Area, Length and Mineralization Content of New Bone at Bone–Tendon Junction Predict Its Repair Quality

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ABSTRACT: We investigated the hypothesis that if the area, length, and mineralization of newly formed bone could be used to predict the healing quality of patella–patellar tendon (PPT) junction after partial patellectomy. Twenty-four rabbits underwent partial patellectomy and their PPT complexes of the operated limbs were harvested at weeks 6, 12, and 18 postoperatively. The area, length, and mineralization of newly formed bone at PPT junction healing interface was evaluated radiographically and peripheral quantitative computational tomographically. The healing quality of PPT complexes in terms of its tensile property was determined by biomechanical testing. The results showed that the area, length, and mineral content of newly formed bone, and its tensile strength increased significantly with follow-up time. The area of newly formed bone was strongly correlated with the failure load, ultimate strength and energy at failure (r = 0.61, 0.54, 0.67, respectively, p < 0.01 for all). The length of newly formed bone was also found to be correlated with failure load, ultimate strength and energy at failure (r = 0.61, 0.54, 0.67, respectively, p < 0.01 for all). In addition, the bone mineral content of newly formed bone but not its bone mineral density was moderately correlated with failure load, ultimate strength and energy at failure (r = 0.44, 0.51, 0.42, respectively, p < 0.05 for all). In conclusion, the area, length, and mineralization of newly formed bone at the PPT junction after partial patellectomy may serve as useful noninvasive indices in assessing the quality of the bone–tendon junction repair. © 2010 Orthopaedic Research Society. Published by Wiley Periodicals, Inc. J Orthop Res 9999:1–6, 2010

Keywords: partial patellectomy; new bone formation; bone mineral density; tensile strength

Injuries to the knee joint involving patella–patellar tendon (PPT) complex resulting from sports injury or vehicular trauma are common.1,2 Both clinical and experimental studies support the concept that whenever possible preserving the entire fragmented patella facilitates fracture repair.1–4 However, in comminuted and displaced fractures of the patella, partial patellectomy is indicated.2,5–7 Clinically, Saltzman et al.6 performed standardized partial patellectomy on patients with displaced patellar fracture and evaluated the clinical outcome results on lateral radiographic follow-up which revealed that the length of the remaining patella gradually increased by 24–28 mm up to 5 years. In their postoperative management, protective passive loading was allowed after cast immobilization and gradually moved to moderate activities and full function in 1 year. Our previous experimental study in rabbits followed a similar postsurgical management regimen although a figure-of-eight wiring was applied to the remaining patella and the proximal tibia to gain additional protection. Animal follow-up data indicated that functional loading was taken place as the fixation wires were gradually broken with time after cast removal. In addition, the radiographic enlargement of the remaining patella next to the healing interface was trabecular bone outgrowth histologically, with an ex vivo measured length on the anterior–posterior radiographs ranging from 0.93 to 2.5 mm between postoperative weeks 8 and 24.8,9 Such difference in the length of new bone outgrowth was explained by potential variations in the extent of the excised patella, that is, it was not always possible to perform a uniform transverse osteotomy. Such variation might also be related to the fixation technique and postoperative activities experienced by each animal. Thus, the biomechanical loading-related remodeling should also play a role in the length of the new bone formed. These findings suggested the possibility of using the length or area of the new bone formation to predict the quality of the bone–tendon junction repair.

This study was designed to investigate the hypothesis that the area, length, and mineralization of the new bone formed from the remaining patella could be used as the predictive factors in assessing the quality of the repair in terms of its tensile properties of the healing PPT complex after partial patellectomy and reconstruction.

