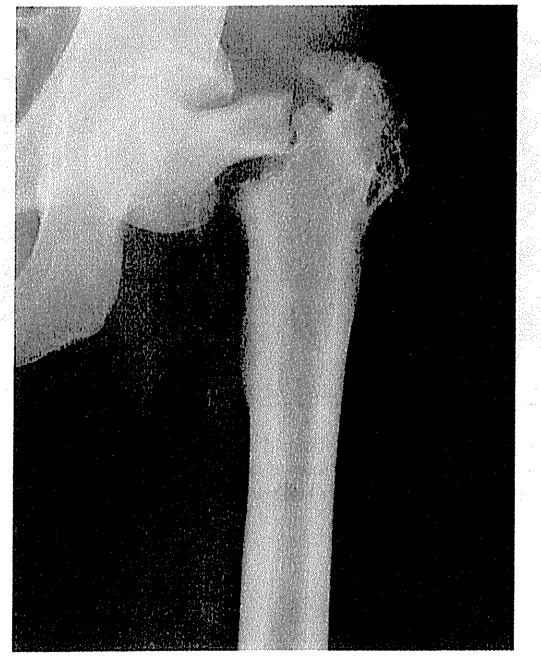

Fig. 1 Preoperative radiograph (A-P view): a displaced femoral neck fracture with scalloping around the femoral neck is apparent. An osteolytic and hypertrophic lesion with bony fragments was detected in the greater trochanter. Cortical thickening was present in the subtrochanter. The lesser trochanter was absent

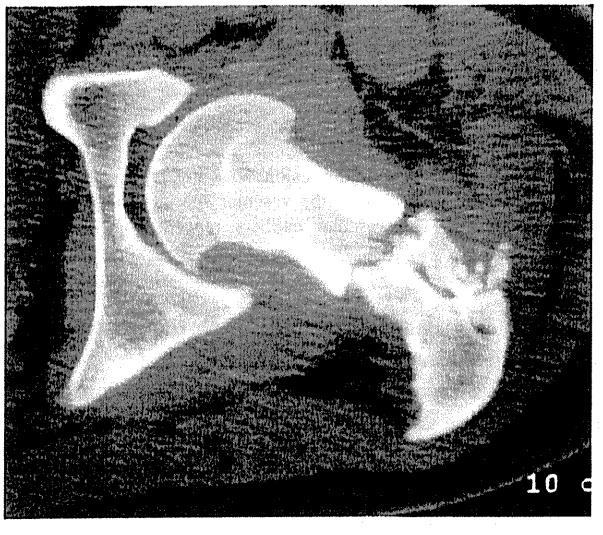

Fig. 2 Computed tomography (CT) images showed a dense area of soft tissue with scalloping of the femoral neck. Irregular osteolytic and sclerotic changes with bony fragments were present at the trochanteric region

Coronal T2-weighted images showed high signal intensity regions extending into the adductor muscles. This finding was interpreted as hemorrhage from the femoral neck fracture (Fig. 3).

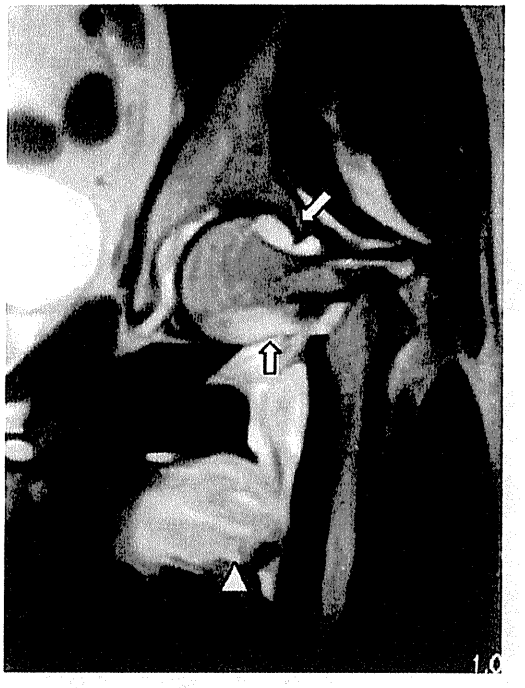

Fig. 3 Coronal T2-weighted image (3600/125). An intra-articular mass lesion surrounding the femoral neck was present (*arrow*). The signal intensity of the lesion was higher than that of bone marrow. High signal intensity in the adductor muscles was also noted (*arrowhead*)

An axial T2-weighted image showed a high-intensity lesion at the caudal aspect of the femoral neck and peri-trochanteric region, suggesting extra-articular extension. Hemorrhage and edematous change was also evident in the peri-trochanteric region.

Open biopsy using a posterior approach was performed. Histopathological examination revealed synovium with fibroconnective tissue but without obvious tumor tissue in the sample. The results of bacterial cultures of the synovial tissue samples were negative.

Under the working diagnosis of pathological fracture caused by fibrous and synovial proliferation of an unknown cause, we performed internal fixation with the Hansson Twin Hook system and implanted a bone graft.

The follow-up plain radiograph showed osteolytic changes in the femoral neck. At 10 months after surgery, nonunion pseudoarthrosis at the fracture site resulted in screw breakage and implant failure (Fig. 5).

We performed revision surgery for femoral head resection and plate fixation of the greater trochanter. The fracture site was covered with fibrous and synovial tissue. The femoral head was displaced, and the cartilage of the femoral head was thinned with discoloration and ulcerations (Fig. 6).

Synovium with connective tissue around the fracture site was surgically excised and specimens were submitted for

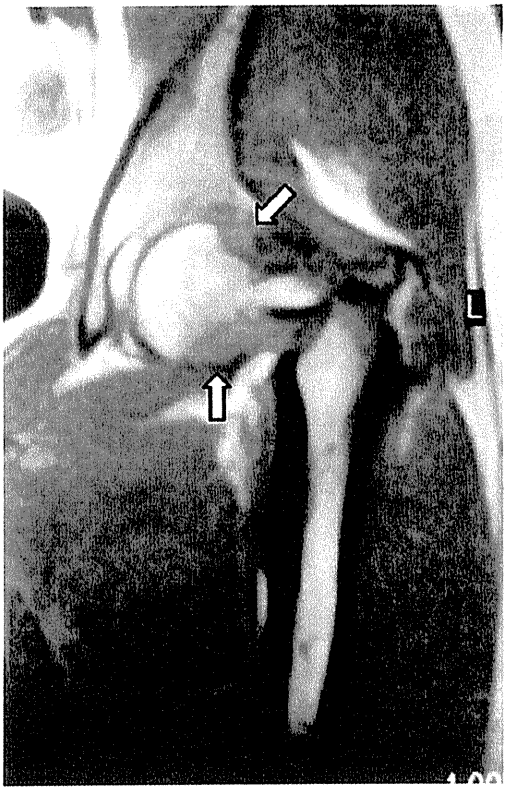

Fig. 4 Coronal T1-weighted image (400/15) showed an intermediate signal intensity lesion surrounding the femoral neck (*arrow*)

Fig. 5 Plain radiograph (A-P view) 10 months after surgery. Screw breakage and implant displacement occurred. The femoral head and greater trochanter were displaced

histopathological examination, together with the femoral head.

Macroscopically, the resected fermoral head showed marked deformity with an irregular articular surface exhibiting eburnation and fibrillation. Avascular osteonecrosis was observed at the cut surface of the specimen.

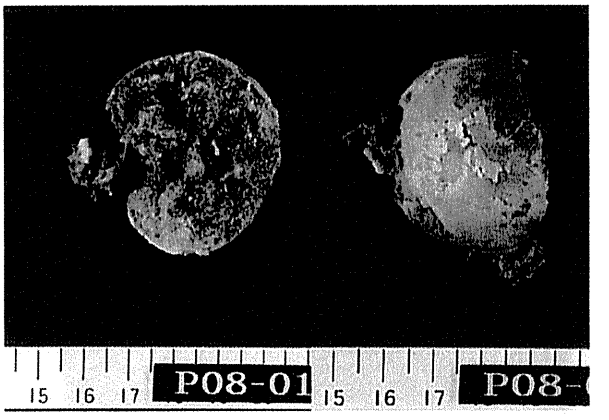

Fig. 6 Image of the excised femoral head. A deformed femoral head is seen with an irregular surface of articular cartilage. Subchondral trabecular bone and medullary bone were replaced with fibrous tissue

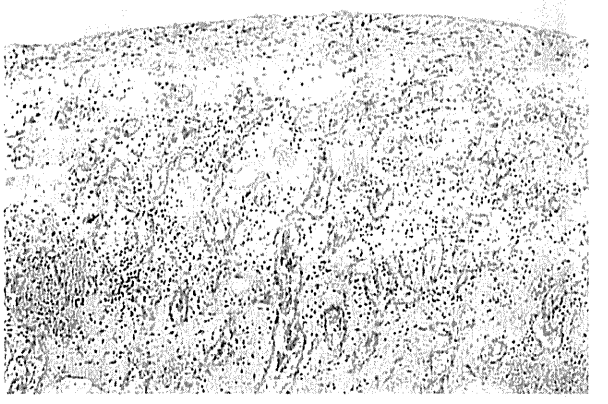

Fig. 7 A high-power view of the lesion. Vascular proliferation was seen in the lesion. The sizes of the vascular spaces containing red blood cells varied. The vascular calibers ranged from small to moderate

Histologically, subchondral osteonecrosis of trabecular bone with medullary fibrosis was evident.

In the histological examination of the synovium with connective tissue around the fracture site, the proliferation of capillary vessels of various sizes were observed, with focal hemorrhage and hemosiderin deposition, which diffusely involved the synovium and adjacent connective tissue (Figs. 7, 8).

Based on the aforementioned findings, a pathological diagnosis of synovial hemangioma was made.

After 3 years of follow-up, no recurrence was detected (Fig. 9).

The functional results for the patient were evaluated by the Enneking score system [10].

