LOW-INTENSITY PULSED ULTRASOUND ACCELERATES OSTEOSTENOSIS AT BONE-TENDON HEALING JUNCTION

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Abstract—This study was designed to evaluate low-intensity pulsed ultrasound (LIPUS) in acceleration of mineralization and remodeling of the new bone formed at the healing interface of bone-tendon junction. Thirty-two mature New Zealand white rabbits underwent partial patellectomy and direct repair of the patellar tendon and proximal patella. Animals were then divided into LIPUS treatment group (20 min/d, 5 times/wk) and placebo control group and were euthanized at week 8 and 16 postoperatively (n = 8, for each group and time point). The main outcome measures included new bone size and its bone mineral density (BMD). Results showed that the size of new bone was found to be 2.6 and 3.0 times significantly greater in the LIPUS group compared with that of the control group at weeks 8 and 16, respectively. In addition, the LIPUS group showed significantly higher BMD at week 8 than controls, but not at week 16. In conclusion, this was the first experimental study to show that LIPUS was able to enhance osteogenesis at the healing bone-tendon junction, especially before the postoperative week 8. Findings of this study formed a scientific basis for future clinical trials and establishment of indication of LIPUS for enhancing bone-tendon junction repair. (E-mail: Lingqin@cuhk.edu.hk) © 2006 World Federation for Ultrasound in Medicine & Biology.

Key Words: Low-intensity pulsed ultrasound, Bone-tendon junction repair, Bone remodeling, Bone mineral density, Histology.

INTRODUCTION

Vehicular trauma and sports injury are common causes of injuries around the knee joint (Bennell and Brukner 1997; Hou et al. 2002; Kaye and Jick 2004). The patella is an important functional component of the knee extensor mechanism. Therefore, the treatment options selected for repair of the patella-patellar tendon complex may influence the functional outcome of the knee joint (Kaufer 1971; Scheinberg and Bucholz 1994; Sutton et al. 1976).

In comminuted and displaced fractures of the patella, the clinical treatment can be fracture repair or partial patellectomy (Hung et al. 1993; Saltzman et al. 1990; Scheinberg and Bucholz 1994; Sutton et al. 1976). Clinically, Saltzman et al. (1990) performed a standardized partial patellectomy on patients with displaced patellar fracture and found a significant postoperative increase in length of the remaining patella evaluated using lateral radiographs. The exact factors involved in this process were not clear, but the process appeared to be quantitatively different from the phenomenon of calcification or ossification within the extensor mechanism after total patellectomy (Burton and Thomas 1972; Wilkinson 1977). Our previous experimental study in rabbits revealed that postoperative enlargement of the remaining patella after partial patellectomy reported by Saltzman et al. (1990) corresponded to the trabecular bone outgrowth from the remaining patella radiologically and histologically (Qin et al. 1999a). The size of the remaining patella after partial patellectomy was found to be associated with an increased patellofemoral contact area and a diminished patellofemoral contact pressure (Marder et al. 1993), which may have the potential advantage in minimizing development of postoperative osteoarthritis (Buckwalter et al. 1994; Feller and Bartlett...
Therefore, how to promote bone formation and mineralization at the healing interface and to achieve greater enlargement of the remaining patella after partial patellectomy would be of clinical importance. One of the possible approaches is to use biophysical intervention, such as low intensity pulsed ultrasound (LIPUS) (Einhorn 1995; Klassen and Trousdale 1997; Leung et al. 2004b).

LIPUS is a noninvasive form of mechanical energy transmitted transcutaneously as high-frequency acoustical pressure waves in biologic tissues, which provides a direct mechanical stimulation on osteoblast proliferation, endochondral ossification and mineralization reported in many in vitro and in vivo studies (Einhorn 1995; Klassen and Trousdale 1997; Leung et al. 2004a; Nelson et al. 2003). 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 (Einhorn 1995; Klassen and Trousdale 1997; Leung et al. 2004b).

As a better bone-tendon repair with healing over time was associated with the progressive ingrowth of collagen fibers, mineralization and maturation of the healing tissue at the bone-tendon reattachment experimentally (Arnoczky et al. 1988; Leung et al. 2002; Qin et al. 1999a; Rodeo et al. 1993), we hypothesized that the LIPUS was not only able to accelerate the growth of new bone and increase its mineralization. This study was designed to employ our established partial patellectomy model in rabbits (Leung et al. 1999, 2002; Qin et al. 1999a) to confirm the above hypothesis by using quantitative radiographic imaging technique, multilayer peripheral quantitative computed tomography (pQCT) and histology.