MATERIALS AND METHODS
Twenty-four skeletal mature female New Zealand white rabbits (18-week old, weighed 3.5 ± 0.3 kg) were used for partial patellectomy according to a previously established experimental protocol.5,8,9 Briefly, under general anesthesia with sodium pentobarbital (0.8 ml/kg, i.v.; Sigma Chemicals Co., St. Louis, MO), one of the knees was approached through an anterolateral skin incision. Transverse osteotomy was performed between the distal 1/3 and the proximal 2/3 of the patella using an oscillating hand saw (SYNTHERES, Mathys AG, Bettlach, Switzerland) and guided by a fine caliper. The distal patella was resected without preserving the fibrocartilage zone at the patellar tendon junction. After excising the distal patella, two 0.8 mm diameter drill holes were made vertically along the remaining patella. The patellar tendon was then directly sutured to the proximal 2/3 of the patella via these two drilled holes and fixed with a figure-of-eight tension band wire drawn...
around the superior pole of the patella and the tibia tuberosity to protect the reconstruction and facilitating repair. After suturing the skin, the knee was immobilized with a long leg cast at a resting position of the knee joint. Pain relief drug (TEMGESIC, Reckitt & Colman Pharmaceuticals, Hull, UK) was given to the animals subcutaneously at a dose of 0.01 mg/kg for 3 days after the operation. The immobilization lasted for 4 weeks and then the cast was removed for free cage activity. Daily check-ups were conducted for cast and skin conditions as well as animal lameness caused by pain associated with the surgery. Weekly radiographs were obtained to examine the wire fixation and repair progress. Before weekly radiographs, animals were sedated with ketamin (i.m. 0.25 ml/kg, ALFASAN, Alfasan International BV, Holland). Animal ethical approval had been obtained prior to the experiment (Ref No 01/023/ERG).

After euthanizing the animals with an overdose of sodium pentobarbital, the PPT complexes were harvested at 6, 12, and 18 weeks postoperatively. There were eight animals in each healing time point. The anteroposterior X-ray film of PPT complex was taken using a contact microradiograph machine (Faxitron cabinet X-ray system model; Faxitron X-Ray Corporation, Wheeling II, NJ). The PPT complexes were then wrapped in 0.9% saline-soaked gauze, sealed in airtight plastic freezer bags, and stored at −20°C freezer.

New Bone Area and Length Measurements
After digitizing the X-ray films into an image analysis system (Metamorph image analysis system, version 4.5; Universal Imaging Corp., Downingtown, PA), the new bone size, that is, the enlarged bony part from the proximal remaining patella, was quantified for its area and length from the anteroposterior X-ray film using a previously established measurement protocol by a single examiner (Fig. 1a–c).8,9

New Bone Mineralization Measurements
A multi-slice high-resolution peripheral computed tomography (pQCT, Deniscan 2000; Scanco, Bassersdorf, Switzerland) with a spatial resolution of 0.3 mm and a slice thickness of 1 mm was used to determine the volumetric bone mineral density (BMD) and bone mineral content (BMC) of the newly formed bone. A phantom was used in each scan to standardize the gray-scale level prior to BMD and BMC measurements.10

Biomechanical Testing
After the hind limbs were thawed overnight at room temperature for temperature equilibration, the quadriceps–patella–patellar tendon–tibia complex (QPPTT complex) was carefully dissected by removing all periarticular connective soft tissues around the knee, suture material and metal tension band wire, and disconnecting it from the femur. The QPPTT complex was then mounted on a custom-made tensile testing jig, which consisted of an upper clamp and a lower clamp to fix the distal quadriceps plus the proximal patella and the proximal tibia, respectively. The distal quadriceps, its tendon, and the proximal patella were clamped directly in line with the axis of loading (Fig. 2). A Hounsfield Test Machine (Hounsfield H25KM, Hounsfield Test Equipment LTD, Surrey, UK) with a load cell of 2 KN was used for the loading test. Tensile force was applied to the QPPTT complex to failure at a rate of 20 mm/min.3,9 The failure mode of the test specimen was determined by observing the specimen during and after the mechanical testing and confirming it by radiographic and histological analyses. The maximum tensile force and energy at failure were determined. The maximum tensile force was then divided by the cross-sectional area (CSA) measured at the site of the transverse osteotomy at the distal patella using pQCT transverse slice to obtain the tensile strength at failure, that is, the ultimate strength at failure.

Descriptive Histology
After tensile testing, the PPT complex was prepared for undecalified histology using previously established protocols.9 Briefly, the PPT complex was embedded in methyl methacrylate without decalcification. Mid-sagittal sections in a thickness of 200 µm were cut using a microtome (Leica Sp1600; Leica Instruments, Nussloch, Germany), polished to a thickness of 150 µm with a grinding machine (Phoenix 4000; Wirtz Buehler, Germany), and then stained with toluidine blue for observation under both transmitted light and polarized
The newly formed bone with the healing over time. The due to remodeling. No differences were found in BMD of weeks 6 to 12 but its value decreased at week 18 probably time. The CSA increased at the early healing stages from broken.