The total score was 16/30 and the rating was 53.3% (pain 5, function 1, emotional acceptance 3, supports 1,

Fig. 8 Hemangioma involving the intertrabecular space. Vascular proliferation around the necrotic trabecular bone was seen

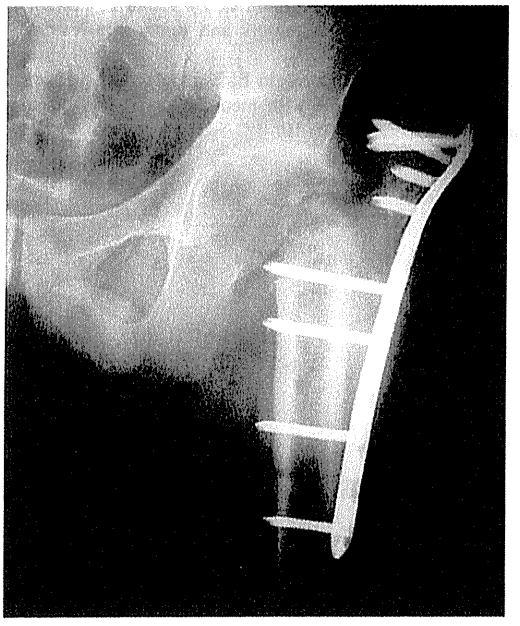

Fig. 9 Plain radiograph (A-P view) 3 years after revision surgery. The femoral head was resected, and the greater trochanter was united

walking 5, gait 1). We are planning hip reconstruction surgery using the Ilizarov method or total hip replacement in the future.

The patient and his family allowed us to submit his data for publication.

Discussion

Preoperative diagnosis of synovial hemangioma of the knee is very difficult [5, 11–13]. The differential diagnosis of synovial hemangioma includes pigmented villonodular synovitis (PVNS), synovial osteochondromatosis, hemophilic arthropathy, and joint infection (cf. tuberculosis, Lyme arthritis) [4, 5, 13]. In many cases, MR imaging findings aid in establishing a differential diagnosis of synovial hemangioma; an intra-articular lobulated mass of intermediate signal intensity on T1-weighted images and hyperintense regions on T2-weighted images are indicative of synovial hemangioma [1, 3, 4, 14, 15].

In the present case, pressure erosion was clearly observed on plain radiograph and CT scan. Although this finding strongly suggested the existence of an extraskeletal mass lesion, we did not find specific signals from synovial hemangioma in the intra-articular mass on MR images. A preoperative diagnosis could not be established based on imaging and histopathological findings.

Due to the anatomical characteristics of the hip joint, it is often difficult to obtain adequate tissue samples for histopathological examination. If an intra-articular mass lesion is evident on MR images, CT-guided biopsy can be performed to establish the pathological diagnosis. If a small lesion is detected on CT or MR images, arthroscopy can be an alternative diagnostic tool [7].

A high level of suspicion is a prerequisite for diagnosing synovial hemangioma of the hip, because this condition is very rare and it is difficult to obtain adequate tissue samples.

Synovial hemangioma of the knee may lead to progressive joint destruction due to repeated bleeding, which is also observed in hemophilia [2, 8]. In some cases of synovial hemangioma of the knee, destructive changes in the adjacent femur have been reported [1, 3, 4, 9]. The aggressive growth of the tumor leads to the destruction of juxta-articular structures [3, 4, 16]. There have been no reports of synovial hemangioma with severe bone destruction resulting in pathological fracture.

In the present case, a pre-existing synovial hemangioma extended around the femoral neck and intertrochanteric region, which weakened the structure of the femur and resulted in pathological femoral neck fracture and avascular necrosis of the femoral head. Because the joint cavity of the hip is narrow and small, intra-articular synovial hemangioma can erode the femoral neck and easily extend into extra-articular soft tissue. Therefore, synovial hemangioma of the hip joint can cause more severe complications than synovial hemangioma of the knee joint.

Conclusion

Synovial hemangioma of the hip joint is a very rare condition that can extend into extra-articular structures and cause pathological fracture of the femoral neck.

Conflict of interest The authors did not receive any outside funding or grants in support of this work. Neither they nor any member of their immediate families received payments or other benefits or an agreement to provide such benefits from a commercial entity.

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Endoscopic Surgery for Young Athletes With Symptomatic Unicameral Bone Cyst of the Calcaneus

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Investigation performed at Teikyo University, Itabashi, Japan

Background: Open curettage with bone graft has been the traditional surgical treatment for symptomatic unicameral calcaneal bone cyst. Endoscopic procedures have recently provided less invasive techniques with shorter postoperative morbidity.

Hypothesis: The authors' endoscopic procedure is effective for young athletes with symptomatic calcaneal bone cyst.

Study Design: Case series; Level of evidence, 4.

Methods: Of 16 young athletes with symptomatic calcaneal bone cyst, 13 underwent endoscopic curettage and percutaneous injection of bone substitute under the new method. Three patients were excluded because of short-term follow-up, less than 24 months. For the remaining 10 patients, with a mean preoperative 3-dimensional size of 23 × 31 × 35 mm as calculated by computed tomography, clinical evaluation was made with the American Orthopaedic Foot and Ankle Society Ankle-Hindfoot Scale just before surgery and at the most recent follow-up (mean, 36.2 months; range, 24-51 months), and radiologic assessment was performed at the most recent follow-up, to discover any recurrence or pathologic fracture. Furthermore, the 10 patients—all of whom returned to sports activities—were asked how long it took to return to initial sports activity level after surgery.

Results: Mean ankle-hindfoot scale score improved from preoperative 78.7 ± 4.7 points (range, 74-87) to postoperative 98.0 ± 4.2 points (range, 90-100) ($P < .001$). Pain and functional scores significantly improved after surgery ($P < .01$ and $P < .05$, respectively). Radiologic assessment at most recent follow-up revealed no recurrence or pathologic fracture, with retention of injected calcium phosphate cement in all cases. All patients could return to their initial levels of sports activities within 8 weeks after surgery (mean period, 7.1 weeks; range, 4-8 weeks), which was quite early as compared with past reports.

Conclusion: Endoscopic curettage and injection of bone substitute appears to be an excellent option for young athletes with symptomatic calcaneal bone cyst for early return to sports activities, because it has the possibility to minimize the risk of postoperative pathologic fracture and local recurrence after early return to initial level of sports activities.

Keywords: calcaneal cyst; endoscopic surgery; bone substitute; percutaneous injection

Unicameral bone cyst, which is a benign fluid-filled lesion, occurs most commonly in long bones, such as the humerus and femur, and its occurrence in the calcaneus is relatively rare.[23,30,35] Although about 60% of patients with calcaneal bone cyst complain of pain in the heel or foot and ankle, some patients are asymptomatic and are identified radiologically as an incidental finding.[24] Unlike with cysts of long bone, pathologic fracture due to calcaneal bone cyst is rare, and only a few such cases have been reported.[7,14,23] Therefore, there is still controversy about how to deal with unicameral bone cyst of the calcaneus. Most surgeons agree about the need for surgical intervention for cases with persistent pain or a risk of impending or pathologic fracture.[1,12,23,27,30] Furthermore, some reports have supported surgical intervention for cases with high occupational or sports activities for fear of pathologic fracture of this weightbearing bone.[1,29] Traditionally, open curettage with bone graft has been the main procedure for unicameral bone cyst of the calcaneus, and good prognosis has been reported.[12,20,23,30] However, it is problematic to indicate this surgical method for young athletes who desire early return to sports activities, because such invasive procedures include long-term morbidity. Yet some types of less invasive surgery have been reported.§ Steroid injection can be useful for bone cyst of long bones,[4,5,28] but past studies

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The authors declared that they had no conflicts of interest in their authorship and publication of this contribution.

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§References 1, 2, 4, 5, 8, 19, 22, 26-28.

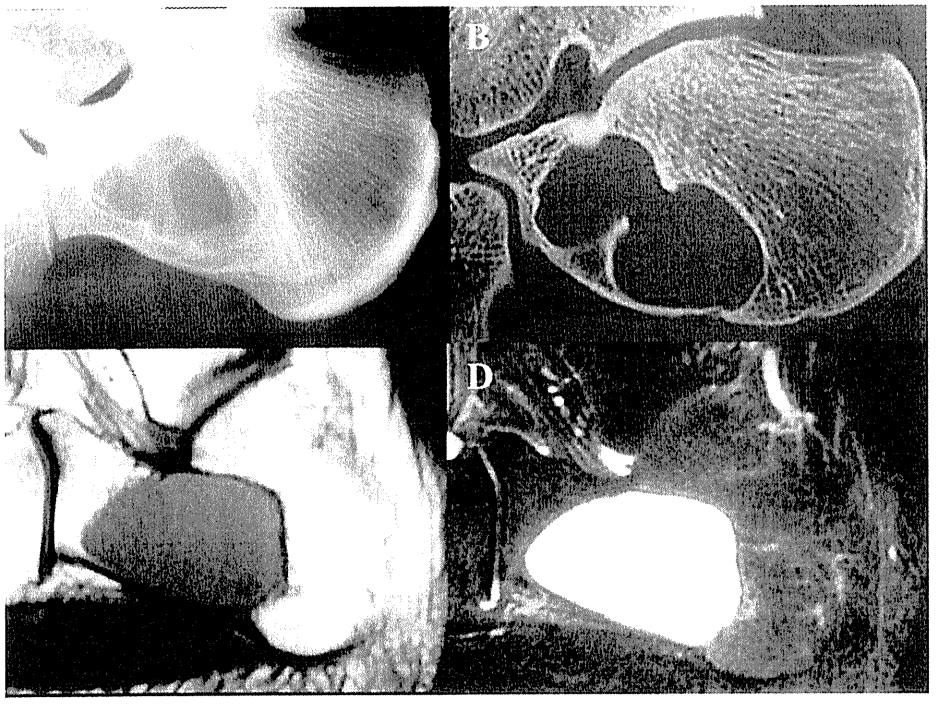

Figure 1. Radiologic examination for diagnosis. Plain radiograph (A) and computed tomography (B) showing cystic lesion in the calcaneus. Sagittal T1-weighted magnetic resonance image (C) showing a hypointense mass and T2-weighted image (D) showing a hyperintense mass, which is brighter than fat tissue.

have confirmed the inefficiency of this procedure for calcaneal bone cyst.[12,16] Although other less invasive surgeries have been indicated for calcaneal bone cyst with good prognosis—namely, multiple drilling and/or continuous decompression using a cannulated screw,[1,27] as well as minimal or endoscopic curettage and injection of bone substitute[2,8,19,22,26]—no study has determined whether these surgical methods have brought about early return to sports activities for young athletes with symptomatic calcaneal bone cyst. The purpose of this study was to evaluate the feasibility, effectiveness, and clinical outcome of our less invasive procedure for young athletes with symptomatic calcaneal bone cyst.

