Extracorporeal Shock Wave Therapy in Treatment of Delayed Bone-Tendon Healing
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Delay in repair of musculoskeletal injuries affects the performance of athletes in routine training and participation in sports. Among sports injuries, bone-tendon junction (BTJ) injury is not uncommon. The repair of BTJ is difficult and delayed because of limited regeneration capacity of its interface fibrocartilage zone experimentally and clinically.

To evaluate potential therapeutic means for enhancing BTJ repair, we adapted biophysical interventions indicated for fracture enhancement, such as low-intensity pulsed ultrasound (LIPUS), which has demonstrated positive effects on acceleration of BTJ repair experimentally. However, LIPUS is a daily treatment modality. Nonsurgical, high-energy extracorporeal shock wave therapy (ESWT) has been tested and shown to be effective for treatment of delayed union and nonunion in fracture repair. Extracorporeal shock wave therapy produces pressure waves that generate high positive pressures and cavitations at the focal treatment region of musculoskeletal tissues, and it has been reported to be able to accelerate healing by modifying the local intracellular and extracellular biological environment. The acceleration of
healing occurs through up-regulation of growth factors and activation of osteoblasts and fibroblasts to accelerate injury repair.4,19,46 Extracorporeal shock wave therapy has also been adapted into orthopaedic sports medicine for treatment of many chronic conditions, such as lateral humeral epicondylitis, Achilles tendinitis, rotator cuff calcifying tendinopathy, and plantar fasciitis.8,14,15,20,41 Recently, ESWT has been investigated for enhancement of BTJ healing experimentally. It is thought to work by triggering neovascularization and improving local blood supply and tissue regeneration.48,50,51 However, no study has been conducted to evaluate the treatment efficacy of ESWT for delayed BTJ healing. The present study was designed to study the treatment efficacy of ESWT for delayed BTJ repair using a BTJ delayed healing model based on an established partial patellectomy model in rabbits.27,38 with emphasis on osteogenesis, regeneration of BTJ fibrocartilage zone, and the tensile properties of the healing BTJ.

MATERIALS AND METHODS
Animals and Model Establishment

Twenty-eight mature female New Zealand rabbits (18 weeks old; 3.82 ± 0.42 kg) were used for establishing the delayed BTJ healing model. Standard partial patellectomy was performed on 1 of the hindlimbs using our established surgical protocol.27,37,38 Briefly, with the animal under general anesthesia with sodium pentobarbital (0.8 mL/kg, intravenous injection; Sigma Chemicals Co, St Louis, Mo) and aseptic technique, 1 of the knees of each rabbit was approached using an anterolateral skin incision. A partial patellectomy was performed through a transverse osteotomy made between the proximal two thirds and the distal one third of the patella with knee flexion at 90°. After the distal third (lower pole) of the patella and its fibrocartilage zone to the patellar tendon were removed, 2 evenly spaced tunnels of 0.8-mm diameter were drilled longitudinally through the remaining proximal patella. Before the patellar tendon was sutured to the proximal patella via the figure-of-8 tension band wiring (0.4-mm-diameter stainless steel wire, Biomet Ltd, Waterton, United Kingdom) was fixed between the remaining proximal patella and patellar tendon (Figure 1). A latex slice (left arrow) is interposed for shielding the bridge of tendon and patella (right arrow) for the first 4 postoperative weeks.

Figure 1. A latex slice (left arrow) is interposed for shielding the bridge of tendon and patella (right arrow) for the first 4 postoperative weeks.

Extracorporeal Shock Wave Therapy

The rabbits were randomly divided into a delayed healing (DH) group (n = 14) and DH with ESWT (DH + ESWT) group (n = 14). With the animal under sedation with ketamine (0.25 mL/kg, intramuscular), 2 weeks after removal of the immobilization cast and the interposed shielding sheet, a single ESWT treatment was delivered to the healing BTJ interfaces of the DH + ESWT group using the shock wave machine (Dornier MedTech Epos, Wessling, Germany). The treatment protocol was composed of energy flux 0.43 mJ/mm² at a frequency of 4 Hz for 1500 impulses. These physical parameters were reported to be effective to induce tissue microdamage, fast remodeling, and repair.19,20,43 The shock wave was delivered with a laser focus indicator and perpendicular to the healing interface of the proximal patella with knee flexion at 90°.