All animals survived the experimental periods without major complications. After 4 weeks postoperatively, the cast was removed and all rabbits resumed near-normal cage activities. The patella fixation wires to the proximal tibia showed signs of loosening with a few breakages in the follow-up radiographs. During dissection of the harvested specimens, all fixation wires were found to be broken.

The results demonstrated that the area, length, and BMC of newly formed bone, the CSA of B–T healing interface, failure load, ultimate strength and energy at failure increased significantly with the healing over time. The CSA increased at the early healing stages from weeks 6 to 12 but its value decreased at week 18 probably due to remodeling. No differences were found in BMD of the newly formed bone with the healing over time. The results also showed larger individual variation in the area and length of newly formed bone measured on the anteroposterior contact microradiograph and that of the BMC from the pQCT measurements at each time point (area: 11.7–56.2%; length: 21.1–47.1%, and BMC: 34.2–52.9%). Comparatively, the variations in BMD and CSA were smaller (ranged 16.9–25.6% and 23.1–26.2%, respectively). The tensile properties also showed large individual variation at all time points (failure load: 20.7–49.0%; ultimate strength: 33.6–49.2%; energy at failure: 35.1–73.3%; Table 1).

The correlations were found between new bone formation in terms of its area, length, and BMC and its mechanical properties (failure load, ultimate strength and energy at failure). The area of newly formed bone highly correlated with its length ($r = 0.83, p < 0.01$) and its BMC ($r = 0.68, p < 0.01$) but not its BMD. The length of newly formed bone also correlated with its BMC ($r = 0.46, p < 0.05$). BMD of newly formed bone correlated with its BMC ($r = 0.55, p < 0.01$). Tensile properties (failure load, ultimate strength and energy at failure) correlated with each other ($r$ ranged 0.62–0.84; $p < 0.01$ for all). Most importantly, when the comparison was made to correlate between the area, length, or the mineralization of newly formed bone and tensile properties of the healing PPT complex regardless of the healing time, the area of newly formed bone was found to be highly correlated with failure load, ultimate strength and energy at failure ($r = 0.75, 0.76$, and 0.70, respectively, $p < 0.01$ for all). The length of newly formed bone was also found to be correlated at lower $r$-value with failure load, ultimate strength and energy at failure ($r = 0.61, 0.54, 0.67$, respectively, $p < 0.01$ for all). The BMC of newly formed bone but not its BMD was also found to be correlated with failure load, ultimate strength and energy at failure ($r = 0.44, 0.51, 0.42$, respectively, $p < 0.05$ for all). The results showed that the area, length, and mineralization of newly formed bone from the remaining patella after partial patellectomy can reliably predict the healing quality of PPT complex (Table 2).

Undecalcified sections stained with toluidine blue were observed under both bright light and polarized light microscopy (Fig. S2a–f), which show that the radiographic appearance of calcified region next to the remaining patella after partial patellectomy was indeed the new trabecular bone formation, and it exhibited the remodeling process of transforming from woven to lamellar bone with healing over time. As compared with week 6 specimens, the week 18 sample revealed more advanced remodeling from woven bone to lamellar bone with better collagen alignment of the bone matrix and the formation of more marrow cavities. The failure line resulting from the tensile testing with slow deformation rate occurred between the newly formed bone and the residual proximal patella at both weeks 6 and 12 (Fig. S2c,e). At week 18, some failure lines were found to traverse the junction zone reflecting a more advanced healing stage.

**RESULTS**

All animals survived the experimental periods without major complications. After 4 weeks postoperatively, the cast was removed and all rabbits resumed near-normal cage activities. The patella fixation wires to the proximal tibia showed signs of loosening with a few breakages in the follow-up radiographs. During dissection of the harvested specimens, all fixation wires were found to be broken.

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**Figure 2.** Quadriceps–patella–patellar tendon–tibia (QPPTT) structure complex was mounted on a custom-made tensile testing jig for tensile test, which consisted of an upper clamp and a lower clamps to fix the distal quadriceps and the proximal tibia, respectively.
The present model is indeed a unique one to study the healing of the PPT junction after partial patellectomy and reconstruction. Although it was difficult to quantify animals’ functional activities, their general health condition and absence of major complications reflected the appropriate postoperative care extended to them. The application of wire and cast immobilization assured the early reconstruction protection and the subsequent cast removal allowed gradual loading of the PPT complex to minimize joint contracture and B–T rupture.

