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Exploration of the Optimal Shape for Bone Tumour Biopsy

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Abstract. *Background/Aim: Biopsy hole for bone tumour biopsy may cause pathological fractures. This study aimed to identify the optimal shape of bone tumour biopsy hole using the rabbit femoral head compression test to avoid pathological fracture. Materials and Methods: A compression test with no defect was performed to identify bone fracture location. Three shapes of biopsy holes (same size) were made artificially. Sixty rabbit femurs were randomly divided (n=15 each) into control (no defect), Shape 1 (round), Shape 2 (square), and Shape 3 (rectangular) groups. Results: Twelve out of fifteen femurs were fractured on the femoral shaft; the femoral shaft was targeted for the compression test. Compressive maximum load and fracture energy were significantly higher for Shape 3 than for the other Shapes. Conclusion: A rectangular biopsy hole helps minimise reduction in bone strength. The defect width may be related to fragility of the affected bone.*

If malignant bone tumour is suspected, orthopaedic oncologists generally require a pathological diagnosis to determine the treatment strategy. It is necessary to make a biopsy hole on the cortex wall of the affected bone and then obtain a piece of the tumour tissue (1). However, sometimes this procedure leads to several severe complications, such as massive bleeding, expansion of tumour contamination, and, ultimately, pathological fracture due to weakening of the bone (2, 3).

Some previous papers on pathological fractures of malignant tumours are available (4, 5). Pathological fracture before the treatment of a malignant bone tumour has a negative effect on the quality of life because of the necessity of much wider resections to remove possible tumour contamination (2). The width of the sacrificed tissue will be larger than the surgical margin originally planned, resulting in poor limb function and poor survival rate of these patients (5, 6).

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To the best of our knowledge, there have been only a few similar experimental investigations concerning the optimal shape of bone biopsy holes using human cadavers or animal bone models to avoid pathological fracture due to the bone biopsy procedure (7). The aim of this study was to identify the optimal shape of the bone tumour biopsy hole using the rabbit femoral head compression test under the condition of same defect sizes.

Materials and Methods

Animals. The experimental animals used in this study were 60 female New Zealand White rabbits (NZWRs) aged 1-2 years and weighing between 3500 g and 4500 g. They were purchased from SLC Japan (Shizuoka, Japan) and housed individually, with free access to food and water. They were sacrificed with an overdose of pentobarbitone sodium (300 mg/kg) intravenously, and their hind limbs were dissected. The present study was approved by the institutional review board (No. 17031) and was performed in accordance with the ethical standards laid down in the Declaration of Helsinki. Furthermore, the animal experiment complied with the ARRIVE guidelines. The animal experiments were performed strictly in accordance with the regulations of the Guidelines on Animal Experiments of our university.

Experiment 1. A femoral head compression test was conducted using EZ Graph (Shimadzu Corp., Kyoto, Japan) (Figure 1a). Femur specimens were kept in a freezer at –20˚C for 2 weeks after harvesting and were stored in saline solution before the test. A femoral head compression test was set up using upper and lower jigs and a cylindrical holder, oriented so that the femur was vertical in the sagittal plane, in valgus in the frontal plane, and slightly extroverted in the coronal plane. The lower part of the femur was completely fixed in a polyvinyl chloride pipe using epoxy resin. The upper jig that contacted the femoral head was designed to be flat. The upper jig was designed in such a way that any interference with the femur diaphysis during the compression test was avoided (Figure 1a).

Before mechanical testing, the femurs were removed from frozen storage and saturated in tepid saline while keeping them moist (for consistency). A compression load was applied at a rate of 10 mm/min. The compression direction was parallel to the mechanical axis. The compression test was performed until the femur specimens fractured (Figure 1b). The magnitude of the applied load and displacement was continuously recorded. From the test results, maximum load (N), displacement (mm), elastic modulus

