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REVIEW ARTICLE

A novel hyperthermia treatment for bone metastases using magnetic materials

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Abstract Patients with bone metastases in the extremities sometimes require surgical intervention to prevent deterioration of quality of life due to a pathological fracture. The use of localized radiotherapy combined with surgical reinforcement has been a gold standard for the treatment of bone metastases. However, radiotherapy sometimes induces soft tissue damage, including muscle induration and joint contracture. Moreover, cancer cells are not always radiosensitive. Hyperthermia has been studied since the 1940s using an experimental animal model to treat various types of advanced cancer, and studies have now reached the stage of clinical application, especially in conjunction with radiotherapy or chemotherapy. Nevertheless, bone metastases have several special properties which discourage oncologists from developing hyperthermic therapeutic strategies. First, the bone is located deep in the body, and has low thermal conductivity due to the thickness of cortical bone and the highly vascularized medulla. To address these issues, we developed new hyperthermic strategies which generate heat using magnetic materials under an alternating electromagnetic field, and started clinical application of this treatment modality. The purpose of this review is to summarize the latest studies on hyperthermic treatment in the field of musculoskeletal tumors, and to introduce the treatment strategy employing our novel hyperthermia approach.

Keywords Hyperthermia · Bone metastasis · Pathological fracture · Magnetic material · Electromagnetic field

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Introduction

Bone is the most common site of cancer metastasis, and is particularly important in breast and prostate cancers because these diseases have a high prevalence of bone metastases. At postmortem examination, ~70% of patients dying of these cancers have evidence of metastatic bone disease. Cancers of the thyroid, kidneys, and lungs also commonly give rise to bone metastases with an incidence of 30–40% at postmortem examination [1].

Patients with bone metastases in the extremities sometimes require surgical intervention to prevent deterioration of quality of life due to pathological fractures, which commonly occur as a result of lytic lesions in weight-bearing bones. Destruction of both cortical and trabecular bone is structurally important. Fractures are highly unlikely to occur (2.3%) when less than 50% of the cortex is destroyed, and are most likely to occur (80%) when over 75% of the cortex is destroyed [2].

Radiotherapy is generally a safe and effective treatment modality, and is well established for patients with bone metastases. However, radiotherapy without surgical reinforcement cannot prevent pathological fractures in patients presenting with impending fractures of long tubular bones. The addition of internal fixation before localized radiotherapy can reduce the risk of further bone destruction,

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which leads to increased pain, loss of fixation, and the need for additional orthopedic procedures [3, 4]. Radiotherapy also sometimes induces soft tissue damage, including muscle induration and joint contracture [4]. Moreover, cancer cells are not always radiosensitive. Better methods are therefore needed to treat patients with bone metastases. We have developed a novel hyperthermic strategy which generates heat using magnetic materials under an alternating electromagnetic field to treat bone metastases, and have started clinical applications of this treatment modality.

The purpose of this review is to summarize the use of hyperthermic treatment in the field of musculoskeletal tumors, and to introduce our new treatment strategy using hyperthermia.

The history of therapeutic hyperthermia and the mechanisms of cancer cell death

Although there have been many references to the use of heat to treat human cancer, dating back to the writings of Hippocrates, the scientific approach to hyperthermia has been studied since the 1940s [5]. Hyperthermia can be applied by several methods: local hyperthermia by external or internal energy sources, regional hyperthermia by perfusion of organs or limbs, or by irrigation of body cavities, and whole body hyperthermia [5].

There is a clear rationale for using hyperthermia in cancer treatment. Recent progress in cell biology has revealed that hyperthermia, variously reported between 40 and 45°C, triggers tumor cell death by apoptosis, although the exact temperature differs depending on the individual conditions [6, 7]. Treatment at temperatures between 40 and 45°C is cytotoxic for cells in an environment with a low pO_2 and low pH conditions, which are usually found within tumor tissue due to the insufficient blood supply [5]. It is now well-known that hyperthermia-induced apoptosis is characterized by the occurrence of intra-nucleosomal DNA cleavage [8]. Furthermore, recent experiments revealed that hyperthermia can increase tumor immunogenicity by stimulating antigen-presenting cells through heat shock proteins secreted from lysed tumor cells [5].

