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Low Intensity Pulsed Ultrasound Increases the Mechanical Properties of the Healing Tissues at Bone-Tendon Junction

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Abstract—The re-establishment of bone-tendon junction (BTJ) tissues is involved in many trauma and reconstructive surgeries. A direct BTJ repair requires a long period of immobilization which may be associated with a postoperative weak knee. In this study, we investigated if low-intensity pulsed ultrasound treatment increases the material properties of healing tissues at bone-tendon junction (BTJ) after partial patellectomy using rabbit models. Standard partial patellectomy was conducted on one knee of twenty four rabbits which were randomly divided into an ultrasound group and a control group. The bony changes of BTJ complexes around the BTJ healing interface were measured by anteroposterior x-ray radiographs; then the volumetric bone-mineral density (BMD) of the new bone was assessed using a peripheral computed tomography scanner (pQCT). The stiffness of patellar cartilage, fibrocartilage at the healing interface and the tendon were measured in situ using a novel noncontact ultrasound water jet indentation system. Not only significantly more newly formed bone at the BTJ healing interface but also increased stiffness of the junction tissues were found in the ultrasound group compared with the controls at week 18. In addition, the ultrasound group also showed significantly 44% higher BMD at week 6 than controls.

I. Introduction

Bone-tendon junction (BTJ), such as patellar tendon-patella junction, is a unique structure composed of a transitional fibrocartilage zone, characterized by calcified fibrocartilage connecting to the bone and noncalcified fibrocartilage connecting to the tendon. This unique structure is thought to have material property intermediate between that of bone and tendon, providing a gradual transition in stiffness, which diminishes stress concentration, and tearing or shearing at the interface [1]. Many trauma surgeries and reconstructive surgeries involve re-establishment of bone-tendon junction (BTJ). Investigators have established a partial patellectomy model in rabbits to study interventions for accelerating BTJ repair [2-5]. However, BTJ healing is slow due to the poor regenerative capacity of the fibrocartilage zone between bone and tendon, poor alignment of patella tendon scar tissue and bone loss of the patella at the BTJ healing interface [5]. Therefore, BTJ healing requires a longer resting and immobilization period before limb loading activities are permitted, which may result in adverse effects and complication of immobilization for the affected joints, including muscle and tendon atrophy, bone loss, and articular cartilage degeneration [6-7]. Therefore, it would be of clinical importance for discovering methods to enhance the healing of the BTJ interface after partial patellectomy. One of the biophysical approaches is to use low intensity pulsed ultrasound (LIPUS) to accelerate the repair of muscular skeletal tissues [8-10].

LIPUS is a noninvasive form of mechanical energy transmitted transcutaneously as high-frequency acoustical pressure waves into biological tissues. In experimental and clinical situations, LIPUS has been successfully proven to stimulate fracture healing and bone growth [11-13]. It has also been demonstrated that influencing the expression of proteins relevant to cartilage generation which may result in enhanced mechanical stability [14]. LIPUS is recommended for a daily application of about 20 to 30 min for acceleration of fracture healing, treatment of delayed or nonunion and bone lengthening [8-9, 15].

The positive effects of LIPUS on a variety of connective tissues and related cellular and molecular mechanisms have been assessed radiographically, histo-morphologically, and biomechanically. In this study, we investigated the potential effect of LIPUS on acceleration of BTJ healing using an established partial patellectomy model in rabbits [4, 16]. The BTJ healing was not only assessed by using quantitative radiographic imaging technique, multilayer peripheral quantitative computed tomography (pQCT), but also the material properties of BTJ tissues were measured by a novel noncontact ultrasound water jet indentation system [17-18], which have been usually difficult to evaluate in situ..