Table I. *Results of rabbit femoral head compression test.*

	Control	Shape 1	Shape 2	Shape 3
Maximum Load (N)	449 (394-692.5)	244 (195-308.5)	182(133.5-213)	334 (276-422.5)
Displacement (mm)	2.53 (2.225-2.82)	1.12 (0.805-1.38)	0.82 (0.665-1.15)	1.28 (1.12-1.685)
Elastic Modulus (N/mm^2)	29782.9 (21221.5-41285.2)	22198.2 (14803.7-28406.8)	26035.7 (12929.9-36438.6)	27628.4 (22725.1-35983.3)
Fracture Energy (N·mm)	943.98 (762.77-1074.1)	142.74 (106.57-224.2)	66.34 (57.98-105.64)	237.53 (164.55-306.6)

(N/mm^2), and fracture energy (N·mm) were calculated (Figure 1c). Elastic modulus was calculated between 20% and 80% of the maximum fracture load using the load–displacement curve.

The maximum load was the maximum value of loading increase during mechanical testing. We could deduce that the higher the numerical value of the maximum load, the more difficult was the fracture of the femur. Displacement was the distance of the site of the femoral head from the start of the experiment to the moment of fracture. Again, the higher the numerical value of the displacement, the more difficult was the fracture of the femur, as it was easy to bend. The elastic modulus was highly correlated with stiffness, so we could deduce that the numerical value of the elastic modulus depicts material properties. Fracture energy was equal to the area under the load–displacement curve. We observed that the higher the numerical value of fracture energy, the more difficult was the fracture of the femur.

Experiment 2. Before mechanical testing, both femurs were removed from frozen storage and saturated in tepid saline to drill the bone while keeping the femur moist. We used the results from experiment 1 to decide the location of bone fenestration. A Stryker Total Performance System high-speed drill (Stryker Instruments, Kalamazoo, MI, USA) set at 60,000 rpm with a diamond burr was used to perforate the bone to create holes of the same size as far as possible. The femurs were randomly assigned to three equal groups of 15 each. The three groups included various kinds of defects of the same size at the same site on the femoral shaft. In each of the rabbit femurs, holes of three different shapes were artificially created at the distal 1.5 cm of the lesser trochanter on the front surface of the femoral shaft. Shape 1 was a round hole, 7 mm in diameter (3.14×3.5^2). Shape 2 was a 6.2 mm square hole (6.2×6.2). Shape 3 was a 3.1 mm by 12.4 mm rectangular hole (3.1×12.4). All drilled areas had the same size of 38.4 mm^2 (Figure 2).

Statistical analysis. The Kruskal–Wallis test with the Steel–Dwass test for multiple comparisons was performed to compare the four groups in terms of the following four parameters: maximum load (N), displacement (mm), elastic modulus (N/mm^2), and fracture energy (N·mm). Statistical analysis was performed using Excel statistics software (version 2015; SSRI Co., Ltd) for Windows. p-Values <0.05 were considered statistically significant.

Results

Experiment 1. In the femoral head compression test, 12 of 15 femurs were fractured at the femoral shaft, whereas only three femurs were fractured at the proximal end.

Experiment 2. The median compressive maximum load (N) of the Control (no defect), Shape 1 (round), Shape 2 (square), and Shape 3 (rectangular) groups recorded during mechanical testing were 449 (394-692.5), 244 (195-308.5), 182 (133.5-213), and 334 (276-422.5), respectively (Table I, Figure 3a). The compressive maximum load of Shape 3 was significantly higher than those of Shape 1 ($p=0.042$) and Shape 2 ($p=0.003$) (Figure 4a).

The median displacements (mm) of the Control, Shape 1, Shape 2, and Shape 3 were 2.53 (2.225-2.82), 1.12 (0.805-1.38), 0.82 (0.665-1.15), and 1.28 (1.12-1.685), respectively (Table I, Figure 3b). The displacement of Shape 3 was significantly higher than that of Shape 2 ($p=0.018$, Figure 4b). There was no significant difference between the displacements of Shape 3 and 1 ($p=0.33$, Figure 4b) or between Shape 1 and Shape 2 ($p=0.33$, Figure 4b).