The clinical value of hyperthermia, in addition to other treatment modalities, has been shown in randomized trials. For example, significant improvements in clinical outcome have been demonstrated for tumors of the head and neck, breast, brain, bladder, cervix, rectum, lung, esophagus, prostate, vulva and vagina, and also for melanoma, especially in conjunction with radiotherapy or chemotherapy [5, 9, 10]. In the field of bone metastases, Fan et al. [11] reported that 57 of 62 patients treated with intra-operative microwave-induced hyperthermic treatment had shown excellent local control. Sakurai et al. [12] reported that hyperthermic treatment combined with external radiation therapy improved local control in thirteen patients with primary non-small cell lung cancer directly invading to bone.

However, the bone has several special properties which discourage oncologists from developing hyperthermic therapeutic strategies for bone metastasis. Heating of the tumor is usually achieved by means of external sources such as microwaves, ultrasound or a water bath [5]. However, even if these external sources are applied for bone metastases, it is difficult to achieve enough heat conduction to the tumor because the bone is located deep in the body and has low thermal conductivity, with a highly vascularized medulla. However, hyperthermia for bone tumors can be achieved using the polymerization heat of polymethylmethacrylate bone cement as a hyperthermic treatment [13, 14]; however, the generated heat tends to be unreliable and insufficient to reduce bone tumor growth [15]. Microwave-induced hyperthermia [11], laser-induced thermotherapy [16], and radiofrequency ablation [17] have been recently used, especially for spinal and pelvic metastasis. However, these therapeutic modalities are unsatisfactory for lesions located in the long tubular bones of the limbs, because pathological fractures cannot be prevented without surgical reinforcement of the bone lesion.

We therefore developed a new hyperthermic therapeutic strategy that uses magnetic materials for metastatic bone tumors based on experimental studies [15, 18–20], and have also started clinical investigations [21].

Novel hyperthermia induced using magnetic materials

The unique feature of magnetic materials is their reaction to a magnetic field. Physical energy conversion occurs in an alternating magnetic field, and hysteresis loss is a very important feature of magnetic materials, because it enables effective hyperthermia.

The concept of hyperthermic cancer therapy that utilizes magnetic materials and an alternating magnetic field has been proposed by many researchers [22–24]. Yan et al. showed that treatment using Fe_2O_3 nanoparticles combined with magnetic field hyperthermia could inhibit not only the proliferation of cultured liver cancer cells, but also induce apoptosis of cultured liver cancer cells. Moreover, they showed that this hyperthermic strategy has a significant inhibitory effect on the weight and volume of xenograft liver cancer [25]. Similarly, Hilger et al. [26] showed the feasibility of thermal ablation of breast cancer with magnetic nanoparticles using an animal model. Johannsen et al. [27] published the first report of the clinical application of hyperthermia for human cancer using magnetic

nanoparticles. They injected magnetic nanoparticle suspensions into the prostate under ultrasound and fluoroscopy guidance, and showed that hyperthermia using magnetic nanoparticles was feasible and well tolerated for a patient with previously irradiated and locally recurrent prostate carcinoma.

Localized hyperthermic treatment for musculoskeletal tumors with ferromagnetic ceramics was first reported by Kokubo [28] using an animal model. He made a bioactive and ferromagnetic glass ceramic by heat treatment of a Fe_2O_3–$CaO.SiO_2$–B_2O_3–P_2O_5 glass, and showed that this glass ceramic was useful as a thermoseed for hyperthermic treatment of cancer [28–31].