Sampling and Evaluation

Animals for each treatment were euthanized with 25% sodium pentobarbital at postoperative week 8 (n = 7) or 12 (n = 7), with 2 rabbits for histologic analysis and 5 rabbits for biomechanical testing. The latter were also prepared for histologic evaluation after mechanical testing. The quadriceps–patella–patellar tendon–tibia (QPPTT) complex of the operated knee was then harvested for taking anteroposterior high-resolution radiographs (Medical Radiograph, Fuji Photo Film Co, Ltd, Tokyo, Japan) with an experimental animal radiograph machine (Faxitron 43855C cabinet x-ray system model, Faxitron X-ray Corp, Wheeling, Ill), with an exposure time of 2 seconds, a tube voltage of 60 kVp, and a radiograph source-object distance of 40 cm. The radiographs were then digitized into an image analysis system.40

Radiographic Measurement of New Bone Size. After digitizing the radiographic films into an image analysis system (Image Pro Plus, version 5.1, Media Cybernetics Inc, Bethesda, Md), the new bone size (ie, the enlarged bony part from the remaining proximal patella) was quantified.
for its area from the radiograph films using our previous measurement protocol by a single examiner (Figure 2).40

Bone Mineral Status of New Bone. A multislice, high-resolution peripheral CT (Densiscan 2000, Scanco, Bassersdorf, Switzerland) with a spatial resolution of 0.3 mm and a slice thickness of 1 mm was used to measure the mineral status of the new bone, including bone mineral content (BMC) and volumetric bone mineral density (BMD), using our established protocol.6,31

Mechanical Testing. After removal of the suture material and the metal tension band wire, the QPPTT complexes were prepared for the following measurements: (1) Cross-sectional area measurement of QPPTT complex: Under a constant tensile load of 5 N applied to the QPPTT complex fixed on a custom-made preloading jig, the thickness and width at the BTJ were measured using a fine caliper, repeated 3 times, and its mean value was used to calculate the cross-sectional area of the bone-tendon healing interface.27,31 (2) Tensile strength of QPPTT complex: The QPPTT complex was then mounted on a custom-made tensile testing jig consisting of an upper clamp to fasten the patella together with the distal quadriceps and a lower clamp to grip the proximal tibia; the proximal tibia was fixed longitudinally at a 10° angle with the tibial axis. A Hounsfield Test Machine (H25K-S, Hounsfield Test Equipment LTD, Surrey, United Kingdom) with a load cell of 2.5 kN was used and tested at a speed of 50 mm/min up to failure.27,31 Normal saline soaks were applied throughout the testing procedure to avoid dehydration of the QPPTT complex. Ultimate stress was calculated by dividing the failure load over the cross-sectional area of the BTJ. Specimens after mechanical testing were further processed for histologic evaluations as described below.

Histologic Evaluations. The harvested samples of patella-patellar tendon (PPT) complexes were decalcified and embedded in paraffin; 5-μm-thick sections from the mid-sagittal plane of each specimen were stained with H&E by 1 musculoskeletal pathologist of our investigation team who was not blinded to grouping for the following evaluations: (1) General morphology and collagen fiber alignment: BTJ healing between tendon and bone was evaluated using a transmission polarized light microscope by a LEICA Q500MC microscope (Leica Cambridge Ltd, Cambridge, United Kingdom) using our established protocols.31,38,57 (2) Evaluation of tenocyte density and thickness of the fibrocartilage zone: tenocyte density within the healing tendon next to the regenerated fibrocartilage zone was calculated by counting the number of cells in 5 standardized rectangular fields (100 × 100 mm) on H&E sections at a magnification of 100 times using an image analysis system (Image Pro Plus, version 5.1) (Figure 3A). The thickness of the fibrocartilage zone was calculated by our recently established protocol in which the area of the fibrocartilage zone was first quantified by drawing 2 lines, respectively, along the identifiable chondrocytes next to the distal, newly formed bone and the proximal tendon and the deepest and the most superficial boundaries along the longitudinal extension lines of the top, newly formed bone. The distance between the lower and upper boundary points in the middle region of the fibrocartilage zone at the PPT interface was measured as the length of the fibrocartilage zone. The thickness of the fibrocartilage zone was then calculated by dividing its area by the corresponding length (Figure 3B).