The median elastic modulus (N/mm^2) of the Control, Shape 1, Shape 2, and Shape 3 were 29782.9 (21221.5-41285.2), 22198.2 (14803.7-28406.8), 26035.7 (12929.9-36438.6), and 27628.4 (22725.1-35983.3), respectively (Table I, Figure 3c). There were no significance differences between the Control and the other shapes ($p=0.283$, Figure 4c).

The median fracture energy (N·mm) of the Control, Shape 1, Shape 2, and Shape 3 were 943.98 (762.77-1074.1), 142.74 (106.57-224.2), 66.34 (57.98-105.64), and 237.53 (164.55-306.6), respectively (Table I, Figure 3d). The fracture energy of Shape 3 was significantly higher than those of Shape 1 ($p=0.033$) and Shape 2 ($p<0.001$) (Figure 4d).

Discussion

According to the result of experiment 1, 12 of 15 rabbit femurs were fractured on the femoral shaft, whereas only three femurs were fractured at the proximal end. Therefore, the compression test was applied on the femoral shaft in experiment 2. Three types of artificially created biopsy holes, namely, round, square, and rectangular, were compared. Compared to the other shapes, the rectangular shape had significantly higher numerical value of the maximum load, displacement, and fracture energy.

Biopsy is essential in the diagnosis of bone tumours. Specifically, it is a key step in the diagnostic strategy and

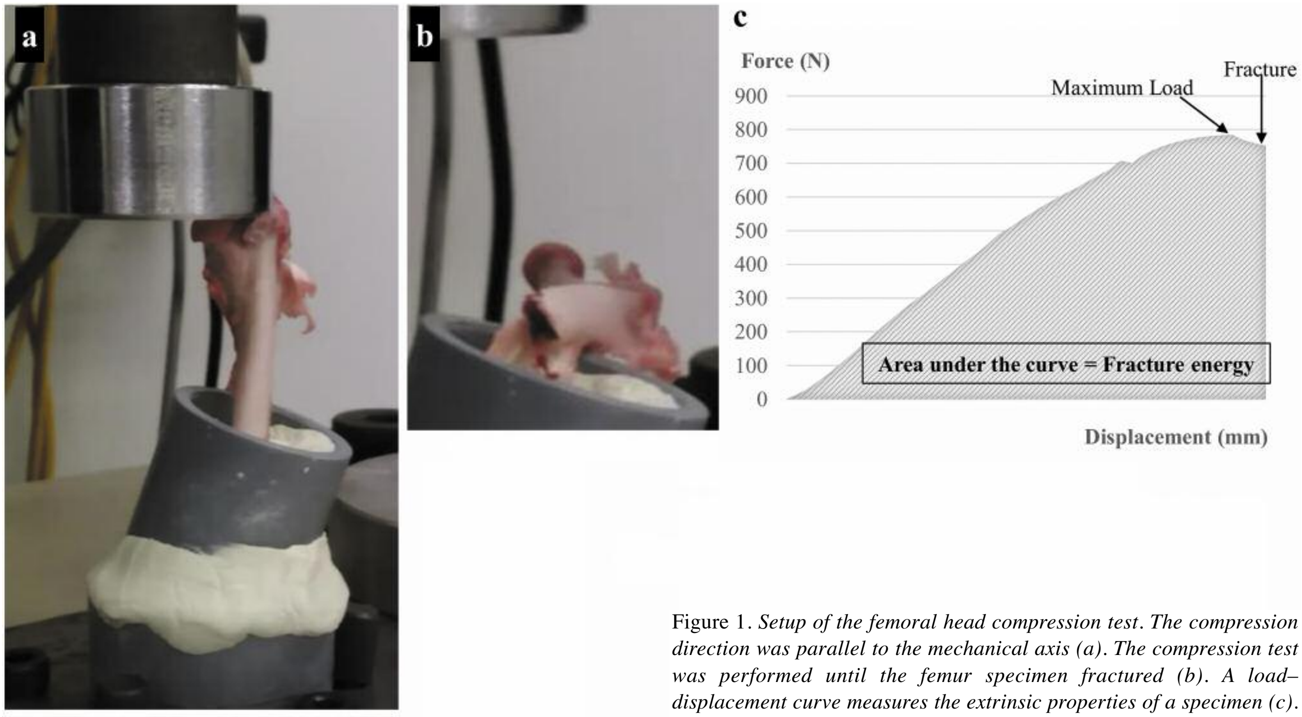

Figure 1. *Setup of the femoral head compression test. The compression direction was parallel to the mechanical axis (a). The compression test was performed until the femur specimen fractured (b). A load–displacement curve measures the extrinsic properties of a specimen (c).*