We modified these new hyperthermic strategies for use in treating bone metastases. First, we developed an alternating electromagnetic field generator (Yamamoto Vinita Co., Ltd., Osaka, Japan) [15, 18, 20]. The output power of the electromagnetic field was 7 kW, at a fixed frequency of 1.5 MHz. Exposure of the affected limb to the electromagnetic field can be achieved by inserting the limbs into a cylindrical coil of the generator (Fig. 1).

Fig. 1 Hyperthermia was postoperatively applied by inserting the affected limb into a cylindrical coil of the electromagnetic field generator

Next, we created a new bone cement made of glass ceramic that was partly replaced by magnetite (Fe_3O_4) powder [15, 18, 19], and examined the heat induction generated by hysteresis loss [18]. The composition of this material resembles the bioactive bone cement described by Kawanabe et al. [32], with a portion of the bioactive glass ceramic component replaced by magnetite. The temperature of this thermoseed rose in proportion to the weight ratio of magnetite powder, the volume of the thermoseed, and the intensity of the magnetic field. Furthermore, the heat induction in this thermoseed implanted into rabbit and human cadaver tibias was investigated by applying a magnetic field with a maximum of 300 Oe and 100 kHz. This system could easily produce heat in the thermoseed in bone beyond 50°C. When the temperature of the thermoseed in rabbit tibias was maintained at 50–60°C, the temperature at the bone surface rose to 43–45°C; but at a 10-mm distance from the thermoseed in the medullary canal, the temperature did not exceed 40°C (Fig. 2). These results demonstrate that ferromagnetic bone cement may be applicable for the hyperthermic treatment of bone tumors.

We next examined the antineoplastic effects of this treatment using an animal model. VX-2 tumors were transplanted into the tibia of rabbits. One week later, bone cement containing magnetite at a 60% weight ratio was implanted into the same site, followed by exposure to an alternating magnetic field for 50 min. We observed that the temperature of the tibia could rise to over 43°C, and that tumor growth was inhibited without any systemic adverse effects [20] (Fig. 3).

Since our first magnetite cement has not yet been approved by the Ministry of Health, Labour and Welfare of Japan, we decided to use a calcium phosphate cement

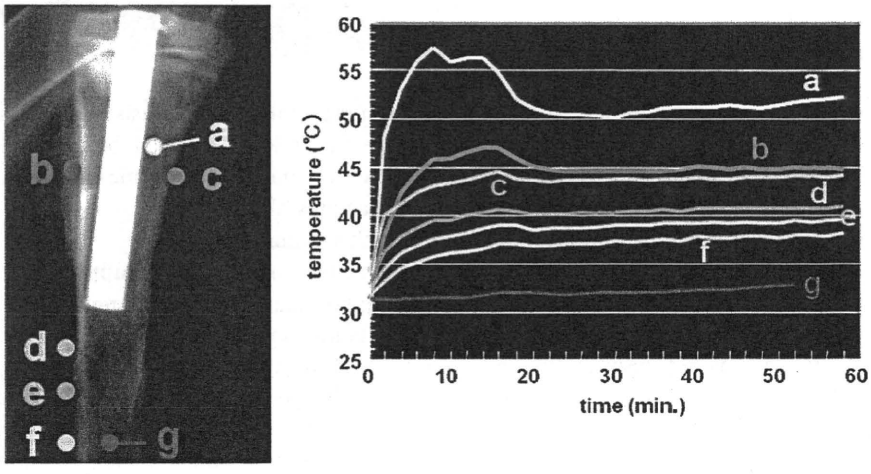

Fig. 2 Monitoring of the temperature at the various portions of a rabbit tibia. These data show the following: heat is easily generated on the surface of the magnetic material, the cortical bone has good heat conductivity, and the cancerous bone has poor heat conductivity