At animal euthanasia, all fixation wires in different groups were broken which strongly indicated that the rabbits’ knee extensor mechanisms were functioning. This also suggested that the load-induced remodeling was involved during the healing process to enhance the size changes of the newly formed bone.

The large variation in the data might have been in part due to the difficulties in controlling the surgical location of the osteotomy site and the area or length fluctuation among the experimental animals as a result of the varying status of their knee functional loaning. This variation might also be explained by the surgical procedure mentioned above. This was also true for the experimental studies using a standard osteotomy, where it was technically not always possible to perform a precise osteotomy at the distal one-third of the patella, apart from the potential changes in surgical technique, the status of immobilization and postoperative weight bearing. However, these variations did not affect the outcome of the present study in examining the proposed hypothesis that the area, length, and mineralization of the new bone formation could predict the quality of the PPT junction repair strength.

### Table 1. The Healing Properties and Time-Related Changes of Patella–Patellar Tendon (PPT) Junction at Weeks 6, 12, and 18.

<table>
<thead>
<tr>
<th>Measurements</th>
<th>Healing Time (Weeks)</th>
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<tr>
<td></td>
<td>6</td>
</tr>
<tr>
<td>Newly formed bone</td>
<td></td>
</tr>
<tr>
<td>Area (mm²)</td>
<td>2.17 ± 1.22 (56.2%)</td>
</tr>
<tr>
<td>Length (mm)</td>
<td>0.85 ± 0.40 (47.1%)</td>
</tr>
<tr>
<td>BMD (mg/cm³)</td>
<td>0.65 ± 0.14 (21.5%)</td>
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<tr>
<td>BMC (mg)</td>
<td>0.0075 ± 0.0027 (36.0%)</td>
</tr>
<tr>
<td>CSA (mm²)</td>
<td>29.15 ± 6.74 (23.1%)</td>
</tr>
<tr>
<td>Tensile properties</td>
<td></td>
</tr>
<tr>
<td>Failure load (N)</td>
<td>87.23 ± 42.70 (49.0%)</td>
</tr>
<tr>
<td>Ultimate strength (MPa)</td>
<td>2.89 ± 0.97 (33.6%)</td>
</tr>
<tr>
<td>Energy at failure (J)</td>
<td>0.15 ± 0.11 (73.3%)</td>
</tr>
</tbody>
</table>

Data presented as mean ± SD. The coefficient of variation of the parameters are shown in parenthesis (CV = SD/mean in %). CSA, cross-sectional area measured at the site of transverse osteotomy; BMD, bone mineral density; BMC, bone mineral content.

*p < 0.05, the effect of the healing time. **p < 0.01, the effect of the healing time.

### DISCUSSION

The present model is indeed a unique one to study the healing of the PPT junction healing after partial patellectomy and reconstruction. Although it was difficult to quantify animals’ functional activities, their general health condition and absence of major complications reflected the appropriate postoperative care extended to them. The application of wire and cast immobilization assured the early reconstruction protection and the subsequent cast removal allowed gradual loading of the PPT complex to minimize joint contracture and B–T rupture. At animal euthanasia, all fixation wires in different groups were broken which strongly indicated that the rabbits’ knee extensor mechanisms were functioning. This also suggested that the load-induced remodeling was involved during the healing process to enhance the size changes of the newly formed bone.

### Table 2. Correlation (Expressed in Spearman’s Correlation Coefficient, r) between Area, Length, and Mineralization of New Bone and the Tensile Properties of the Patella–Patellar Tendon Complex at Weeks 6, 12, and 18.

