basic Appl Myol* 2006;16:9-15.
10. Wronski TJ. Skeletal effects of systemic treatment with basic fibroblast growth factor. *J Musculoskel Neuron Interact* 2001;2:9-14.
11. Dunstan CR, Boyce R, Boyce BF, et al. Systemic administration of acidic fibroblast growth factor (FGF-1) prevents bone loss and increases new bone formation in ovariectomized rats. *J Bone Miner Res* 1999;14:953-9.
12. Shimizu S, Miyasaka Y, Yamamoto S, Ishii M, Kiuchi Y. Stimulation of Tetrahydrobiopterin Synthesis by Basic Fibroblast Growth Factor in Vascular Endothelial Cells. *Pteridines* 2003;14:9-12.
13. Hu C, Ding Y, Chen J, Liu D, Ding M, Zhang Y. Treatment of corneal epithelial wounds in dogs using basic fibroblast growth factor. *Veterinarni Medicina* 2009; 54:280-6.
14. Oryan A, Goodship AE, Silver IA. Response of a Collagenase-Induced Tendon Injury to Treatment with a Polysulphated Glycosaminoglycan (Adequan). *Connect Tissue Res* 2008;49:351-60.
15. Oryan A, Silver IA, Goodship AE. Effects of Serotonin S2-receptor blocker on healing of acute and chronic tendon injuries. *J Invest Surg* 2009;22:246-55.
16. Oryan A, Moshiri A, Meymani-Parizi A. Effects of Sodium-hyaluronate and Glucosamine-chondroitin sulfate on remodeling stage of tenotomized superficial digital flexor tendon in rabbits: a clinical, histopathological, ultrastructural and biomechanical study. *Connect Tissue Res* 2010a;1-11, Doi: 10.3109/03008207.2010.531332.
17. Oryan A, Silver IA, Goodship AE. Metrenperone enhances collagen turnover and remodeling in the early stages of healing of tendon injury in rabbit. *Arch Orthop Trauma Surg* 2010b;130:1451-7.
18. de Wit T, de Putter D, Tra WM, et al. Auto-crosslinked hyaluronic acid gel accelerates healing of rabbit flexor tendon *in vivo*. *J Orthop Res* 2009;27:408-15.
19. Bigham AS, Shadkhist M, Nourani H, et al. Fresh autogenous and allogeneous tendon graft in rabbit model. *Comp Clin Pathol* 2010;20:109-13.
20. O'Sullivan CB. Injuries of the flexor tendons: focus on the superficial digital flexor tendon. *Clin Techniq Equine Pract* 2007; 6:189-97.
21. Sharifi D, Kazemi D, Latifi H. Evaluation of tensile strength of the superficial digital flexor tendon in horses subjected to transcutaneous electrical neural stimulation therapeutic regimen. *Am J Appl Sci* 2009;6:816-9.
22. Dyson SJ. Medical management of superficial digital flexor tendonitis: a comparative study in 219 horses (1992-2000). *Equi Vet J* 2004;36: 415-9.
23. Thomopoulos S, Kim HM, Das R, Silva MJ, et al. The Effects of Exogenous Basic Fibroblast Growth Factor on Intrasyovial Flexor Tendon Healing in a Canine Model. *J Bone Joint Surg (Am)* 2010;92:2285-93.
24. Chan BP, Fu S, Qin L, Lee KK, Rolf CG, Chan KM. Effects of basic fibroblast growth factor (bFGF) on early stages of tendon healing: a rat patellar tendon model. *Acta Orthop Scand* 2000;71:513-18.
25. Ide J, Kikukawa K, Hirose J, et al. The effect of a local application of fibroblast growth factor-2 on tendon-to-bone remodeling in rats with acute injury and repair of the supraspinatus tendon. *J Shoulder Elbow Surg* (2009);18:391-398.
26. Conzemius MG, Brown TD, Zhang Y, Robinson RA. A new animal model of femoral head osteonecrosis: one that progresses to human-like mechanical failure. *Journal of Orthopaedic Research* 2002; 20(2): 303-309.
27. Kim SB, Kwon DR, Kwak H, et al. Additive effects of intra-articular injection of growth hormone and hyaluronic acid in rabbit Model of collagenase-induced osteoarthritis. *J Korean Med Sci.* 2010; 25(5):776-780.
28. Gibson CJ, Donnelly TM. Lameness in a rabbit. *Lab Anim* 2011;40(2):39-41.
29. Oryan A, Shoushtari AH. Histology and ultrastructure of the developing Superficial Digital Flexor Tendon in rabbits. *Anat Histol Embryol* 2007;37:134-40.
30. Oryan A, Shoushtari AH. Biomechanical properties and dry weight content of the developing superficial digital flexor tendon. *Comp Clin Pathol* 2009;18:131-7.
31. Sha DF, Xin CT, Yang XX. Experimental study on basic fibroblast growth factor combined slow-releasing degradable membrane to prevent tendon adhesion. *Chinese Journal of Reparative and Reconstructive Surgery* 2004;

- 18:148-51.
32. Sheng J, Zeng B, Jiang P. Effects of exogenous basic fibroblast growth factor on in-sheathed tendon healing and adhesion formation. *Zhongguo Xiu Fu Chong Jian Wai Ke Za Zhi* 2007;21:733-7.
  33. Wurgler-Hauri CC, Dourte LM, Baradet TC, Williams GR, Soslowsky LJ. Temporal expression of 8 growth factors in tendon-to-bone healing in a rat supraspinatus model. *J Shoulder Elbow Surg* 2007;16:198-203.
  34. Gospodarowicz D, Ferrara N, Schweigerer L, Neufeld G. Structural characterization and biological functions of fibroblast growth factor. *Endocrine Rev* 1987;8:95-114.
  35. McNeil PL, Muthukrishnan L, Warder ED, D'Armore PA. Growth factors are released by chemically wounded endothelial cells. *J Cell Biol* 1989;109:811-22.
  36. Hideki S, Hideki T. The Effects of Basic Fibroblast Growth Factor on the Healing of Flexor Tendons *in vitro*. *J Japan Soc Surg Hand* 1999;15:809-17.
  37. Hamada Y, Katoh S, Hibino N, Kosaka H, Hamada D, Yasui N. Effects of Monofilament Nylon Coated With Basic Fibroblast Growth Factor on Endogenous Intrasynovial Flexor Tendon Healing. *J Hand Surg* 2006;31:530-40.
  38. Tang JB, Cao Y, Zhu B, Xin KQ, Wang Xt, Liu PA. Adeno-Associated Virus-2-Mediated bFGF Gene Transfer to Digital Flexor Tendons Significantly Increases Healing Strength. *J Bone Joint Surg (Am)* 2008;90:1078-89.
  39. Gulotta LV, Rodeo SA. Growth Factors for Rotator Cuff Repair. *Clin Sports Med* 2009;28:13-23.