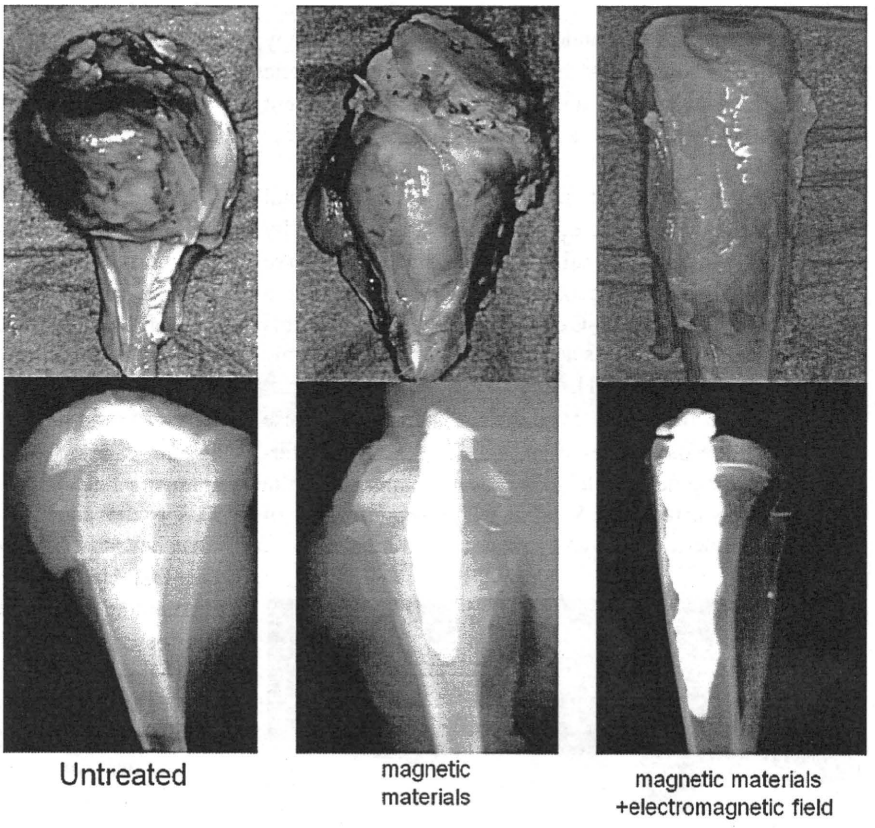

Fig. 3 The effects of hyperthermia with magnetic materials under an electromagnetic field. VX2 sarcoma cells were implanted into the tibias of rabbits. In radiographs, massive bone destruction was observed in rabbits treated with neither magnetic materials or the electromagnetic field. However, when the magnetic materials were implanted and the electromagnetic field was applied, the bone destruction was strikingly inhibited. This suggests that our new hyperthermic therapy has a prominent anti-tumor effect on metastatic bone tumors

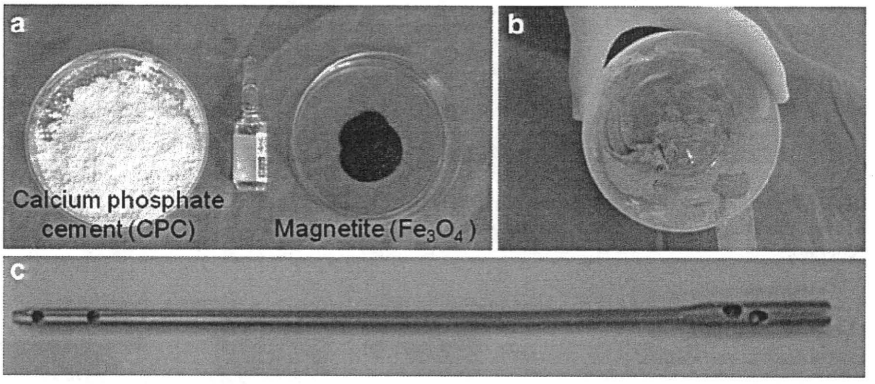

Fig. 4 The magnetic materials used for clinical appreciation of the novel therapeutic hyperthermia. **a**, **b** When the lesion was located at the metaphysis, curettage of the lesion was performed, followed by implantation of calcium phosphate cement containing magnetite into the cavity. **c** In contrast, when the lesion was located at the diaphysis, only reinforcement with an intramedullary nail was performed