Statistics

A 2-way analysis of variance with post hoc test was used for analysis of differences in new bone size, BMD, BMC, failure load, ultimate strength, tenocyte density, and thickness of fibrocartilage zone between the DH + ESWT and DH groups and between week 8 and week 12 measurements. Significance level was set at $P \leq .05$. All data were analyzed with SPSS version 13.0 (SPSS Inc, Chicago, Ill), and results were interpreted in mean ± SD.

RESULTS

New Bone Size at the BTJ Healing Interface

Radiographic measurements showed 193.4% and 92.5% significantly more new bone formation in the DH + ESWT group when compared with the DH group at both week 8 (6.19 ± 2.22 mm$^2$ vs 2.11 ± 1.25 mm$^2$, $P < .01$) and week 12 (9.47 ± 2.92 mm$^2$ vs 4.92 ± 2.42 mm$^2$, $P < .01$), respectively (Table 1). To compare the new bone formation between 2

Figure 2. Representative anteroposterior radiographs of the remaining proximal patella after partial patellectomy with newly formed bone at the healing interface, that is, the bony region below the white dotted line; white dotted line is the initial osteotomy line. A and B, week 8 DH group and DH + ESWT group, respectively. C and D, week 12 DH group and DH + ESWT group, respectively. Bone formation of DH group (A, C) was less than that of DH + ESWT group (B, D) at the same healing time point. DH, delayed healing; ESWT, extracorporeal shock wave therapy.
the DH groups between week 8 and week 12 (0.43 ± 0.11 g/mm³ vs 0.48 ± 0.18 g/mm³, \( P > .05 \)). In DH + ESWT groups, BMD was 97.5% higher at week 12 than at week 8 (0.79 ± 0.16 g/mm³ vs 0.40 ± 0.10 g/mm³, \( P < .05 \)). As compared with the DH groups, only the week 12 DH + ESWT group was 64.6% higher than the DH group in BMD (\( P < .01 \)) (Table 1).

Histology Analysis

Descriptive Histology. At postoperative week 8, extensive fibrous scar tissue was shown in the BTJ healing interface with an intensive cellularity (cell density) at the tendon side (Figure 4A) and poor alignment of the collagen fibers in the healing interface (Figure 4C) in the DH group. In the DH + ESWT groups, the regions around the BTJ healing interface were integrated via parallel aligned collagen fibers (Figures 4B and D) as compared with the DH group at the same healing time point. At week 12, the lamellar bone became the dominant structure in both groups; the alignment of collagen fibers was found to be better in the DH + ESWT group (Figure 5D) compared with the DH group (Figure 5C). The tidemark in the regenerated fibrocartilage zone appeared in the DH + ESWT group (Figure 5B) but was not observable in the DH group (Figure 5A).

Quantitative Evaluations for Both Density of Fibroblast-like Cells and Thickness of the Regenerated Fibrocartilage zone. The cellularity was significantly less at week 12 than at week 8 in both the DH + ESWT group (22.14 ± 5.64 cells/100 \( \mu \text{m}^2 \) vs 33.43 ± 2.23 cells/100 \( \mu \text{m}^2 \), \( P < .01 \)) and the DH group (30.88 ± 7.53 cells/100 \( \mu \text{m}^2 \) vs 37.57 ± 2.57 cells/100 \( \mu \text{m}^2 \), \( P < .05 \)). However, no significant difference in tenocyte density was found between the DH + ESWT and DH groups at week 8 (\( P > .05 \)). At week 12, 28.30% significantly lower density was shown in the DH + ESWT group as compared with that of the DH group (\( P < .05 \)). For the thickness of the fibrocartilage zone, the DH + ESWT group was found to be thicker than was the DH group by 46.2% at week 8 (0.38 ± 0.10 mm vs 0.26 ± 0.08 mm, \( P > .05 \)) and 63% at week 12 (0.49 ± 0.12 mm vs 0.30 ± 0.08 mm, \( P < .01 \)) (Table 1).