histological confirmation and is particularly valuable in patients who may have a solitary bone metastasis or unusual radiological features suggesting malignant bone tumours (8). During biopsy, orthopaedic oncologists usually make a biopsy hole on the cortex wall of the affected bone. This procedure sometimes induces the risk of weakening the bone (1). When the fragile bone undergoes pathological fracture, tumour contamination may become expansive, especially if the bone tumour is malignant (1). It is difficult to radiologically determine the surgical margin safely, so wider safety tissues, including the tumour, must be sacrificed (9, 10). Additionally, it may be complicated to remove the malignant tumour along with the pathological fracture. Physical performance after surgical treatment of the pathological fracture in malignant tumours is reduced, so pathological fractures have a negative effect on the quality of life (Figure 5) (4). Previous reports have shown that pathological fracture is closely related to poor survival rates of patients (6). Therefore, biopsy must be performed as safely as possible. However, there have been few reports that showed experimental investigations of bone tumour biopsy to avoid pathological fractures. Herein, we made a biopsy hole on the cortical wall of the rabbit femur to identify the optimal shape of the bone tumour biopsy hole using the rabbit femoral head compression test.

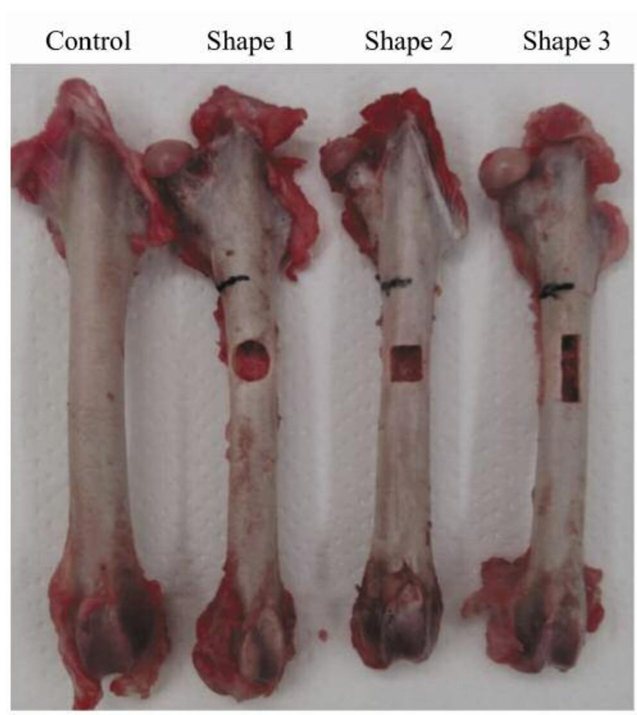

Figure 2. *Representative specimens demonstrating the artificially created bone biopsy hole in groups. Control: No defect. Shape 1: Round hole. Shape 2: Square hole. Shape 3: Rectangular hole.*