II. Methodology

A. Animal Model and Surgery

Twenty-four mature female New Zealand White rabbits (18-weeks-old, weight: 3.5±0.3 kg) were prepared for the experiments. The standard and established partial patellectomy and surgical reconstruction between the patella and patellar tendon were applied to the animals [4, 19]. Briefly, under general anesthesia with sodium pentobarbital (0.8 mL/kg, i.v., Sigma Chemical Co., St. Louis, MO) and aseptic technique, one of the knees was shaved and

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approached through an anterolateral skin incision. After excising the distal 1/3 of the patella (35.8 ± 8.2% of the original patella length of all 24 experimental patellae), two holes with 0.8 mm in diameter were drilled vertically along the patellar. The patellar tendon was sutured directly to the proximal 2/3 of the patella via the two drilled holes with nonabsorbable suture and protected with figure-of-eight tension band wire which was drawn around the superior pole of the patella to the tibia tuberosity to reinforce the ligament repair [20]. After closing the incision, antibiotic spray was used for disinfection. A custom-made tension meter was used to standardize the tension of the tension band fixation at the knee flexion angle of 90 degrees before closing the wound. Intramuscular analgesic was given daily for 3 days after surgery. Immobilization for 6 weeks was followed using an established cast – splints immobilization device with an "open window" on the upper cast which allowed placement of ultrasound transducer on the surface of the patella for ultrasound treatment. This study was approved by the Animal Research Ethics Committee of the Chinese University of Hong Kong (Reference CUHK: 4098/01).

The rabbits were randomly divided into the LIPUS treatment group and control group without any postoperative treatment. Each group was further divided into three subgroups euthanized at postoperative week 6, 12 or 18. The patella-patellar tendon complex of the operated knee of both LIPUS and control group were harvested and prepared for radiographic and BMD measurements, and the stiffness values of each tissues involved in BTJ complexes were also measured in situ using a noncontact ultrasound water jet indentation system.

B. The LIPUS Treatment

Under sedation with ketamine (intramuscular 0.25 ml/kg) (Alfasan International BV, Utrecht, the Netherlands), LIPUS stimulation was delivered to the animals by a 2.5-cm-diameter ultrasound transducer (SAFHS, Exogen, Inc, USA) via the "open window" of the cast and placed against the anterior surface of the healing junction of the operated knee via a thin layer of coupling gel (Acoustix, CONMED Corp., USA). The ultrasound signal composes a 200 μs burst of 1.5 MHz and 30.0 ± 5.0 mW/cm^2 spatial average and temporal average incident intensity [9, 21-22]. The ultrasound treatment was started at 3 days after operation, kept 20 minutes for each day and ended at the end of week 6. At postoperative week 6, fixation devices for immobilization were removed. The animals were euthanized at week 6, 12 or 18 for in situ measurement of tissue stiffness.

C. Radiographic Measurements

High-resolution X-ray films of the anterior-posterior view of patella-patellar tendon complex were taken by an X-ray machine (Faxitron X-ray Corp., Wheeling, IL, USA) with exposure time 6 s, a tube voltage 60 kVp, and the X-ray source-object distance is 40 cm. After digitizing the X-ray films into an image analysis system (Metamorph image analysis system, version 4.5, Universal Imaging Corp., PA, USA), the anterior-posterior area of new bone can be measured. The initial osteotomy line was identified to separate the newly formed bone from the remaining patella for quantification of new bone size, that is, the area of the enlarged bony part from the proximal remaining patella, using the previous measurement protocol by a single examiner [4]. All the samples of week 6, 12 and 18 groups were measured.

D. BMD Measurement

A multilayer high-resolution peripheral computed tomography scanner (pQCT) (Densiscan 2000, Scanco, Bassersdorf, Switzerland) with a spatial resolution of 0.3 mm and a CT-slice thickness of 1 mm was used to measure volumetric BMD of the new bone where it was defined for measuring its size on x-ray films.

E. Tissue Stiffness Measurement

A noncontact ultrasound water jet indentation system composed of a high-frequency focused ultrasound transducer and a water beam eject system was used to quantitatively measure the effective stiffness of the BTJ tissues, especially the remaining patellar cartilage, the junction (newly formed fibrocartilage zone) and the tendon [17-18].