Mechanical Testing

The failure load and the ultimate strength were found to be improved significantly more with remodeling over time in the DH + ESWT group than in the DH group, with a 67.7% and 45.1%, respectively, higher failure load in the DH + ESWT group than in the DH group at week 8 (216.82 ± 75.26 N vs 129.32 ± 27.96 N, \( P < .05 \)) and week 12 (232.66 ± 53.01 N vs 160.30 ± 42.27 N, \( P < .05 \)) (Table 1). The ultimate strength was shown to be 98.2% and 78.2%, respectively, significantly greater in the DH + ESWT group for both week 8 (\( P < .05 \)) and week 12 (\( P < .05 \)) when compared with that of the corresponding DH groups (Table 1).

DISCUSSION

The present study is the first experimental investigation to demonstrate that ESWT is effective for treatment of DH at
the BTJ through facilitation and promotion of osteogenesis and regeneration of the fibrocartilage zone at the healing BTJ in a DH BTJ rabbit model. The mechanical properties of the BTJ were used as the end point for evaluation of repair quality. The significantly greater failure load and ultimate strength after ESWT revealed a better repair of the DH BTJ complex.

In the present study, a DH BTJ model was established for studying the augmentation effect of ESWT using an

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TABLE 1

<table>
<thead>
<tr>
<th>Group</th>
<th>Healing Bone-Tendon Junction</th>
<th>DH: 8 Weeks (n = 7)</th>
<th>DH + ESWT: 8 Weeks (n = 7)</th>
<th>DH: 12 Weeks (n = 7)</th>
<th>DH + ESWT: 12 Weeks (n = 7)</th>
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<tr>
<td>New bone</td>
<td></td>
<td></td>
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<tr>
<td>Area, mm²</td>
<td></td>
<td>2.11 ± 1.25</td>
<td>6.19 ± 2.22b</td>
<td>4.92 ± 2.42c</td>
<td>9.47 ± 2.92de</td>
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<td>Length, mm</td>
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<td>0.86 ± 0.37</td>
<td>1.75 ± 0.49f</td>
<td>2.01 ± 0.99f</td>
<td>2.33 ± 0.66f</td>
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<td>Bone mineral density, g/mm³</td>
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<td>0.40 ± 0.10</td>
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<td>Bone mineral content, g</td>
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<td>0.32 ± 0.26</td>
<td>0.23 ± 0.11</td>
<td>1.28 ± 0.43e</td>
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<td>Fibrocartilage zone</td>
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<td>Thickness, mm</td>
<td></td>
<td>0.26 ± 0.08</td>
<td>0.38 ± 0.10</td>
<td>0.30 ± 0.08</td>
<td>0.49 ± 0.12e</td>
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<td>Tendon</td>
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<td>Cell density, No./100 mm²</td>
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<td>37.57 ± 2.57</td>
<td>33.43 ± 2.23</td>
<td>30.88 ± 7.53d</td>
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<td>32.54 ± 2.05f</td>
<td>33.73 ± 6.91</td>
<td>28.04 ± 1.94f</td>
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<td>Failure load, N</td>
<td></td>
<td>129.32 ± 27.96</td>
<td>216.82 ± 75.26c</td>
<td>160.30 ± 42.27</td>
<td>232.66 ± 53.01f</td>
</tr>
<tr>
<td>Ultimate strength, mPa</td>
<td></td>
<td>3.41 ± 0.66</td>
<td>6.71 ± 2.54c</td>
<td>4.37 ± 1.08</td>
<td>8.41 ± 2.34f</td>
</tr>
</tbody>
</table>