MATERIALS AND METHODS

Between January 2003 and February 2010, 16 young athletes with unicameral bone cyst of the calcaneus were followed at our institution. All patients were introduced to us by their practitioners because of heel pain that prevented them from participating in their sports activities for a few months; there was no case of incidental discovery without symptom. The diagnosis of unicameral bone cyst was made by plain radiographs, computed tomography, and magnetic resonance imaging in all patients (Figure 1) and confirmed by histologic examination for patients who underwent our surgical procedure. Our indication for surgical intervention was (1) persistent heel pain that disturbed patients' daily activity or sports activity and did not respond to nonoperative therapy, including sports restriction, heel support, and partial or nonweightbearing with crutches during 6 months, or (2) a risk of impending fracture, which we defined according to Fidler's study,[9] as destruction of the cortical wall by more than 50% of its thickness by extension of the large cyst, which could be confirmed by radiologic assessment and supposed as a cause of persistent pain. Three patients with small cysts responded to nonoperative therapy and were not included in this study. Thirteen patients were treated surgically by means of endoscopic curettage and percutaneous injection of calcium phosphate cement by a senior surgeon; however, 3 of these patients were excluded from this study because of short-term follow-up, less than 24 months, after surgery. As a result, the remaining 10 patients were included in the study, with a mean preoperative 3-dimensional size of 23 × 31 × 35 mm at the position of neutral triangle as calculated by computed tomography. No case with pathologic fracture had been clearly revealed by preoperative radiologic examination. The right hindfoot was affected in 6 patients and the left hindfoot in 4 patients, and there was no case of bilateral involvement. There were 7 male and 3 female patients, with an average age of 18.7 years (range, 16-24 years). All patients had engaged in sports activity before occurrence of symptoms, and all strongly desired to return to their sports activity without pain (Table 1).

TABLE 1
Descriptive Data of 10 Patients

No.	Sex	Age, y	Follow-Up, mos	Affected Side	Sport	Return to Sport, wks
1	Male	16	51	Right	Sprints	8
2	Male	23	39	Left	Marathon	8
3	Male	21	30	Right	Marathon	8
4	Male	16	40	Right	Basketball	6
5	Male	18	32	Left	Soccer	8
6	Male	17	24	Right	Soccer	4
7	Female	24	30	Left	Basketball	6
8	Male	17	48	Left	Soccer	8
9	Female	18	32	Right	Sprints	8
10	Female	17	36	Right	Badminton	7
Mean		18.7	36.2			7.1

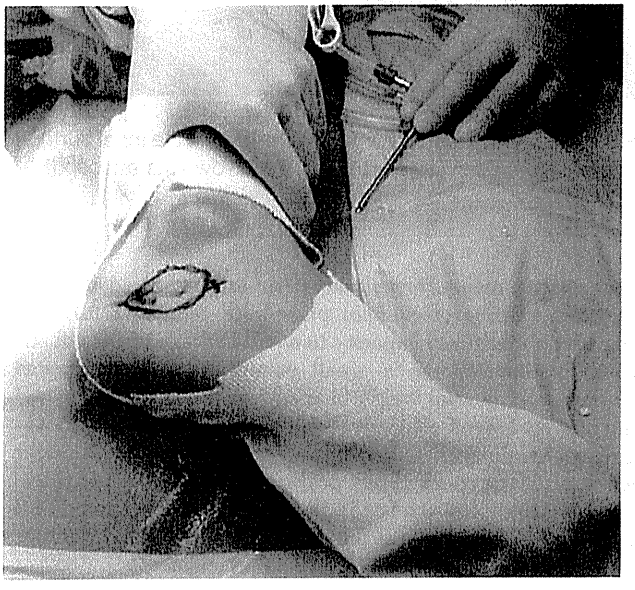

Figure 2. The configuration of the calcaneal bone cyst is marked on the lateral aspect of the hindfoot.

Surgical Technique

Surgery was conducted under lumbar spinal anesthesia with the patient in the lateral position and with no application of a pneumatic tourniquet. First, the configuration of the calcaneal bone cyst was confirmed under image intensifier and marked on the lateral aspect of the hindfoot (Figure 2). Two guide wires were inserted at 2 points—the most anterior and posterior points of the oval-like, marked configuration of the cyst, in the direction toward the center of the cyst—and 2 small portals were made at each insertional point of the guide wires. The lateral calcaneal wall was fenestrated via 2 portals by means of a cannulated drill with a diameter of 5 mm (Figure 3). Next, a 2.7-mm arthroscope was inserted via the posterior portal (Figure 4), and aspirated cyst fluid via the anterior portal was sent for bacterial and fungus culture. After a detailed inspection of

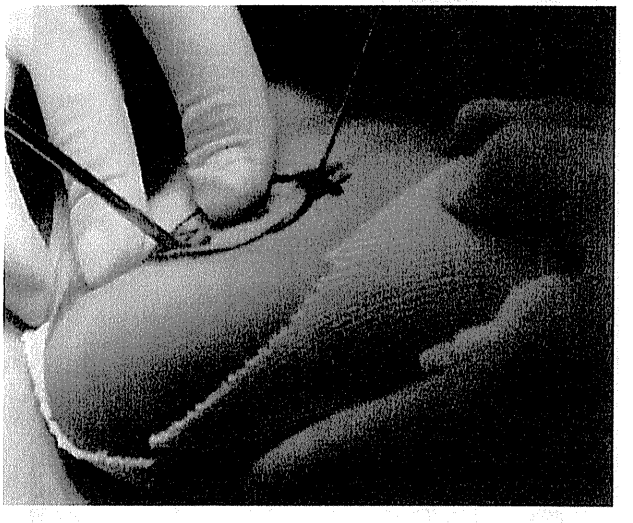

Figure 3. Fenestration of the lateral wall by means of a cannulated drill with a 5-mm diameter.

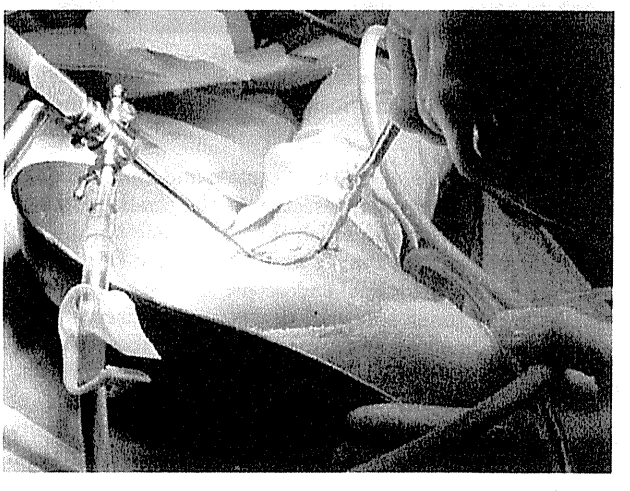

Figure 4. Insertion of the arthroscope and forceps via 2 portals.

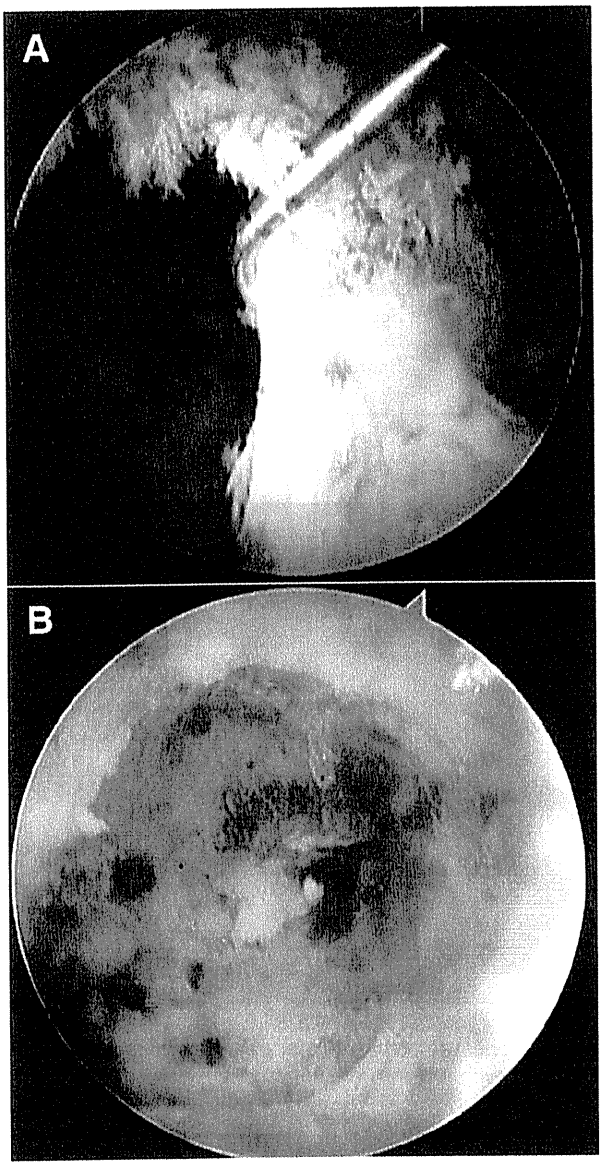

Figure 5. Endoscopic view showing the inner bony septum in the cyst (A) and cavity after resection of the bony septum (B).

the structure in the cyst via the 2 portals and biopsy sample for histologic examination, resection of the inner bony septum was performed to diminish the endoscopic blind area, using a small suction shaver and an abrader (Figure 5). Under the resulting optimal endoscopic visualization, curettage of fibrous inner surface was performed circumferentially using a small curette through either of the 2 portals. Special care was taken at the wall, with cortical thinning to prevent iatrogenic fracture. Sufficient curettage of the inner surface was then confirmed endoscopically (Figure 6). After irrigation, calcium phosphate cement (Biopex, Mitsubishi Pharma Corp, Osaka, Japan) was injected percutaneously via either of the 2 portals under image intensifier. Before this procedure, we injected saline into