<table>
<thead>
<tr>
<th>Measurements Variables</th>
<th>New Formed Bone</th>
<th>Tensile Property</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Area</td>
<td>Length</td>
</tr>
<tr>
<td>Newly formed bone</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Length</td>
<td>0.83* (0.001)</td>
<td>0.31 (0.139)</td>
</tr>
<tr>
<td>BMD</td>
<td></td>
<td></td>
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<tr>
<td>BMC</td>
<td>0.68** (0.001)</td>
<td>0.33 (0.113)</td>
</tr>
<tr>
<td>CSA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Failure load</td>
<td>0.75** (0.001)</td>
<td>0.61** (0.002)</td>
</tr>
<tr>
<td>Ultimate strength</td>
<td>0.76** (0.001)</td>
<td>0.54** (0.006)</td>
</tr>
<tr>
<td>Energy at failure</td>
<td>0.70** (0.001)</td>
<td>0.67** (0.001)</td>
</tr>
</tbody>
</table>

Values are presented in parentheses.
CSA, cross-sectional area measured at the site of transverse osteotomy; BMD, bone mineral density; BMC, bone mineral content.
*p < 0.05. **p < 0.01.
The time-related change in all data studies was a direct indication of the PPT junction repair progress. The increase of the new bone area, length, BMC as well as its tensile property may be positively related to the bone regeneration and the biomechanical load-induced remodeling process. In consistency with our previous findings, the radiographic enlargement of the remaining proximal patella after partial patellectomy was histologically new bone formation. This finding may explain the underlying mechanism of radiographic enlargement of the remaining patella after partial patellectomy in patients reported by Saltzman et al., who showed that the length of the remaining patella in 11 patients gradually increased by 5 years follow-up after a standardized partial patellectomy. The increase in bone area, length, and BMC with corresponding increase in tensile properties of the PPT junction is a natural outcome of load and function-induced remodeling process. From the radiographic and pathologic findings of broken patella fixation wires in many animals followed more than 6 weeks, this is a strong evidence of functional usage of the quadriceps–patella–patella tendon mechanism among these animals. These results also help to support the conclusions of this study.

The strong correlation results suggested that the area, length, and BMC but not its BMD of the new bone formed from the remaining patella after partial patellectomy could be used as the non-invasive indicators to estimate the quality of PPT junction repair. However, the BMD of the newly formed bone did not show the predictive power for the tensile properties of the healing PPT complex. Furthermore, a slightly lower BMD value was found in week 18 specimens as compared with that of the week 12. This may be explained by the false-negative property of BMD measurement using the pQCT, there the volumetric BMD (mg/cm³) would be calculated by dividing the BMC over the total volume of the newly formed bone containing both bone matrix and marrow spaces. This provides information on degree of bone mineralization only at the organ level, that is, the BMD calculation used the bulk bone volume, which related to the bone mineralized phase, the marrow spaces, osteon canals, lacunae, and canaliculi, without providing the true degree of BMD at matrix level. As bone marrow cavities gradually formed and enlarged with remodeling over time from woven to lamellar bone (refer to Fig. S2a–d), advancement in bone matrix mineralization would be expected with healing over time in the newly formed bone. Since newly formed bone contained an enlarged marrow cavity, which would decrease the BMD, this explained why we could not find a positive association between tensile strength and BMD.

The postoperative enlargement of the remaining patella may bear special clinical significance in increasing patellofemoral contact area, accompanied by the diminished patellofemoral contact pressure and hence preventing the possibility of chondromalacia and osteoarthritis. Therefore, it is important to use the area or length of the new bone formation to predict healing quality at PPT healing junction in assessing the healing progress in order to recommend early mobilization and start effective postoperative rehabilitation programs to prevent joint contracture and enhancing normal function recovery. Whether these findings could be generalized from the PPT healing complex to other anatomical regions such as the Achilles–Calcaneus and rotator cuff, additional experimental and clinical investigations must be conducted.

It was postulated that through tensile loading of the PPT junction, the repairing patella was able to achieve adaptive remodeling under the biomechanical requirements of this important functional unit. Significant increase in newly formed bone area, length, its BMC but not its BMD, and the tensile strength of the PPT junction is a strong indication of successful repair and remodeling after partial patellectomy and reconstruction. Unfortunately, this study still cannot answer the question whether the bone–tendon junction healing using the current reconstructive technique would ever become normal after rupture due to insufficient follow up and the lack of the necessary controls (e.g., the prolonged immobilization of the animals/limbs). Future investigations on any form of enhancement would need this baseline data in order to address this important problem in sports medicine field.

CONCLUSIONS
In conclusion, the area, length, and mineralization of the newly formed bone at the PPT junction after partial patellectomy may serve as useful noninvasive indices in assessing the quality of the bone–tendon junction repair. These findings will have significant basic science as well as clinical impact in sports medicine.

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REFERENCES