All the BTJ tissue samples were stored at -20^{o}C before use. The sample was first thawed in normal saline solution (0.15 M NaCl) at room temperature 20^{o}C for 1 hour, and then it was fixed by a fixation device with the articular surface facing the ultrasound transducer perpendicularly (Fig. 1). The sample was first scanned along the patella-junction-tendon direction with a scan step at 50 μm under a preloading of 2.12 ± 0.07 kPa by the water jet ultrasound indentation system. The scan line was carefully selected by obtaining the maximal ultrasound reflection echoes from the interface of water/AC surface, usually at the most prominent convex site of the patella. The scan distance was typically 5 mm for a postoperative sample. After the first scan was finished, the sample was quickly scanned again along the identical line at a pressure of 135.32 ± 0.36 kPa.

The stiffness was calculated by the force applied to the tissue by the water jet divided by the tissue deformation. The tissue deformation was measured from two consequent ultrasound B-scans. Three sites from each part, including patellar cartilage, fibrocartilage zone and tendon were selected, and the deformation at each site was calculated from the deflection of the flight of time of ultrasound echoes. The speed of ultrasound used for the calculation of tissue deformation was assumed as 1636 m/s in cartilage [24] and 1580 m/s in tendon [25]. The averaged values calculated from the three sites were used as the stiffness value for each tissue part.

F. Statistical Analysis

Data were expressed as mean ± SD. All the experimental data, including the new bone size, volumetric BMD of new bone, and the stiffness of patellar cartilage, junction tissue and tendon, were statistically analyzed using two-way ANOVA to evaluate the effect of healing time and LIPUS intervention on the healing of BTJ complex. If any significant effect was found, post hoc Bonferroni multiple range tests were used for statistical difference. All the statistical analyses were conducted by using the commercial SPSS software program version 10.0 (SPSS Inc., Chicago, IL, USA). The significance level was set at $P < 0.05$.

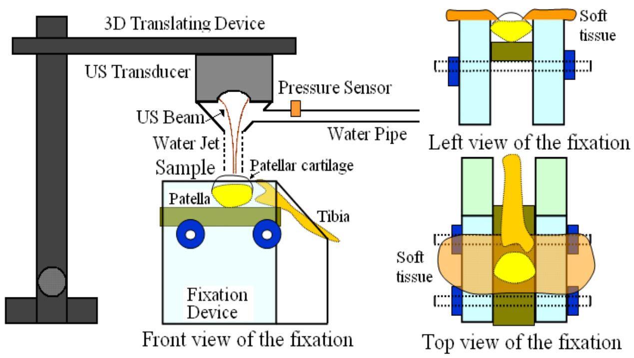

Fig. 1. Diagram of the ultrasound indentation system using the water jet compression. The water jet was used as an indenter and focused high-frequency ultrasound was employed to monitor the deformation of the soft tissue. The 3D translating device facilitated the system to conduct B-scans over tissue surface. By applying different pressures for B-scan sequences, the distribution of the elastic modulus was obtained with the recorded pressure, deformation and tissue thickness.

III. Results

A. New Bone Size Measured on Radiographs

There is a significant postoperative enlargement or outgrowth of the new bone from the remaining proximal patella after the partial patellectomy in both LIPUS group and control group. When the size of radiographic new bone from the remaining patella is compared between both groups, significant more new bone is formed in LIPUS group as compared with controls at week 18 (LIPUS: 8.91 ± 1.31 mm^2 vs. Control: 6.40 ± 1.01 mm^2, $P < 0.05$). Considering the effect of healing time, it is found that significant enlarged new bone formed at week 18 in both LIPUS and control group in comparison with week 6 and 12.