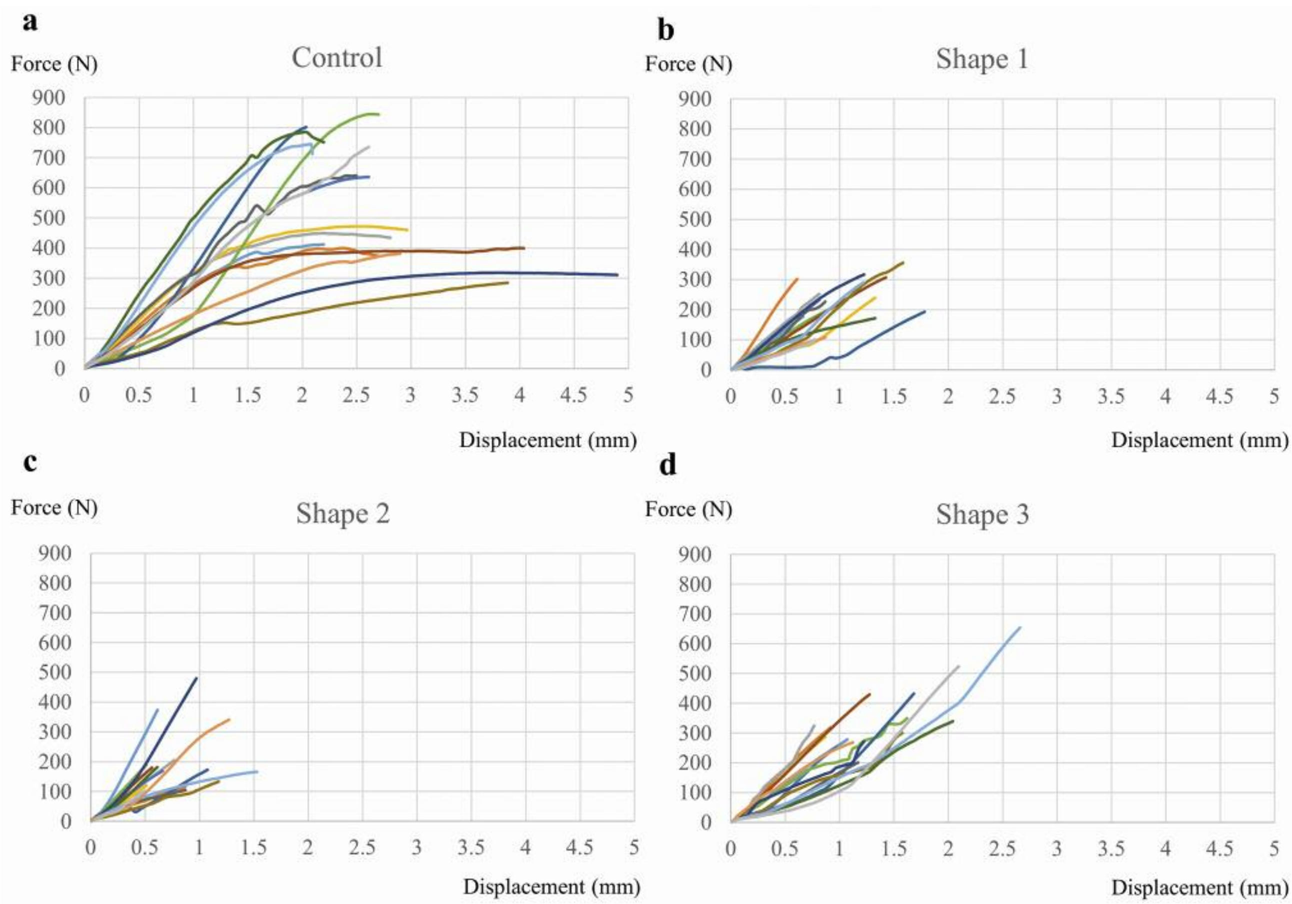

Figure 3. *Load-displacement curves of each femur with Control (no defect) (a), Shape 1 (Round) (b), Shape 2 (Square) (c), and Shape 3 (Rectangular) (d) on the femoral head compression test.*

According to a previous report on the incidence of both primary benign and malignant bone tumours, the femur is the leading tumour site (11). Various studies have revealed that femoral involvement in malignant bone tumours was the most common except for the trunk (12, 13). Moreover, the femur is a weight-bearing bone. Femurs with malignant tumours are of major concern due to the risk of pathological fractures, especially after bone biopsy. Considering the risk assessment of pathological fractures after bone tumour biopsy, the femur is highly likely to be affected. Therefore, we focused on the femur.