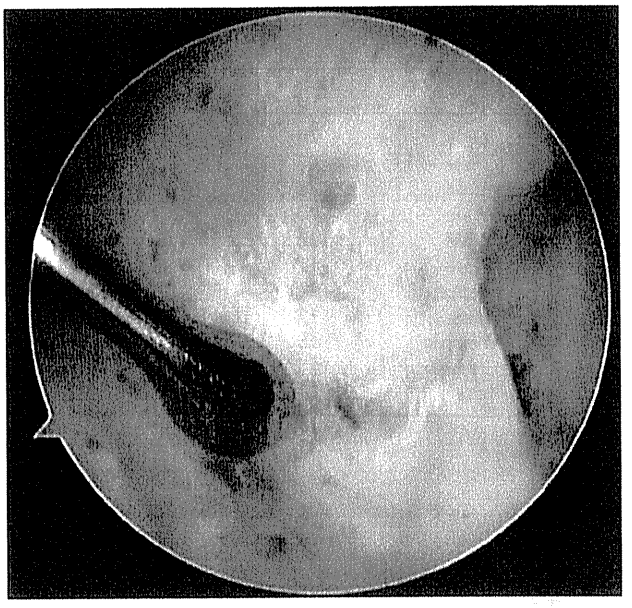

Figure 6. Endoscopic view after curettage of the inner surface of the calcaneal cyst. Healthy bone marrow was exposed.

each cyst to measure each capacity, and we decided on an injective volume of calcium phosphate cement 1 mL less than each measured capacity to avoid leakage of the substitute. Postoperatively, a small compressive dressing was applied, and partial weightbearing was allowed the following day. At 1 week after surgery, full weightbearing and jogging were allowed, and running was allowed at the next week, after the check of gait posture, which was not antalgic and abnormal. Return to the initial sports was allowed at 3 weeks after surgery, and patients joined in sports activities in accordance with their ability to participate.

Evaluation

The clinical results of all patients were assessed by comparing the American Orthopaedic Foot and Ankle Society Ankle-Hindfoot Scale scores[17] before surgery and at the most recent follow-up. Radiologic assessment was based on serial lateral and axial radiographs at the most recent follow-up, as conducted by an orthopaedic surgeon who did not join in the treatment, to reveal whether there was recurrence or pathologic fracture. Furthermore, all patients were asked how long it took to return to their full sports activities after surgery, when they were seen for routine postoperative clinical assessment.

Statistical Analysis

Statistical analysis of the data correlating with the clinical results was done using Wilcoxon signed-rank test, and differences were considered statistically significant when $P \leq .05$.

Figure 7. Lateral (A) and axial (B) radiographs of patient No. 4 at 40 months after surgery, showing neither pathologic fracture nor recurrence.

TABLE 2
Clinical Outcome of 10 Patients

	Max	Preoperative	Final Follow-Up	*P*
AOFAS[a]	100	78.7 ± 4.7	98.0 ± 4.2	.001
Pain	40	22.0 ± 4.2	39.0 ± 3.2	.01
Function	50	45.8 ± 1.5	49.1 ± 1.4	.05

[a]American Orthopaedic Foot and Ankle Society Ankle-Hindfoot Scale score.

RESULTS

The mean follow-up period after surgery was 36.2 months (range, 24-51 months). The mean ankle-hindfoot scale score improved from a preoperative 78.7 ± 4.7 points (range, 74-87) to a postoperative 98.0 ± 4.2 points (range, 90-100) ($P < .001$). In detail, pain and functional scores significantly improved after surgical intervention ($P < .01$ and $P < .05$, respectively) (Table 2). Radiologic assessment at the most recent follow-up revealed no recurrence or pathologic fracture in any patient (Figure 7). There was no complication of surgery, such as infection, delayed wound healing, or sural nerve injury. All patients could return to their initial levels of sports activities within 8 weeks after surgery (mean, 7.1 weeks; range, 4-8 weeks) (Table 1).

DISCUSSION

Although good results have been reported with such conventional surgical management as open curettage and autologous bone graft for the treatment of symptomatic unicameral bone cyst of the calcaneus,[12,20,23,30] this method necessitates an invasive procedure, including large lateral cortical fenestration to achieve good visualization of the lesion, which would be accompanied by long-term morbidity. This method further requires harvesting of autologous iliac crest bone, which has the advantage of the superior nature of osteoinductive and osteoconductive activity but includes the disadvantages of donor site pain and other risks for the long term.[13] To avoid these problems, less invasive techniques have been developed.[1,2,8,19,22,26,27] Recent reports have shown good prognosis and the efficacy of continuous decompression using cannulated screw for calcaneal bone cyst.[1,27] This technique is reasonable because one of the main hypothesized causes of unicameral bone cyst is accumulation of interstitial fluid within the bone due to venous obstruction and blockage.[6,11] Abdel-Wanis et al[1] reported good clinical results with this procedure, with no recurrence or complications among 12 patients. Specifically, they allowed patients to start partial weightbearing with crutches at 3 to 4 weeks after surgery, although they did not offer further information, such as when patients returned to daily activities or sports. Saraph et al[27] described 8 children with 9 unicameral calcaneal

bone cysts who were treated by this procedure, and they reported radiographic assessment, with complete healing of 8 cysts and with residuals in 1 cyst. But their study included no detailed information about postoperative protocol. Although this technique can remove one of the main hypothesized causes, there has been no evidence about how much time is required to achieve sufficient structural support of the calcaneus to tolerate sports activity after the procedure. As another less invasive technique, minimal curettage and injection of some type of bone substitute has been performed.[2,8,19,22,26] Rougraff and Kling[26] applied trephination and percutaneous injection of demineralized bone matrix with autogenous bone marrow for treatment of unicameral bone cyst. In their study, only 1 of 23 patients had an active bone cyst in the calcaneus, and the majority had a cyst in the humerus or femur. Furthermore, their study showed 7 patients with incomplete healing after surgery and 5 who needed a second injection because of recurrence of the cyst. Park et al[22] performed percutaneous injection of demineralized bone powder with autogenous bone marrow in the management of 9 patients with calcaneal cysts. They allowed full weightbearing without support within 1 week after surgery, but there was no further information about when their patients returned to normal or daily activities. Although none of the patients in that study had heel pain at the end of treatment, 5 of 10 cysts were defined as having a residual defect. These methods were accompanied either by no curettage or by indirect curettage of the inner surface without removal of bony septum under fluoroscopic control, which may correlate with an inaccurate radiological outcome.[22,26]

Endoscopic curettage of calcaneal benign lesions has recently been introduced as an effective and feasible technique,[2,10,19,21] similar to the indication of endoscopic curettage for benign lesions of other locations, such as the humerus[25] and the femur.[31,33] Mainard and Galois[19] reported the first case of the treatment of a solitary calcaneal cyst by means of endoscopic curettage and percutaneous injection of calcium phosphate cement, with a successful outcome. They introduced the characteristic of their procedure as provision of optimal visualization, ensuring complete curettage, with the advantage of a small incision, minimal blood loss, and a limited dissection. Futani et al[10] reported successful surgical results of endoscopically assisted tumor resection of a case with bilateral calcaneal intraosseous lipomas. They stated that this approach had the advantage of avoiding the risk of postoperative pathologic fracture, given that the small bony fenestration could preserve the stiffness and strength of the affected bone. We hypothesized that optimal visualization—which makes it possible to achieve complete curettage with minimum violation of the structural integrity of the calcaneal wall by means of endoscopic technique—and percutaneous injection of bone substitute, instead of autologous iliac crest bone grafting, would permit early return to sports activities for young athletes who suffer from symptomatic calcaneal bone cyst, without any postoperative complication.

To avoid donor site morbidity, several graft options have been developed, and satisfactory results have been reported as compared with autologous bone grafting.[7,8,19,22,23,26] We selected calcium phosphate cement as a bone substitute for our surgery because of its favorable handling properties with an endoscopic procedure such as injectability and because of its ability to complete contoured filling of the cavity. Calcium phosphate cement was introduced into dental surgery in the early 1980s, and it has been brought into orthopaedic surgery during the past decade.[18] Especially in orthopaedic surgery, the indication of its use has ranged from filling of metaphyseal fracture void[3] to filling of defect after curettage for benign tumors.[36] In contrast with polymethylmethacrylate, which cannot remodel into bone, animal studies have shown that calcium phosphate cement is gradually remodeled in a manner that is qualitatively similar to normal bone remodeling.[15] Furthermore, that the radiographic appearance at the most recent follow-up in our study showed retention of injected calcium phosphate cement in all 10 patients is not surprising, because change in the radiograph is not usually visible until a substantial part of the material has been replaced by bone.[18] Concerning use of this material in young patients, Thawrani et al[32] reported good clinical results of unicameral cysts treated by a single percutaneous injection of calcium phosphate cement for 13 patients with mean age of 10.5 years (younger than our 10 patients; mean age, 18.7 years) and with a mean follow-up of 35.8 months. Note that none of our 10 patients had open physes during their surgery. These findings enabled us to indicate this procedure to young athletes in this study. As a rare complication, soft tissue necrosis has been reported in only one case following use of calcium phosphate cement in calcaneal bone cyst.[34] The supposed cause was reportedly leakage of the substitute into soft tissue owing to instrument error, no penetration of the injection needle, or a setting time error of the substitute—all of which are amply avoidable by careful technique. As a device to avoid leakage, we injected saline into each cyst to measure each capacity before the procedure, and we decided on an injective volume of calcium phosphate cement 1 mL less than each measured capacity. In our study, no patient had any complication after application of calcium phosphate cement.

There are, however, several limitations in the present study. First, it was a retrospective case series without control cases. Second, we could not make an exact comparison of the surgical results with other surgical methods. Finally, the sample number was limited. Randomized controlled study with a large sample number will be necessary in the future. To our knowledge, this study is the first of endoscopic curettage and injection of calcium phosphate cement for young athletes with symptomatic unicameral bone cyst of the calcaneus reporting the functional results of ankle and foot in detail and the largest sample number. From our successful results with young athletes, endoscopic curettage and injection of calcium phosphate cement has a possibility to become a superlative option for young athletes who suffer from symptomatic calcaneal bone cyst and who desire an early return to their sports activities.