B. BMD Measured by pQCT

LIPUS treatment group shows significant higher volumetric BMD in the new bone at week 6 than controls at 95% significant level (LIPUS: 0.77 ± 0.18 g/cm^3 vs. Control: 0.53 ± 0.09 g/cm^3, $P < 0.05$), but not for week 12 (LIPUS: 0.79 ± 0.09 g/cm^3 vs. Control: 0.75 ± 0.24 g/cm^3, $P > 0.05$) or week 18 (LIPUS: 0.80 ± 0.31 g/cm^3 vs. Control: 0.74 ± 0.16 g/cm^3, $P > 0.05$).

C. Stiffness Measured by Ultrasound Water Jet Indentation System

The stiffness of patellar cartilage of LIPUS group was found to be significantly higher than the controls at postoperative week 6 (LIPUS: 6.75 ± 2.07 N/mm vs. Control: 3.49 ± 1.49 N/mm, $P < 0.05$), but not for week 12 (LIPUS: 6.74 ± 1.98 N/mm vs. Control: 5.20 ± 0.12 N/mm, $P > 0.05$) or week 18 (LIPUS: 6.74 ± 1.55 N/mm vs. Control: 5.78 ± 2.25 N/mm, $P > 0.05$). Regarding the junction tissues, i.e., the new formed fibrocartilage, only significant higher stiffness values were found in LIPUS group than the controls at week 18 (LIPUS: 4.27 ± 3.01 N/mm vs. Control: 1.67 ± 0.58 N/mm, $P < 0.05$), while not for week 6 (LIPUS: 1.69 ± 0.69 N/mm vs. Control: 0.93 ± 0.15 N/mm, $P > 0.05$) or week 12 (LIPUS: 1.26 ± 0.50 N/mm vs. Control: 1.41 ± 1.32 N/mm, $P > 0.05$). However, no significant difference was found in the stiffness of tendon either between the LIPUS and control group, or among the samples at week 6, 12 or 18. Interestingly, significant correlations between the stiffness of fibrocartilage zone and the new bone size ($r = 0.74$, $P < 0.05$) or the volumetric BMD ($r = 0.72$, $P < 0.05$) were found.

IV. Conclusion and Discussion

Bone-tendon junction repair is a long and complex process, especially for the patella-patellar tendon healing. Besides the histo-morphological analysis to investigate the healing process of BTJ repair, this study investigated the effect of LIPUS on accelerating BTJ repair using an established partial patellectomy model in rabbits. Not only the effect on enhancing the new bone formation was radiographically examined, but also the effect on strengthening the stiffness of soft tissue at BTJ was in situ measured using a noncontact ultrasound water jet indentation system for the first time.

Our radiographic measurements provided evidence of the woven bone formation at the patella-patellar tendon healing gap a week 6 postoperatively. Over the healing time, more newly formed bone observed with an enlarged osseous extension from the proximal patella, together with the mineralization of the newly formed bone. However, not the stiffness of all the soft tissues in BTJ complexes increased significantly with healing time.

The LIPUS intervention may result in enhancement of BTJ healing through bony and cartilaginous formation, which showed the progression of newly formed bone at BTJ correlated with the healing quality of BTJ tissue. Significantly more newly formed bone outgrowth at patella-patellar tendon healing junction in the LIPUS group was found as compared with the control group at week 18, and significantly higher volumetric BMD in the newly formed bone were also found in week 6.

Our histology analysis suggested that the morphological transformation of patella-patellar tendon healing junction over time might be more a reflection of functional adaptation rather than simple anatomical replication. However, it was difficult to determine the mechanical properties of the soft tissues in BTJ complexes. We utilized a non-contact ultrasound water jet indentation system with high resolution to measure the compressive elastic properties of the remained patellar cartilage, the newly formed fibrocartilage zone and patellar tendon nondestructively. It was for the first time to evaluate the BTJ healing by investigate the mechanical properties of the soft tissues at patella-patellar tendon junction. Our study suggested that LIPUS helps the remodeling of patellar cartilage and the fibrocartilage zone, but no significant benefit was found for the enhancement of patellar tendon after the standard partial patellectomy.

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