There have been no previous papers about the shape of the biopsy hole in the treatment of bone tumour since Clerks introduced the relationship between biopsy-hole shape and size in 1977 (7). We planned to perform the femoral head compression test from the femoral head to the direction of the mechanical axis by assuming bipedal walking as the method of the study. Before starting the mechanical test, we needed to analyse the difference between humans and rabbits because rabbits walk with four legs while humans walk with two legs. We first investigated the part of the femur anatomically fractured in rabbits under the condition of no defects (experiment 1). There was controversy regarding the preferential anatomical site of the femur during fracture in patients with bone metastases. Miller *et al*. and Mirel *et al*. documented the risk factors of pathological fractures with femoral metastases, and they suggested that the proximal femur was more frequently fractured than the shaft (14, 15). Cadaver femurs with bone metastases were reported to be more frequently fractured in the proximal end than in the femoral shaft (16). According to a previous study that conducted the femoral head compression test using mouse femur, all femurs were fractured in the proximal end (17). On the contrary, Morishige *et al*. indicated that the number of pathological femoral shaft fractures was much higher than that of proximal femoral fractures (18). The femoral head compression test in this study proved that 12 of 15 rabbit femurs without holes were fractured on the femoral shaft.

According to the result of experiment 1, we started to perform mechanical testing on the femoral shaft. In