**MATERIALS AND METHODS**

**Animals and surgery**

Thirty-two 18-week-old skeletally mature female New Zealand white rabbits (3.5 ± 0.3 kg) were used for partial patellectomy according to previously established experimental protocol (Feller and Bartlett 1993; Leung et al. 1999; Qin et al. 1999a). Briefly, under general anesthesia with sodium pentobarbital (0.8 mL/kg, intravenously) (Sigma Chemical Co., St. Louis, MO, USA) and aseptic technique, one of the knees was shaved and approached through an anterolateral skin incision. A caliper was used to measure the length of the patella and then transverse osteotomy was performed between the proximal 2/3 and the distal 1/3 of the patella, using an oscillating hand saw (Synthes, Mathys AG, Bettlach, Switzerland). After excising the distal patella, two 0.8 mm diameter drill holes were made vertically along the patella. The patellar tendon was then directly sutured to the proximal 2/3 of the patella via the two drilled holes with 3/0 nonabsorbable suture (Ethicon Ltd., Edinburgh, UK) and protected with figure-of-eight tension band wire of 0.4 mm in diameter drawn around the superior pole of the patella and the tibia tuberosity to protect the repair. After suturing the skin, the knee was immobilized with a long leg cast at 120° knee flexion, i.e., at a resting position of the knee joint in rabbits (Feller and Bartlett 1993; Leung et al. 1999; Qin et al. 1999a). The immobilization lasted for three weeks and then the immobilization cast was removed for free cage activity. All animal surgery was performed by a single surgeon for avoiding surgical variations. Pain relief drug (Temgesic, Reckitt & Colman Pharmaceuticals, Hull, UK) was given subcutaneously at a dose of 0.01 mg/kg for 3 days after the surgery. The rabbits were individually kept in metal cages in a central animal house and supplied with standard rabbit food and water ad lib. Animal ethical approval was obtained before experiment.

**LIPUS treatment**

The surgical rabbits were randomly divided into LIPUS treatment group and placebo control group. For the LIPUS treatment, animals were sedated with ketamine (0.25 mL/kg, intramuscularly) (Qin et al. 1997) and LIPUS (SAFHS, Smith and Nephew Pharmaceuticals, Memphis, TN, USA) was delivered by a 2.5 cm diameter ultrasound transducer placed against the anterior surface of the operated knee via an “open window” of the immobilization cast.

![Figure 1](https://example.com/fig1.png)

**Fig. 1.** Rabbit under treatment with low intensity pulsed ultrasound: the ultrasound probe is placed against the anterior surface of the healing junction of the operated knee via an “open window” of the immobilization cast.
mW/cm² spatial and temporal average incident intensity (Einhorn 1995; Leung et al. 2004b). The LIPUS treatment started 3 d after operation, 20 min/d, up to postoperative weeks 8 and 16, when the rabbits were euthanized for evaluation. The sample size was $n = 8$ for each group at each time point. For descriptive histology on new bone formation, one rabbit of week 8 and week 16 of both LIPUS and control group was injected (i.m.) with sequential fluorescence labeling with calcein green (10 mg/kg) and xylenol orange (30 mg/kg) (SIGMA, Chemicals Co., St. Louis, MO, USA) in a time sequence of four weeks and two weeks before euthanizing the animals (Parfitt 1983).

**Sampling and evaluations**

Animals were euthanized with an overdose of sodium pentobarbital as scheduled above. Patella-patellar tendon complex of the operated knee was harvested for evaluation of the size, bone mineral density (BMD) and maturation of the new bone formed at the bone-tendon junction healing interface.

**Radiographic measurement.** Anterior-posterior high-resolution x-ray films of the patella-patellar tendon complex were taken using x-ray machine (Faxitron X-ray Corp, Wheeling, IL, USA) with an exposure time of 6 s and tube voltage 60 kVp and an x-ray source–object distance of 40 cm. After digitizing the x-ray films into an image analysis system (Metamorph, Universal Imaging Corp., Downington, PA, USA), new bone size, *i.e.*, the enlarged bony part from the proximal remaining patella, was quantified using our previous measurement protocol by a single examiner (Fig. 2) (Qin et al. 1999a).

**BMD measurements.** 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 (Qin et al. 2003; Siu et al. 2004) was used to measure volumetric BMD of the new bone where it was defined for measuring its size on x-ray films.