aData are means ± SD. DH, delayed healing; ESWT, extracorporeal shock wave therapy.
bP < .01, compared with DH group at week 8 (2-way analysis of variance).
cP < .05, compared with DH group at week 8 (2-way analysis of variance).
dP < .01, compared with DH group at week 12 (2-way analysis of variance).
eP < .05, compared with DH + ESWT group at week 8 (2-way analysis of variance).
fP < .05, compared with DH group at week 12 (2-way analysis of variance).
gP < .05, compared with DH + ESWT group at week 8 (2-way analysis of variance).

Figure 4. Representative micrographs of patella–patellar tendon (PPT) healing interfaces of week 8 samples. A, at postoperative week 8 in the DH group, extensive fibrous scar tissue was seen in the PPT healing interface with an intensive cellularity at the tendon side (H&E, ×20). B and D, in the DH + ESWT group, the regions around the PPT healing interface were integrated via parallel aligned collagen fibers and had good alignment in polarized light microscopy (B, H&E, ×20; D, polarized, ×20) as compared with the DH group at the same healing time point. C, a poor alignment of the collagen fibers was observed under polarized microscopy (polarized, ×20).

Figure 5. Representative micrographs of patella–patellar tendon healing interfaces of week 12 samples. At week 12, the lamellar bone became the dominant structure in both groups. The tidemark (arrow) in the regenerated fibrocartilage zone appears for observation in the DH + ESWT group (B, H&E, ×20) but not in the DH group (A, H&E, ×20). The alignment of collagen fibers was better in the DH + ESWT group (D, polarized, ×20) than that of the DH group (C, polarized, ×20). DH, delayed healing; ESWT, extracorporeal shock wave therapy.
established partial patellectomy. This was achieved by shielding the bridge of bone and tendon at the early stage of BTJ healing, which resulted in less new bone and fibrocartilage zone regeneration and poorer bone and tendon remodeling on the healing interface than in our previous nonshielding normal repair model. This implies that the quality of BTJ injury repair is closely associated with the process of endochondral ossification, regeneration of the fibrocartilage zone, and remodeling and maturation of healing tissue with healing over time. In the present study, the enhanced osteogenesis and accelerated restoration of the fibrocartilage zone in the ESWT group with healing were reflected in the radiographic, densitometric, and histologic evaluations at both cellular and matrix levels. The overall healing quality of the BTJ complex was demonstrated by significantly greater tensile force and strength of healing BTJ in the ESWT group.

The present animal experiment did not address the underlying molecular and cellular mechanisms related to new bone formation and regeneration of the fibrocartilage zone at the BTJ. The underlying mechanisms of ESWT on delayed BTJ healing found in the present study might still share the same pathways reported for its application for treatment of delayed soft tissue healing and fracture repair, including promotion of angiogenesis and neovascularization, osteogenesis (both activation of osteoblasts and up-regulation of related extracellular cytokines and growth factors such as vascular endothelial growth factor and bone morphogenetic protein), collagen synthesis and collagen cross-link, and, consequently, significant improvement in mechanical properties. Our recent in vitro study also supported osteogenic effects of ESWT. Recent application of ESWT for augmentation of BTJ repair also partially confirmed such mechanisms.

It is known that cell proliferation is the characteristic tissue response in the acute stage of healing, and then tenocyte density decreases progressively with advancement in healing over time. The significant decrease in cell density and its accompanied improvement of collagen alignment of the healing tendon and increase in thickness of the fibrocartilage zone were found in the DH + ESWT groups postoperatively. These findings were substantiated by other studies using ESWT on tendinitis to show enhanced proliferation of tenocytes and formation of collagen fibrils. As compared with LIPUS that requires daily treatment of 20 minutes and more than a few weeks to achieve treatment effects—for example, for enhancement of fracture repair, acceleration of bone mineralization in bone lengthening or nonunion, and tendon or ligament repair, cartilage repair, and BTJ repair—clinically only 2 to 3 treatments would be required for ESWT, such as in the present study, which is more convenient than reported daily treatment using LIPUS. Its treatment effects are also reported to be related to osteogenesis. The cellular and molecular mechanisms were reported to be associated with enhancement of differentiation and proliferation of osteogenic cells as well as osteogenic activity of osteoblasts in human and animals.