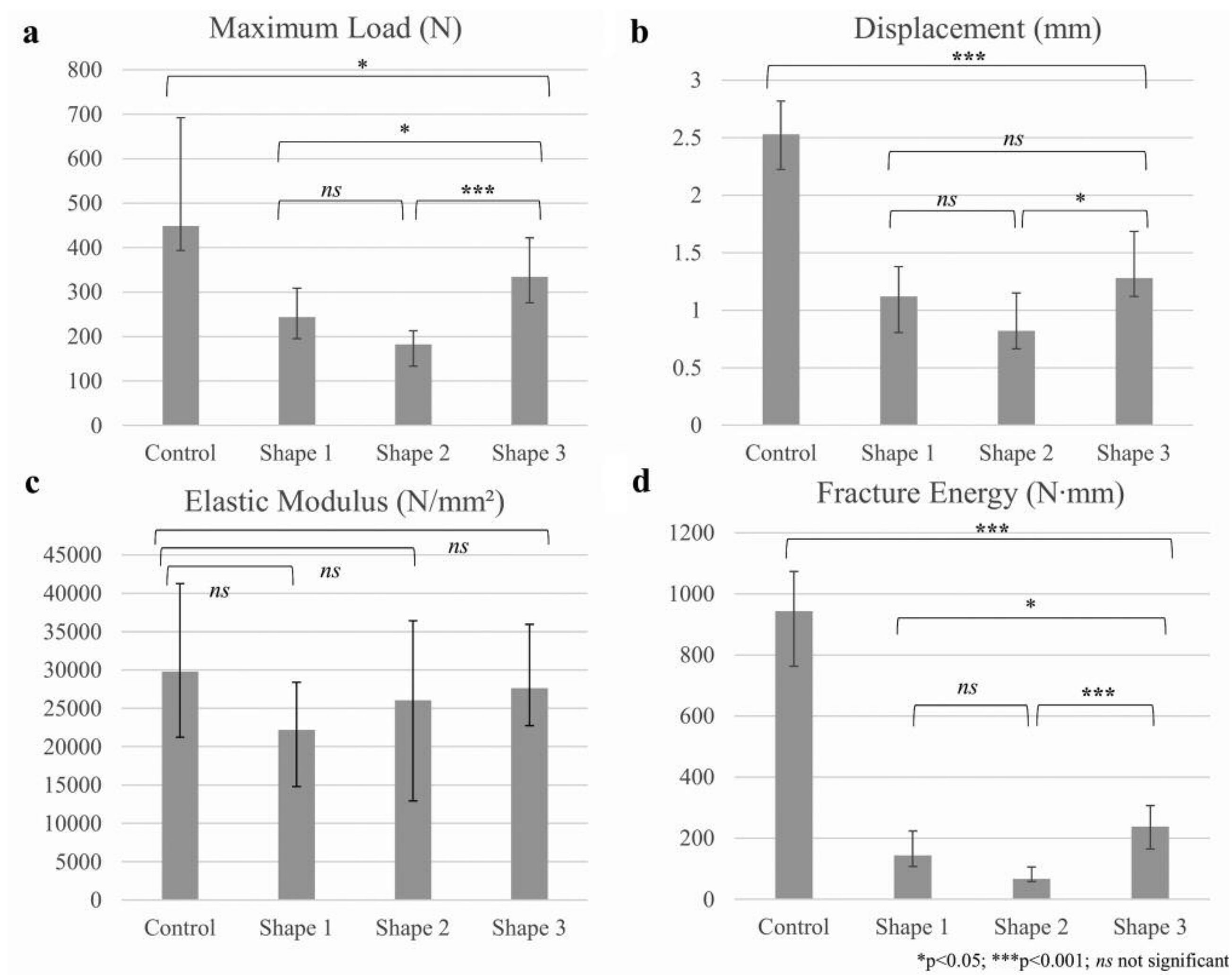

Figure 4. *Distribution of maximum load (a), displacement (b), elastic modulus (c), and fracture energy (d). The data are presented as the median±interquartile range.*

experiment 2, we artificially created three practical biopsy holes (round, square, and rectangular) for bone tumour biopsy and compared the results of the control (no defect) and those of the other three shapes under the condition that the areas of all the drilled holes were the same (38.4 mm^2).

In terms of the maximum load and fracture energy in experiment 2, the bone strength of a femur with a hole was significantly lower than that of a femur without defect (control) (p=0.04 and p<0.001, respectively). Moreover, the maximum load of the Shape 3 was significantly higher than that of Shape 1 (p=0.042) and 2 (Square) (p=0.003). The fracture energy of Shape 3 was also significantly higher than those of Shape 1 (p=0.033) and Shape 2 (p<0.001). There was also no significant difference between Shape 1 and Shape 2 (p=0.48 and p=0.12, respectively). Considering this mechanism, the width of Shape 3 was narrower than that of Shape 1 and Shape 2. Accordingly, the fragility of bone strength appeared to be related to the width of the defect of the cortical bone.

Displacement of the femur with hole was also significantly lower than that of the femur without defect (control) (p<0.001). In our study, the displacement of Shape 3 was approximately half of that of the control. There were no significant differences among the values of elastic modulus (p=0.283), so the elastic modulus of the femur with hole was not significantly lower than that of the femur without defect (control). Therefore, it was deduced that the stiffness of the rabbit femur was maintained after artificially creating the biopsy hole in this study.

In our opinion, if the cortical thickness is thin enough for percutaneous needle biopsy, percutaneous biopsy should be