CONCLUSION

We conclude that endoscopic curettage and percutaneous injection of calcium phosphate cement is a useful treatment for young athletes with symptomatic unicameral calcaneal bone cyst.

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臨床室

緊張性気胸をきたした骨肉腫肺転移の1例*

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[整形外科 62巻12号：1291～1293，2011]

骨肉腫は病期が進行すると肺転移巣をきたすことが多い．肺転移巣は結節性病変であり，時に腫瘍が自壊して出血し，血気胸をきたすことが多い．骨肉腫肺転移巣が薄壁空洞を呈し，化学療法中に緊張性気胸を生じてoncologic emergencyとなった1例を報告する．

症　例．25歳，男．

主　訴：胸部異常影．

既往歴：特記すべきことはない．

喫煙歴：1日数本．

身体所見：体格は中肉中背であった．

現病歴：2005年（20歳）に右大腿骨骨肉腫と診断された（図1）．術前評価で転移はなく，化学療法（シスプラチン/アドリアマイシン＋メトトレキサート大量療法）ならびに腫瘍広範切除を行い経過観察していた．2008年にL2への転移を認め他院で重粒子線治療を行っていたところ，2009年に多発肺転移を認め，イホス

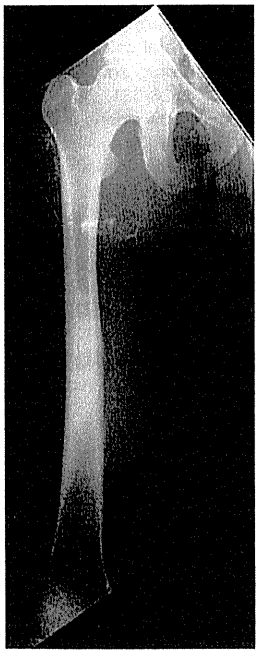

図1．初診時単純X線像．骨幹部の骨膜反応と骨硬化を認める．

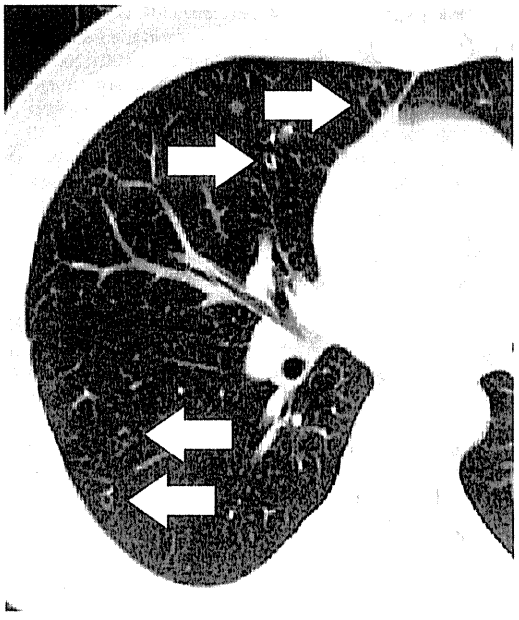

図2．2009年胸部単純CT．多発するリング状陰影を認める（矢印）．

Key words：osteosarcoma, tension pneumothorax, thin-walled cavity, oncologic emergency

* Tension pneumothorax associated with osteosarcoma pulmonary metastases；report of a case
要旨は第651回整形外科集談会東京地方会において発表した．

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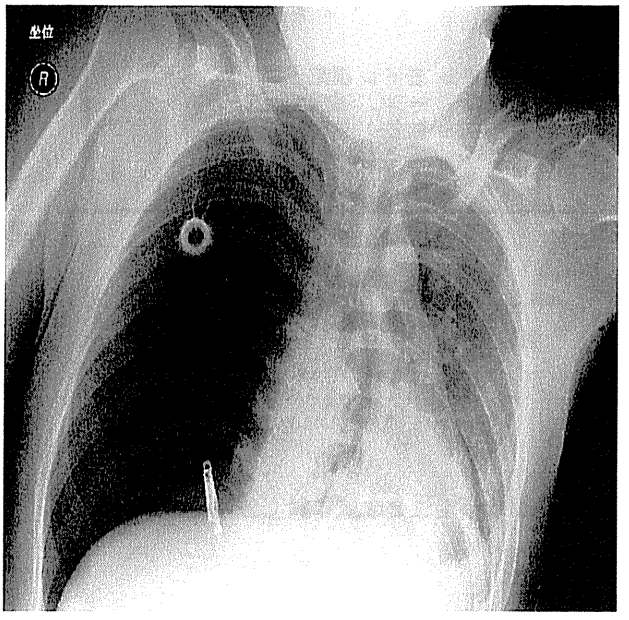

図 3. 緊張性気胸発症時胸部単純 X 線像．気管，縦隔の偏位を認める．

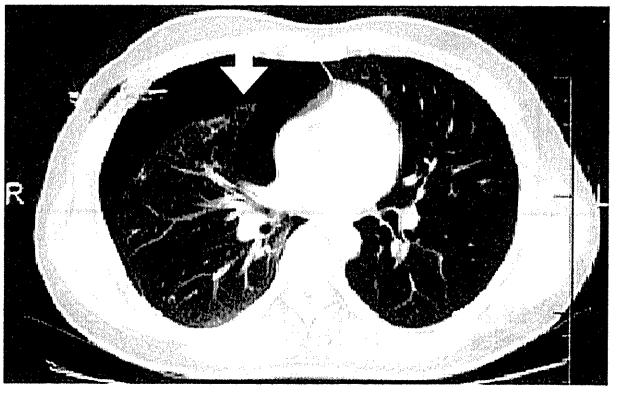

図 4. 発症後 5 日目単純 CT．右上葉に隔壁構造を有する腫瘤を胸膜下に認め（矢印），発症原因と考えられた．

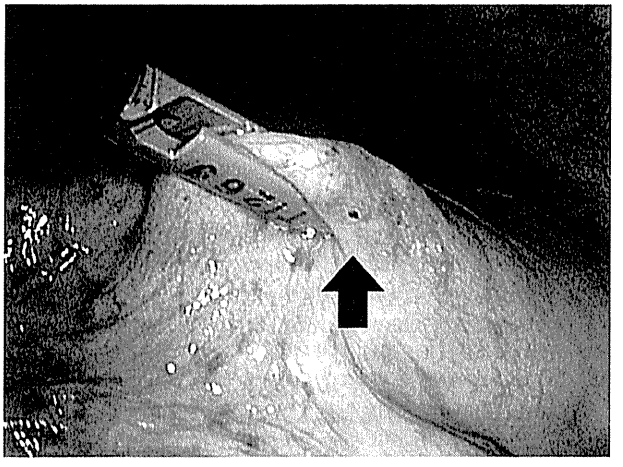

図 5. 発症 15 日目胸腔鏡像．右上葉胸膜下に腫瘍を認める．腫瘍表面は陥凹し，孔を認める（矢印）．

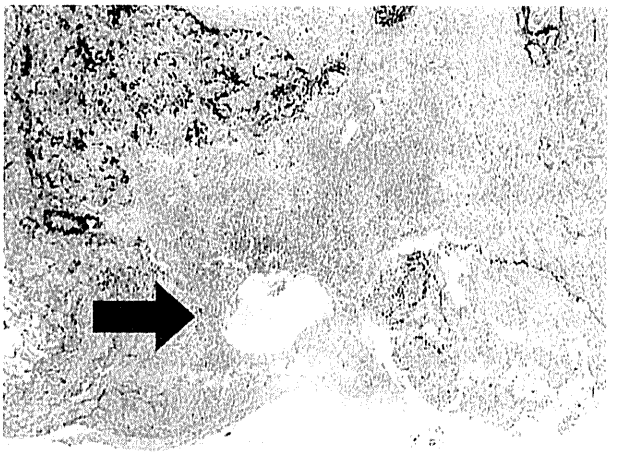

図 6. 病理組織像（Masson 染色，低拡大）．腫瘍塊が胸膜まで浸潤している（矢印）．

ファミド大量療法目的で再入院となった（図 2）．

入院時所見：独歩可能であった．呼吸苦はなく，日常生活への支障はなかった．

入院後経過：開始 2 日目に突然の胸痛と背部痛，心窩部痛，呼吸困難が生じ体動困難となった．血圧の低下と頻脈，右肺野の呼吸音の低下を認めた．胸部単純 X 線像では，右肺野の肺紋理の消失と左方への気管・縦隔の偏位があり（図 3），右緊張性気胸と診断した．ただちに 18 G サーフロー針を 3 本挿入し，続いて胸腔ドレーンを挿入した．

手術所見：気胸の改善が乏しいため（図 4），発症 15 日目に胸腔鏡視下腫瘍摘出術を行った．手術所見および病理組織検査で，胸膜から露出した骨肉腫肺転移巣から air leak のある孔を確認した（図 5，6）．

術後経過：その後も両側気胸を繰り返し，胸腔ドレーン挿入，胸膜癒着療法を繰り返しながら化学療法を行ったが（図 7），2010 年 4 月に呼吸不全により死亡した．

考　　察

本例の報告の意義として，① 骨肉腫肺転移例ではめずらしく薄壁構造を呈する例であったこと，② 非外傷性緊張性気胸を呈したことの 2 点があげられる．

転移性肺腫瘍で内部が疎な構造である薄壁構造を呈す

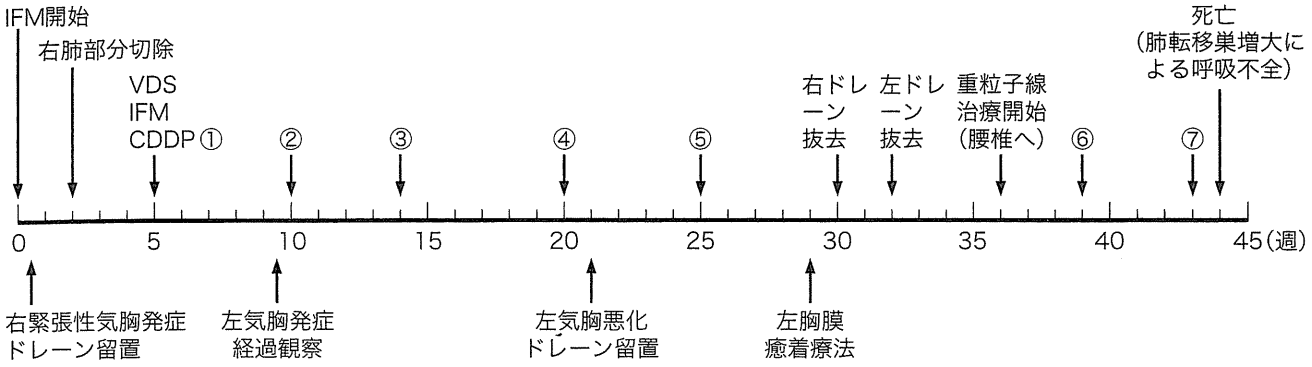

図 7. 再入院後の経過．○内の数字はビンデシン（VDS）＋イホスファミド（IFM）＋シスプラチン（CDDP）療法のクール数を示す．

ることは，ほとんどないとされている[1,2]．また骨肉腫肺転移において報告があるのも，過去4報告のみである[3~5]．さらに緊張性気胸を生じた転移性肺腫瘍は過去に1例しか報告がなく，骨肉腫肺転移での報告例はない[6]．