The present study used end points at postoperative weeks 8 and 12 for evaluation. This was based on our previous studies that these 2 were the relevant time points to assess the quality of tissue repair at both fast remodeling and consolidation phases in the same animal model. Lu et al demonstrated that in normal (nonshielded) PPT junction healing, the osteogenesis, its remodeling, and improvement in tensile strength of the PPT complex were shown between postoperative weeks 4 and 8. As the present study introduced a delay in junction healing for 4 weeks, we proportionally postponed 4 weeks for evaluation of being able to detect larger differences for the selected evaluation parameters, especially related to osteogenesis, bone mineralization, and tensile strength.

The current ESWT application in orthopaedic sports medicine is mainly for treatment of chronic conditions around BTJ. The immediate potential clinical application of our findings for acute treatment of BTJ injury repair may also be related to reattached PPT healing after partial patellectomy or using the midthird of the patellar tendon for ACL reconstruction. Our present and earlier experimental studies demonstrated the association between size of new bone and tensile strength of the healing PPT junction in rabbits. Clinically, Saltzman et al reported variations in new bone formation or enlargement of remaining patella after partial patellectomy in a follow-up study for a mean 8.4 years. They found a significant correlation between the size of new bone and the functional outcome. Later, a human biopsy study demonstrated reconstitution of the tendon into the patellar defect with no evidence of bone formation, and this DH with morphological changes similar to our findings was reported to adversely affect the biomechanical properties of the healed donor site. In addition, postoperative increase in new bone size was suggestive of an increase in patellofemoral contact area and decrease in patellofemoral joint contact pressure after partial inferior patellectomy, resulting in diminished patellofemoral symptoms and accelerated improvement of knee function with healing over time. The findings of our current ESWT study imply its application for the above DH conditions characterized with less or no new bone formation at the PPT healing junction. As ESWT can be focalized onto tissues sitting deep in the body, the expected beneficial effects to be generated from this study might also be generalized for treatment of delayed BTJ repair in other subcutaneous regions, such as hand, foot, ankle (eg, Achilles-calcaneus), and shoulder (eg, rotator cuff). However, before systemic clinical applications, well-designed clinical studies are desirable.

There were a few limitations present in the current study. (1) As an outcome study, we were not able to delineate the underlying biological mechanisms of regeneration of the bone-interface fibrocartilage zone and whether osteogenesis was from the bone marrow of the residual proximal patella or from the scar tissues formed at the BTJ after removal of the distal patella after partial patellectomy. (2) Only a single dose was used, and dosing effects shall be explored in our future studies. (3) The histologic evaluations were not conducted in a blinded fashion as the investigators of this study were also engaged in ESWT intervention and subsequent radiographic monitoring of PPT interface healing, in which we observed statistically significantly more new bone formation in the ESWT group. Although all assessment criteria were objectively defined and conducted, further improvements should be made for...
future studies, including streamline in project planning to guarantee strictly blinded evaluations from the very beginning. (4) As there are differences in knee biomechanics between rabbits and humans, we could only apply experimental findings of this study to patients. However, the knee joint of both rabbit and human shares similar structural features evaluated radiologically and histomorphologically, and we believe that our findings serve as a scientific basis for future clinical trials.

In conclusion, findings of our experimental study suggest for the first time that ESWT is a convenient and effective biophysical intervention for treatment of delayed BTJ healing, which were demonstrated radiologically, by measuring BMD; histologically; and biomechanically. Clinical trials are highly desirable to confirm our experimental findings for establishing a relevant clinical indication of ESWT for treatment of BTJ in orthopaedic sports medicine.

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