**Descriptive histology.** After pQCT BMD measurement, two patella-patellar tendon complexes from each group, *i.e.*, one with and another without fluorescence labeling, were prepared for undecalcified and decalcified histology using our established protocols (Qin et al. 1999a, 1999b). Briefly: (1) Decalcified histology. Specimens were first fixed in 10% neutral buffered formalin for three weeks and then decalcified in 25% formic acid for three weeks. Specimens were processed using a Histo-center (Histokinette 2000, Reichert-Jung GmbH, Nussloch, Germany). After embedding in paraffin using an Embedding-center, histologic sections from the midsagittal plane of each specimen were cut at 5 μm using 1130/Biocut microtome (Reichert-June GmbH, Nussloch, Germany) and stained with hematoxylin and eosin (H&E). Maturity of the new bone in terms of level of its collagen alignment was observed under the polarized light. (2) Undecalcified histology. The patella-patellar tendon complex was embedded in methyl methacrylate without decalcification. Midsagittal sections in a thickness of 10 μm were cut using saw heavy duty microtome (Polycut & Ultramillier system, Reichert-Jung GmbH, Nussloch, Germany). Sequentially labeled calcein green, xylene orange and fluorescence in the new bone were observed under a fluorescence microscopic system (Leica Q500MC, Leica Cambridge Ltd., Cambridge, UK) to reveal dynamic bone remodeling.

**Statistical methods**

Except for the descriptive histology, all the above experimental data were analyzed statistically using two-way ANOVA to evaluate the effect of healing time and LIPUS intervention on new bone size and its bone mineral density. If any significant effect was found, post hoc Bonferroni multiple range tests were used for statistical differences. The significant level was set at $p \leq 0.05$. All statistical analyses were performed with a SPSS 10.0 software program (SPSS Inc., Chicago, IL, USA).
RESULTS

New bone size measured on radiographs

There is a significant postoperative enlargement or outgrowth of the new bone from the remaining proximal patella after partial patellectomy in both LIPUS group and control group (refer to Fig. 2). 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 non-treated controls both at week 8 (4.2 \pm 0.8 \text{ mm}^2 \text{ vs. } 1.6 \pm 0.7 \text{ mm}^2) \text{ and at week 16 (4.6 \pm 0.8 \text{ mm}^2 \text{ vs. } 1.5 \pm 0.5 \text{ mm}^2}) \text{ (} p < 0.01\text{)}. However, no difference in new bone size is found between postoperative weeks 8 and 16 in both groups. **Sample size: n = 8 for each group.**

BMD measured by pQCT

LIPUS treatment group shows significantly higher volumetric BMD in the new bone at week 8 compared with that of the control group (0.97 \pm 0.20 \text{ g/cm}^3 \text{ vs. } 0.81 \pm 0.13 \text{ g/cm}^3, p < 0.05), but not for week 16 (LIPUS: 0.74 \pm 0.26 \text{ g/cm}^3 \text{ vs. control: 0.87 \pm 0.23 \text{ g/cm}^3, p > 0.05}). The volumetric BMD of week 16 LIPUS group is, however, found to be lower than that of the week 8 LIPUS group (p < 0.05) (Fig. 3).

Descriptive histology

H&E sections observed under both bright light (Fig. 5a-d) and polarized microscopy (Fig. 6a-d) show that the radiographic new bone formation from the remaining patella after partial patellectomy (Fig. 2) is trabecular bone, less remodeled at week 8 specimens compared with week-16 samples and also compared between LIPUS treated sample and control sample at each time point. As compared with that of the control specimen, the week-16 LIPUS-treated sample reveals more advanced remodeling from woven bone to lamellar bone.
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DISCUSSION

This was the first experimental study to explore that LIPUS was able to enhance osteogenesis in terms of size of the new bone and acceleration of its mineralization at the healing interface between the patellar tendon and the remaining proximal patella after partial patellectomy.

...mellar bone with better collagen alignment of the trabecular bone matrix (Fig. 6a-d) and formation of more marrow cavities (Fig. 5a-d). Fluorescence microscopic observation reveals more xylenol orange labeling compared with calcein green labeling in the week 8 LIPUS-treated sample as compared with that of the control sample (Fig. 7 ai-bii). Such difference is, however, diminished in the week 16 samples in both groups (Fig. 7 ci iii-div).
The weeks 8 and 16 after partial patellectomy were the time points selected for evaluating the new bone mass, its mineralization and remodeling in the present experimental study. This was based on the observation made in our previous studies, that only showed radiographically measurable new bone outgrowth formed at the healing interface of the remaining proximal patella after 8 weeks of operation (Leung et al. 2002; Qin et al. 1999a). As hypothesized, the digital radiographs showed significant postoperative enlargement or outgrowth of new bone from the remaining proximal patella after partial patellectomy; however, it was only significant in the early weeks, i.e., before week 8, as no further measurable effects were found when compared between week 8 and week 16 samples in both LIPUS and control groups. The volumetric BMD measurement revealed significantly higher BMD in the new bone at week 8 in LIPUS group, but such difference also diminished with healing over time when compared for week 16 samples between both LIPUS group and control group.