西川らは薄壁構造を呈する原因として，①腫瘍の増殖過程での乏血，感染によって中心部に壊死が起こり内容物が吸収，排泄されること，②腫瘍による誘導気管支のチェックバルブ機構によって嚢胞が形成されること，③既存の嚢胞や空洞に腫瘍が浸潤すること，④周囲の肺組織に牽引されることにより空洞が薄く拡張していくことなどをあげている．本例では，チェックバルブ構造をもつ胸膜直下の薄壁腫瘤が破綻した際，それが逆止弁となった結果，緊張性気胸を生じたと考えられる．

緊張性気胸は，一般的にはoncologic emergenciesとしては認識されていない[7]．対応に緊急を要する緊張性気胸を合併症の一つに考えておくことは重要であり，胸膜直下に転移した骨肉腫肺転移巣の治療の際には緊張性気胸発症の可能性を念頭におく必要がある．

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帝京医学雑誌 34(2)：157～165（2011）

■原　著■

骨髄間葉系幹細胞を用いたラット骨欠損モデルの骨再生

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背景・目的：整形外科関連手術における骨欠損に対しては，従来から自家骨移植術や人工骨移植術が行われてきた。しかし自家骨移植術では採骨部の合併症，人工骨移植術では適用に限界がある。近年細胞培養技術の飛躍的向上を背景に，細胞治療による組織再生の研究が盛んに試みられている。本件では自家骨髄由来間葉系幹細胞（以下MSCと略記）に着目し，生分解性の骨再生用scaffoldであるPorous PLGA/HAP compositeブロック（以下PPHCと略記）との組み合せによる治療法の開発を検討した。

対象と方法：最初にラットを用いた偽関節モデルの作製を検討し，骨間隙を5 mmに設定することで再現性の高い偽関節モデルを確立することができることを確認した。次にラットMSCの培養試験を行い，bFGFを添加した増殖培地を用いることで多分化能を有したMSCを培養できる条件を確立した。最後にPPHCにMSCを播種した移植体を用いてラット偽関節モデルの治療試験を試みた。

結果：4，8週でPPHC/MSC群では比較対照群であるPPHC単独群と比較して有意に高い骨再生を確認した。

考察：分化能を維持した状態でMSCを移植したこと，連通多孔性を有し高い気孔径を有するPPHCを用いたことで良好な骨再生が実現できたものと考えられる。再生された骨組織は生体骨に近い組織であり，本治療法は新しい骨再生治療法として有用であると考えられる。

キーワード：間葉系幹細胞，骨再生，ラット，偽関節，再生医療

はじめに

骨折後合併症としての偽関節および整形外科関連手術における骨欠損に対しては，従来から自家骨移植術や人工骨移植術が行われてきた[1]。生物活性のある細胞を骨組織とともに局所に導入できるという利点を有するため，特に偽関節の治療においては，自家海綿骨移植術は最も有用な治療法であると考えられている。自家海綿骨の採取部位としては腸骨が最も一般的であるが，約10％の頻度で深部感染・骨髄炎・出血および血腫形成・神経損傷・血管損傷・医原性の腸骨骨折や仙腸関節損傷・疼痛の持続・骨盤不安定性・美容上の問題といった採骨部位の合併症があると報告されている[1]。また，近年，骨組織へ置換されていく人工骨も開発され臨床応用され一定の評価を受けている[2]が，偽関節治療には限定的な使用に限られ自家海綿骨移植術を凌駕するものではない。

自家骨髄間葉系幹細胞（Mesenchymal stem cell，以下MSCと略記）は，各種組織への分化能を有することが知られている。これまでの基礎研究の結果，MSCは塩基性線維芽細胞増殖因子（basic fibroblast growth factor，以下bFGFと略記）存在下で培養することにより，分化能を高いレベ

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ルで維持した状態で効率よく増殖培養させることが可能となっており[3]，さまざまな分野での活用が期待されている。歯科領域ではこのMSC移植をヒトにも臨床応用し，GTR（Guided Tissue Regeneration）法[4,5]や豚歯胚抽出物（エムドゲイン）[6]の改良方法として適応され，これらは既存の治療方法と同等の優れた結果が示されている[7]。

もし，MSCの骨分化能を偽関節治療に応用できれば，自家海綿骨移植に伴う採骨部の合併症を回避できると考える。そこで，生分解性ポリマーであるpoly（DL-lactic-co-glycolic acid）（以下PLGAと略記）と人工骨材料として広く使用されているHydroxyapatite（以下HAPと略記）とを組み合わせた骨再生用scaffoldであるPorous PLGA/HAP compositeブロック（以下PPHCと略記）とMSCとの組み合せによる偽関節治療の可能性を検討することを目的として本研究を行った。

最初にラットを用いた偽関節モデルの作製を検討し，再現性の高い偽関節モデルの作製条件の確立を検討した。次にラットMSCの培養試験を行い，bFGFを添加した増殖培地を用いることで多分化能を有したMSCを培養できる条件を検討した。最後にPPHCにMSCを播種した移植体を作製し，ラット偽関節モデルへの移植試験を試み，その骨再生能力の評価を行った。

材料と方法

帝京大学医学部の動物実験に関する倫理委員会の承認を得た後に本研究を実施した。対象動物として同種移植に適しているFisher近交系ラット（F344/Jcl, 日本クレア）を用いた。飼育および試験は帝京大学医学部中央動物実験施設内にて実施した。scaffoldはHAP微粉末を分散させたPLGAブロックであるPorous PLGA(LA：GA＝50：50, Mw. 50,000)/HAP composite block ϕ5×5 mm気孔率85％（ジーシー）を特注し，試験に供した（**図1**）。本実験では，ラット骨髄MSCの骨再生能をより観察しやすくするために，HAPは微粉末としてscaffoldに分散させ早期吸収するようにした。

1．ラット偽関節モデルの作製

11～13週齢の雄ラット10匹の左大腿骨を用いて偽関節モデルの作製を検討した。イソフルラン吸入麻酔（2％，0.4 L/min）による全身麻酔下にて，大腿部外側を切開して大腿骨を露出させた。近位部および遠位部に各2カ所ずつ手動でドリリングしてϕ1 mmの骨孔を作製した。この4カ所の骨孔にϕ1.2 mmのKirschner wireを徒手的に刺入した後に，ラット用にデザインしたユニラテラル型延長機構付き創外固定器（**図2A**）で固定した。続いて，近位および遠位のKirschner wire間の中心部をϕ1 mmのドリルで数カ所骨孔を作製した後に，徒手的に骨折させた。創外固定器の延長機構を用いて主骨片間の骨間隙が5 mmとなるよう調整した（**図2B, C**）。術野を十分に洗浄

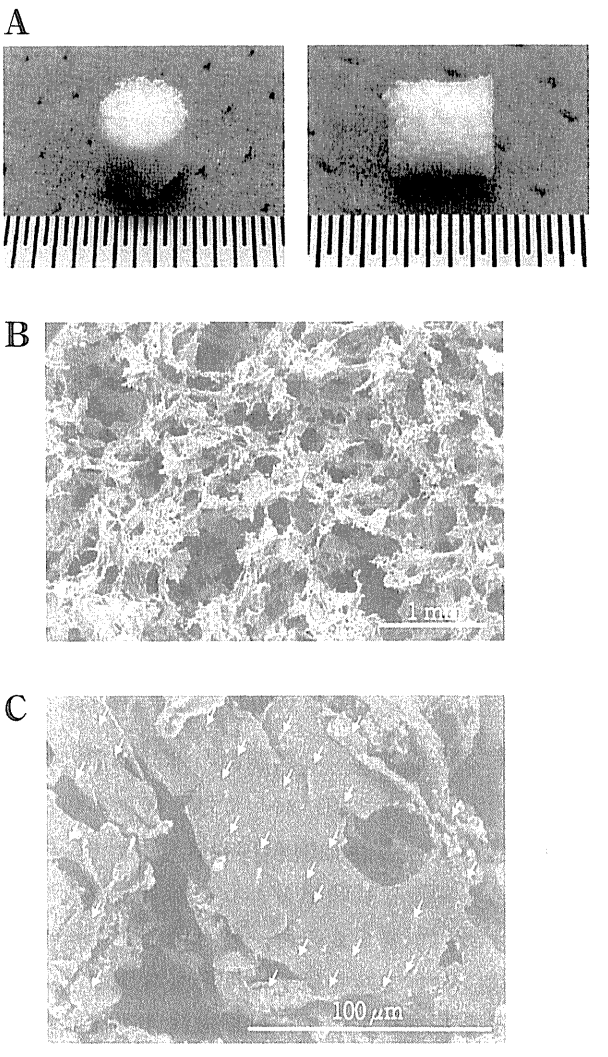


図1 骨再生用scaffold
骨再生用scaffoldとして用いたPorous PLGA/HAP compositeブロック（PPHC）。外観（**A**）とSEM像（**B** x30, **C** x1000）。矢印はHAPの微粉末を示している。