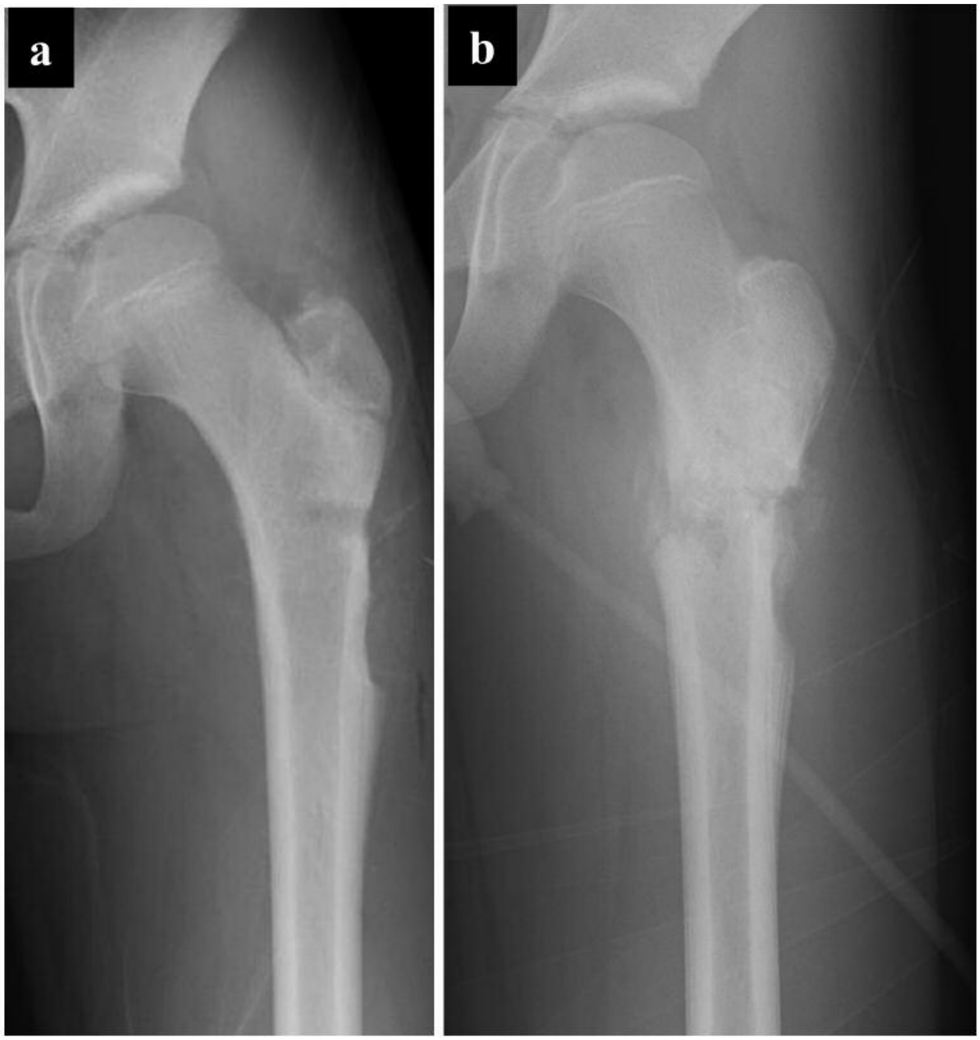

Figure 5. *Radiograph of a 12-year-old male patient with Ewing Sarcoma who underwent bone biopsy of the left femur (a). Unfortunately, femur fracture occurred on the site of the biopsy hole (b).*

preferred over incisional biopsy to avoid pathological fractures, as it is safer (1). On the contrary, if it is difficult to perform needle biopsy because of insufficient cortical thickness, we need to make a biopsy hole of rectangular shape with small width on the cortex wall.

There are some limitations to this study that should be considered. First, we should have chosen human cadavers instead of animal bones based on clinical grounds. Second, surgeons often perform bone biopsy at the lateral side of the femoral bone. Therefore, we should have made holes of the same area on the lateral side of the femoral bone. However, it was difficult for us to make a biopsy hole on the cortex wall on the lateral side because of the narrow surface of the bone, so we created the hole in the front side, on the wider surface of the bone. Third, practical biopsy procedures are performed differently by orthopaedic oncologists, and each shape is dependent on their clinical experience. Hence, a square or rectangular shape created using a bone chisel and a round shape created using a bone drill may be practical for bone tumour biopsy. Fourth, there are many differences between humans and rabbits because rabbits walk on four legs while humans walk on two legs. The results of animal experiments are not always consistent when extrapolated for human beings. Fifth, only the results of the femoral head compression test were used to verify the optimal shape of a drilled hole. The data from torsion tests should have been considered as well.

In conclusion, this study investigated the optimal shape for bone biopsy using a rabbit femoral shaft biopsy model to prevent pathological femoral fracture. Three kinds of artificial biopsy holes (round, square, and rectangular) were compared under same area conditions, and we verified that the rectangular shape was optimal in minimising the reduction in bone strength after bone tumour biopsy. The width of the defect may be related to the fragility of the affected bone.

Conflicts of Interest

The Authors have no conflicts of interests to declare regarding this study.

Authors' Contributions

Tadashi Iwai designed this study, analysed the data, prepared the figures and wrote original draft manuscript. Manabu Hoshi and Hiroaki Nakamura oversaw the study and revised the manuscript. All Authors reviewed the manuscript.

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