Interestingly, the volumetric BMD of week 16 specimens was found to be even lower than that of the week 8 samples in LIPUS treated groups. This “inconsistent” finding between radiographic measurements and volumetric BMD may be explained by the nature of volumetric BMD in mg/cm³ measured by the peripheral quantitative computed tomography (pQCT), which is calculated by bone mineral content (BMC) over the total volume of the new bone. This provides information on degree of bone mineralization only at organ level, i.e., the BMD calculated within its bulk bone volume, with the bone mineralized phase, marrow spaces, osteon canals, lacunae and canaliculi, without providing the degree of BMD at matrix level (Genant et al. 1996; Rauch and Schoenau 2001; Siu et al. 2004). In fact, histologic sections demonstrated that the new bone remodeling of week 16 LIPUS specimen was more advanced in terms of transformation of the woven bone into lamellar bone. This was evidenced with better collagen alignment and more formation of bone marrow cavities. The latter might have resulted in a higher marrow cavity to bone matrix ratio and so a lower pQCT BMD. Fluorescence microscopic observation also supported this assumption that the earlier injected xylene organ was more visualized as compared with the later injected calcine green in the LIPUS treated samples. Similar characteristics of the new bone remodeling at the healing interface between patellar tendon and remaining patella after partial patellectomy was also observed in our early experimental studies (Leung et al. 2002; Qin et al. 1999a; Wong et al. 2003). In addition, LIPUS-induced enhancement of osteogenesis and bone remodeling or microarchitecture were also reported in fracture repair, bone lengthening or distraction osteogenesis in rat and rabbit models (Eberson et al. 2003; Einhorn 1995; Machen et al. 2002; Nelson et al. 2003).

Human studies are not available to show the fine structure of the enlarged bone from the remaining patella after partial patellectomy. Radiographically, Saltzman et al. (1990) performed a standardized partial patellectomy and lateral radiographic follow-up on 11 patients and showed that the length of the remaining patella gradually increased by 2.4 to 2.8 cm at a 5-y follow-up. In accordance with our previous experimental studies (Qin et al. 1999a; Siu et al. 2004), the present radiographic and histologic study also confirmed that the new bone along the sagittal section of healing patella after partial patellectomy was in fact the new bone formation or “outgrowth” of the trabecular bone from the remaining patellar after partial patellectomy. However, the present study did not show further effects of LIPUS on osteogenesis after 8 weeks’ treatment. This may imply the importance of timing and duration of LIPUS treatment to be included for design of cost-effective LIPUS intervention. The potential explanation to be offered for this phenomenon may be related to the decreased cellularity or decrease in number of mechanosensors, accompanied with an increase in bone mineralization with healing over time in the late phase of fracture healing. Similar results were also reported for the LIPUS used for enhancement of bone mineralization and remodeling in the consolidation phase of the distraction osteogenesis (Eberson et al. 2003; Einhorn 1995; Genant et al. 1996; Machen et al. 2002; Nelson et al. 2003).

The significance of postoperative enlargement of the remaining patella may bear special clinical significance. The partial patellectomy shortens the extensor mechanism and decreases patellofemoral contact areas and subsequently results in an increased contact pressure of the patellofemoral joint (Marder et al. 1993) and decreased proteoglycans contents in the cartilage matrix. The latter has been regarded as causative factor in the development of chondromalacia and osteoarthritis (Buckwalter et al. 1994; Donohue et al. 1983; Guilak et al. 1994; Leung et al. 1999). The theoretical advantage of more enlargement of the remaining patella or new bone formation from the proximal patella might reverse the above adverse effects of partial patellectomy (Guilak et al. 1994; Marder et al. 1993). As patellofemoral symptoms are generally stress-related (Burton and Thomas 1972; Feller and Bartlett 1993; Hung et al. 1993; Matthews et al. 1977; Saltzman et al. 1990; Wilkinson 1977), such enlargement may lead to diminished symptoms and contribute to the improvement of knee function with healing over time.

The present study did not address the underlying molecular and cellular mechanism related to the enhanced osteogenesis and remodeling under LIPUS treatment, because all the specimens used in this study were treated for more than 8 weeks, which might have passed the critical period of gene expression or cellular response for evaluations. However, previous in vitro studies (Doan et al. 1999; Reher et al. 2002; Wang et al. 2004)
and experimental studies using fracture healing and bone distraction lengthening models (Azuma et al. 2001; Eber- son et al. 2003; Einhorn 1995; Machen et al. 2002) suggested that such osteogenic effects found in this study may share the common pathways related to inflammatory reaction, angiogenesis, chondrogenesis and osteogenesis under the influence of LIPUS.

In conclusion, LIPUS was able to promote bone-tendon junction healing in a partial patellectomy rabbit model by enhancing osteogenesis in terms of mass of new bone formation, its remodeling and mineralization, especially in the early healing period before postoperative week 8. Clinical trials are suggested, to establish the relevant indications for patients.

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