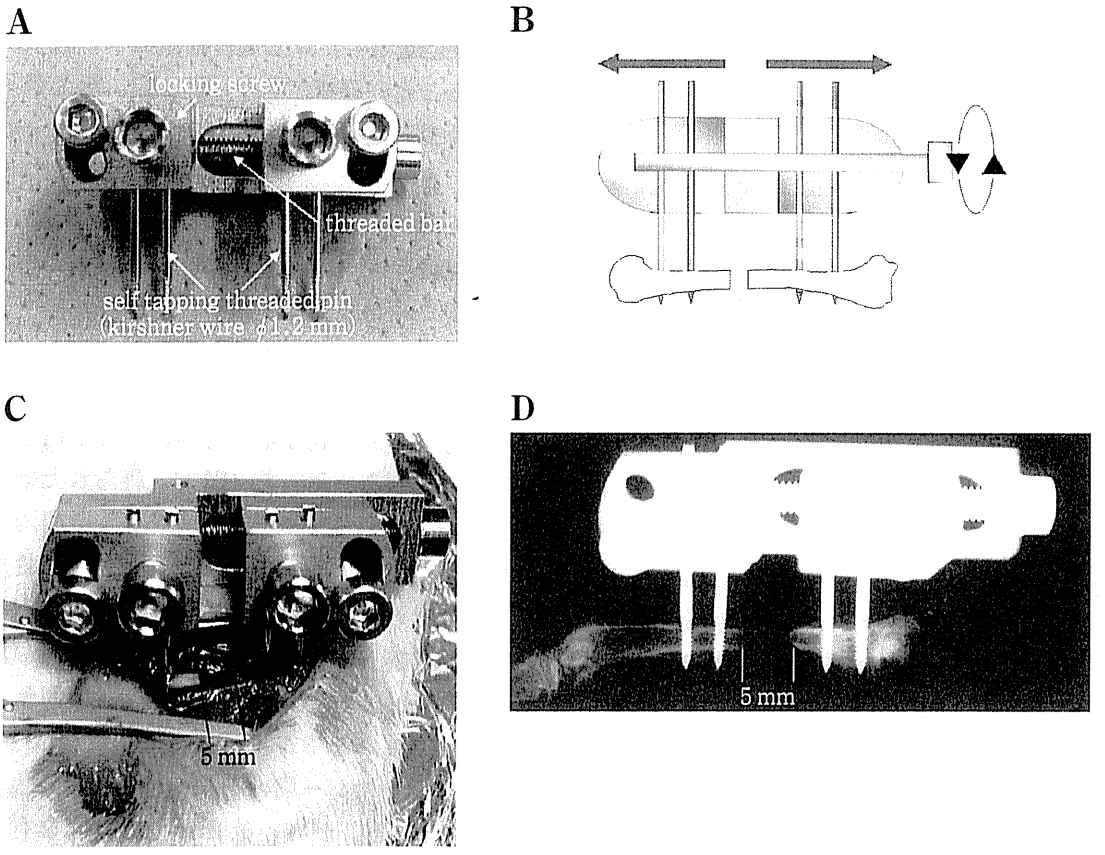


図2　ラット偽関節モデル
延長機構付きの創外固定器を用いて，大腿骨骨幹部に5 mmの骨間隙を作製してこれを保持した。創外固定器（**A**），模式図（**B**），実際に創外固定器を取り付けたところ（**C**），モデル作製後の軟X線写真（**D**）

後に皮膚のみをナイロン糸で縫合した。術後4，8週後にイソフルラン吸入麻酔（2％，0.4 L/min）による全身麻酔下に，頸椎脱臼の手技を用いて安楽死させた後，大腿骨を摘出して軟X線撮影（40 kV，3 mA，20秒）した（**図2D**）。軟X線撮影写真をデジタル画像として取り込み，主骨片間の距離をImage Jにて解析して算出した。

また，大腿骨のHematoxylin-eosin染色（以下HE染色と略記）標本を作製して組織学的に評価した。

2．ラット骨髄MSCの培養

4週齢の雄ラット1匹の両側の大腿骨および脛骨を摘出し，増殖培地（10％FBS含有αMEM培地）にてフラッシュアウトして骨髄液を回収した。得られた骨髄液は増殖培地30 mlに懸濁した後にT75細胞培養フラスコ（BD Falcon）2枚に播種し，37℃，5％CO_2インキュベーター内にて培養した。播種後3日に培地を追加充填（15 ml/T75細胞培養フラスコ）し，6日目に培地を吸引して浮遊細胞を除去した後に培地を追加し，接着細胞のみを得た。9日目以降は増殖培地にbFGFを添加し（3 ng/ml），3日に1回の割合で培地交換を行った。細胞がコンフルエントになったところで，0.05％トリプシン-EDTA溶液（シグマ）にて細胞を剥離し，5,000 cell/cm^{2}となるよう55 cm^{2}細胞培養シャーレに継代した。

MSCの骨・軟骨・脂肪分化誘導は既報の手法[8]にて行った。軟骨分化誘導はMSCを3×10^5cellを15 ml遠沈管に移し，0.5 mLの6.25 μg/mlインスリン，6.25 μg/mlトランスフェリン，6.25 ng/ml亜セレン酸ナトリウム，5.33 μg/mlリノレイン酸，1.25 mg/ml BSA，10 ng/ml TGF-β3，100 nMデキサメタゾン，50 μg/mlアスコルビン酸二リン酸（和光純薬）添加αMEM培地中にて懸濁した後に500 g×5分の遠心を行った。遠沈管のまま37℃，5％CO_2インキュベーター内にて1日静置して細胞ペレットを作製した。播種2日後に培地0.5 mlを追加し，以後2日に1回培地交

換を行って28日間培養した。得られた細胞塊は10％ホルマリン中で1日固定し，パラフィン切片を作製して0.1％トルイジンブルー溶液にて染色を行った。

骨分化誘導は3.8×10⁴cellのMSCを12 well細胞培養プレートに播種し，100 nMデキサメタゾン，50 μg/mlアスコルビン酸二リン酸，10％FBS添加αMEM培地中で28日間培養した。分化誘導した細胞は95％エタノール水溶液にて10分間固定した後に1％アリザリンレッド-S水溶液にて染色を行った。

脂肪分化誘導は3.8×10⁴cellのMSCを12 well細胞培養プレートに播種し，脂肪分化誘導培地（10％FBS，10 μg/mlインスリン，0.2 mMインドメタシン，1 mMデキサメタゾン，0.5 mM 3-イソブチル-1-メチルキサンチン添加DMEM）で培養した。2日目に脂肪分化維持培地（10％FBS，10 μg/mlインスリン添加DMEM）に交換し，以後2つの培地に交互に交換して10日間培養した。分化誘導した細胞は10％ホルマリン固定後に1.8 mg/mlオイルレッドO-ヘマトキシリン2重染色を行った。

3．ラット偽関節モデルへの細胞移植

11～13週齢の雄ラット30匹を3群に分けて実験を行った。MSCは2継代した後にPPHCに1×10⁶cell/ブロックとなるように播種し，3日間培養したものを移植に使用した。PPHCのみを移植した群を比較対照とした。術後4，8週後に大腿骨を取り出し，10％ホルマリン水溶液にて固定後，μCT撮影（TOSCANER-3000 μhd，東芝）および組織学的評価を行った。μCTは空気のCT値（CTa）を-1000，水のCT値（CTw）を0となるようキャリブレーションして計測した。得られたデータは，VGStudio Max（Volume Graphics社，ドイツ）にて3次元化し，CT値1,000以上の領域を石灰化部位として抽出し，大腿骨部に作製した5 mmのギャップ内にできた石灰化領域を新生骨体積（mm^3）として算出した。

結　果

1．ラット偽関節モデル

モデル作製後4，8週の時点で各5匹の大腿骨を摘出し軟X線写真を撮影した。全ての個体において主骨片の間隙には仮骨形成は確認できず，8週経過時点では主骨片断端部は骨吸収が進んでいることが確認できた（**図3A**）。軟X線写真の主骨片間の距離は，4週で5.46±0.39 mm，8週で5.87±0.66 mmと骨間隙部は拡大していた。HE染色標本では骨間隙部位に骨格筋組織および線維性組織の侵入があり，骨の形成が阻害されている様子が確認された（**図3B**）。

2．ラット骨髄MSCの培養

ラット骨髄液からは接着性の線維芽細胞様細胞が分離され（**図4A**），bFGF添加増殖培地中で高い増殖挙動を示した（**図5**）。得られた細胞の骨分化能について確認したところ，4週間誘導を行った細胞層は全面がアリザリンレッド陽性であり，良好な石灰化物の沈着が見られたことから，高い骨分化能を有していることを確認した（**図4B**）。MSCから作製したペレットを4週間軟骨分化誘導したものをトルイジンブルー染色したところ，メタクロマジー陽性の細胞外基質の産生が良好であり，MSCが高い軟骨分化誘導能を有していることを確認した（**図4C**）。脂肪分化誘導については誘導培地と維持培地を用いて10日間誘導培養したところ，細胞質内にオイルレッドO陽性の脂肪滴の形成が確認されたことから，MSCが高い脂肪分化能を有していることを確認した（**図4D**）。

3．ラット偽関節モデルへの細胞移植

μCTによる移植部の画像では，PPHCに播種・培養したMSCを偽関節モデル部位に移植した群（以下PPHC/MSC群と略記）では，4週目から骨間隙の中心部に石灰化組織がわずかに確認でき，8週にかけて石灰化部位が成熟していくことが確認された（**図6**）。一方，PPHC単体で移植した群（PPHC単独群）では，偽関節モデルと同様に骨間隙部には8週の時点でも明らかな石灰化組織は確認できなかった。μCTで撮影された三次元データをもとに骨間隙部位にできた石灰化組織の体積を算出したところ，PPHC/MSC群で有意に高い値を示し，新生骨組織の形成が促進されていることが確認された（**図7**）。皮質骨および海綿骨の平均CT値はそれぞれ2,712±391（皮質骨）および580±244（海綿骨）であり，PPHC/MSC群における移植4週および8週群における新生骨

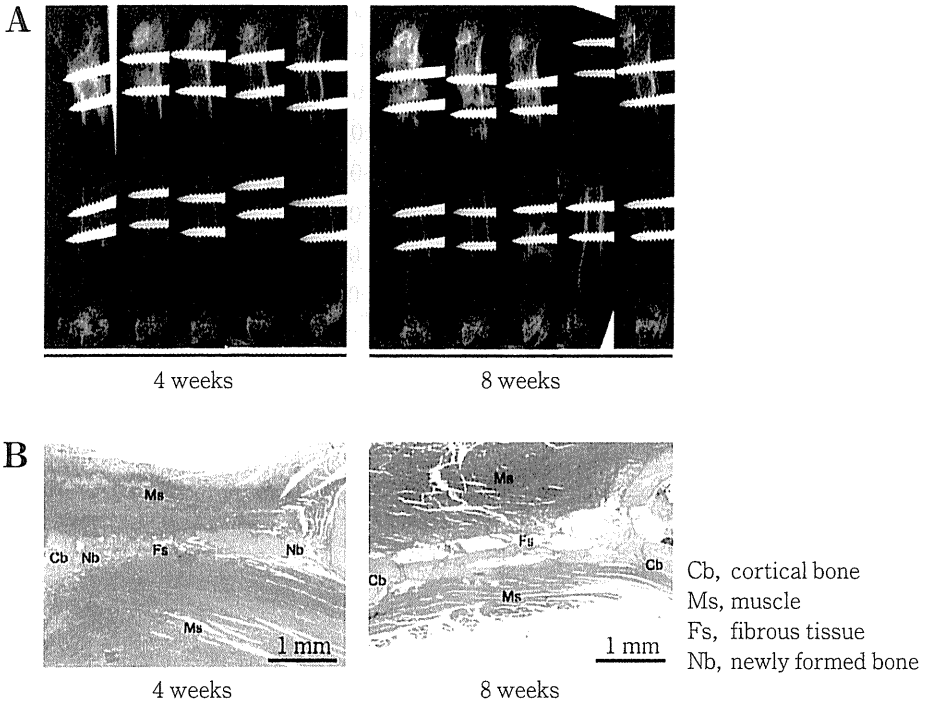


図3　偽関節モデルの経時的変化
モデル作製後4週および8週の軟X線写真（**A**）と，主骨片間隙の組織像（**B**）。主骨片間隙には骨形成を認めない。

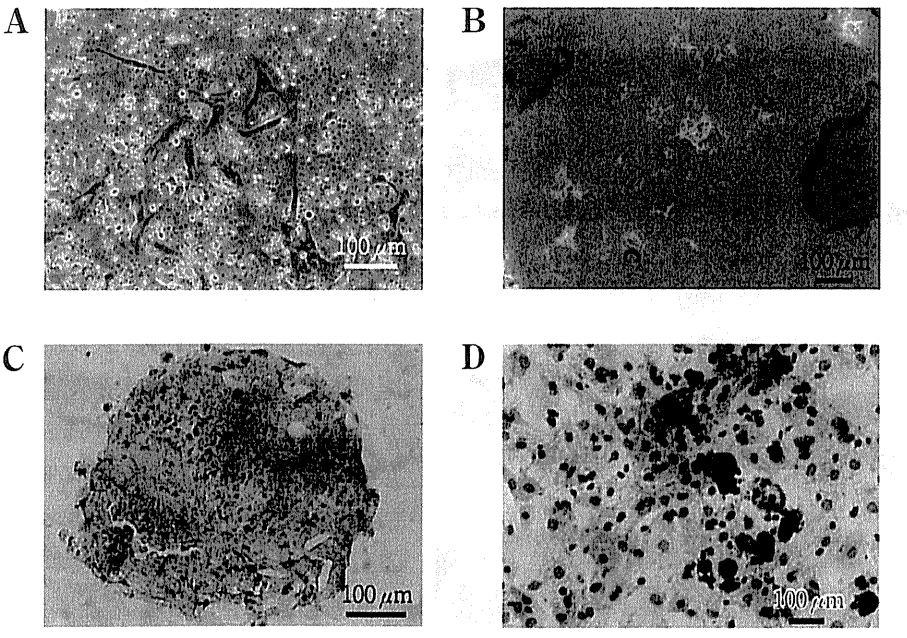


図4　ラット骨髄MSCの分化能
ラット骨髄液から分離した線維芽細胞様細胞（**A**）は，骨分化能（**B**，アリザリンレッド染色），軟骨分化能（**C**，トルイジンブルー染色），脂肪分化能（**D**，オイルレッドO陽性の脂肪滴）を有している。

の平均CT値はそれぞれ1,036±578（4週）および1,061±652（8週）であった。HE標本では比較対照となるPPHC単独群では移植8週において線維性組織の侵入を認めたが，PPHC/MSC群では線維性組織の侵入が見られず，移植4週では幼弱な骨組織が確認でき，8週にかけて成熟して

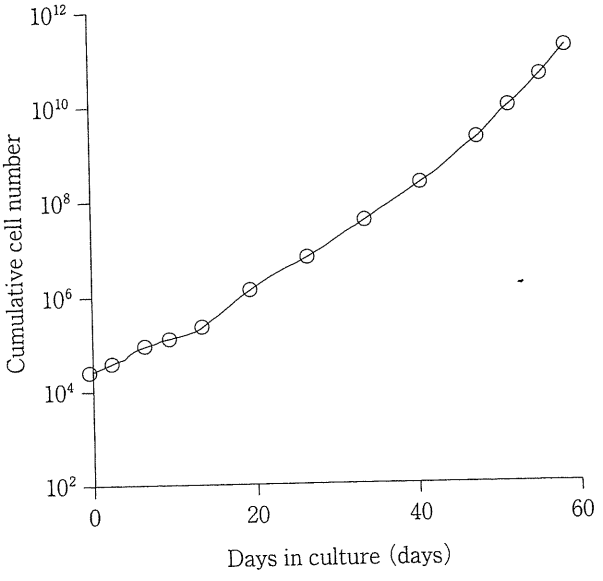
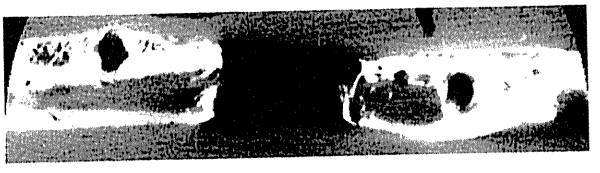
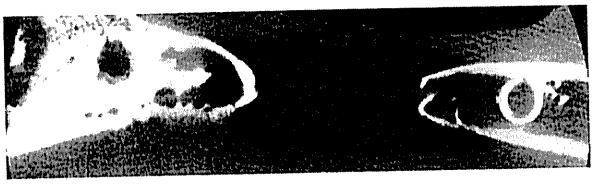


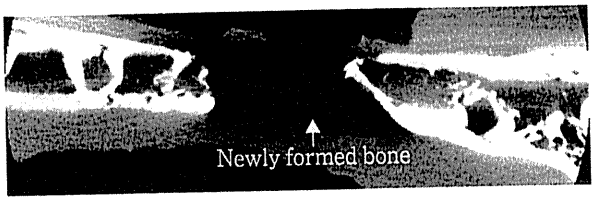
図5　ラット骨髄MSCの増殖活性



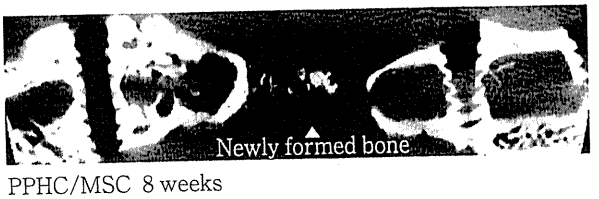
PPHC 4 weeks



PPHC 8 weeks



PPHC/MSC 4 weeks



PPHC/MSC 8 weeks

図6　μCTによる骨再生の変化
PPHC単独群（上2つ）とPPHC/MSC群（下2つ）のモデル作製後4週および8週のμCT像。PPHC/MSC群では8週で骨間隙に石灰化組織が確認できる。

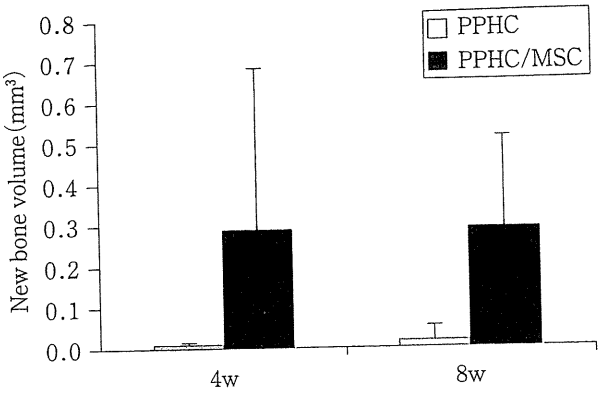


図7　μCTによる新生骨の体積比較

いく様子が観察された（**図8**）。

考　察

ラット偽関節モデルの作製方法についてはこれまで多数の報告がある[9~11]。骨の固定に創外固定器を用いた本研究のモデルは，骨折部に固定材料がないので，プレートや髄内釘を用いた偽関節モデルと比べて骨折部の観察が容易であるという利点がある。

ラットは骨再生能力が高く，主骨片間の間隔を縮めると早い段階で骨癒合し，再現性の良い偽関節モデルを確立するためには骨間隙の長さ設定が重要となる。主骨片間隙の大きさについては5 mm以上とすることで，骨形成が抑制され偽関節となりやすいと報告されている[12]。本実験では延長機構付き創外固定器と骨幹部骨切りにより，5 mmの骨間隙を作製・維持することでラット大腿骨の偽関節モデルを作製できるかどうかを確認した。骨欠損作製後4週のHE標本では，一部に主骨片の断端部から新生骨の形成を認めたが，欠損作製後8週では骨形成は抑制され，骨端が吸収されている様子がHE染色でもμCTの画像からも観察できた（**図3**）。すなわち，骨幹部に5 mmの骨間隙を作製し，これを創外固定器で維持するという方法で再現性のよい偽関節モデルが作製できたといえる。試験数を多く設定できる小動物を用いて偽関節モデルを作製できたことは，今後の偽関節治療の開発研究に有用であると考える。

大腿骨骨欠損モデルに対しては，ナノファイバーマトリックスやBMP，Extracorporeal shock wavesを用いて治療する試みがなされてい