containing powdered Fe_3O_4 (Fig. 4a, b) or an intramedullary nail made of a titanium alloy (Fig. 4c) as the magnetic material [21]. Because intramedullary nails made of titanium alloys have been frequently used to fix fractures all over the world, we had no legal constraints against using these nails for the patients with pathological fractures or impending fractures in Japan [33]. Calcium phosphate cement (CPC) is an injectable biocompatible bone substitute, and has been used to fill the bone defect following resection of bone tumors [34]. We have continually performed experimental studies, and found that our novel hyperthermia strategy promised good local control for bone metastasis without any adverse effects which could be detected by hematological examination and histological examination of the liver, kidneys, lungs and brain [35].

Clinical application of novel hyperthermia for bone metastases

We started clinical applications of this treatment modality from March 2003 after obtaining approval from our Institutional Ethics Investigational Review Board [21]. Our treatment strategy requires two types of treatment modalities:

an electromagnetic field generator (Fig. 1), and the magnetic materials (Fig. 4).

The surgical procedures can be categorized into two types. For the lesions located at the metaphysis, we first perform curettage of the lesion. Calcium phosphate cement containing magnetite is then implanted into the cavity. In contrast, for the lesions located at the diaphysis, only reinforcement with an intramedullary nail was performed. In both cases, hyperthermia was performed after the operation. Hyperthermic treatment was performed postoperatively on days 8, 10, 12, 15, 17, 19, 22, 24, 26 and 29. The exposure time was 15 min per day.

To date, this novel hyperthermic treatment has been performed for 23 patients with 25 metastatic bone lesions. The radiographic outcome was assessed according to the following criteria: "Excellent" indicating "reduced with visible bone formation," "Good" meaning "not progressive for more than 3 months," and "Poor" meaning "progressive". As a result, 8 lesions (32%) showed an "Excellent" outcome, while 16 lesions (64%) showed a "Good" outcome. One lesion (4%) showed a "Poor" outcome. When compared to the historical controls at our institute, the radiographic outcomes were statistically superior to those of the patients who received palliative surgery without either radiotherapy or hyperthermia, and similar to outcomes of the patients who received surgery in combination with postoperative radiotherapy. These results suggest that our novel hyperthermic therapy was as effective as surgery combined with radiotherapy (Figs. 5, 6).

With regard to complications, a sensation of heat during hyperthermia was noted by 3 patients, and tumor recurrence was observed in one patient. To our knowledge, this is the first clinical application of hyperthermia for metastatic bone tumors generated using magnetic materials and an alternating electromagnetic field.

Perspectives

Our hyperthermic strategy has several advantages for the treatment of metastatic tumors of long tubular bones compared to radiotherapy. First, our hyperthermic treatment is minimally invasive to the surrounding soft tissue, because the cooling effect of the intramuscular vascular flow minimizes soft tissue damage to the neurovascular sheath [20]. Second, our hyperthermic strategy can prevent pathological fractures because of the surgical reinforcement of the bone lesion. Moreover, this surgical reinforcement does not require any special techniques other than those routinely used by orthopedic surgeons. Finally, our hyperthermic treatment can be repeatedly applied for recurrent metastatic lesions, in contrast to radiotherapy, which cannot exceed the normal dose limitations.

This hyperthermic strategy may be applicable to other fields. Hyperthermia is already applied for the treatment of soft tissue sarcoma. The combination therapy using both cytotoxic drugs and regional hyperthermia in the treatment of soft tissue sarcoma is based upon experimental and

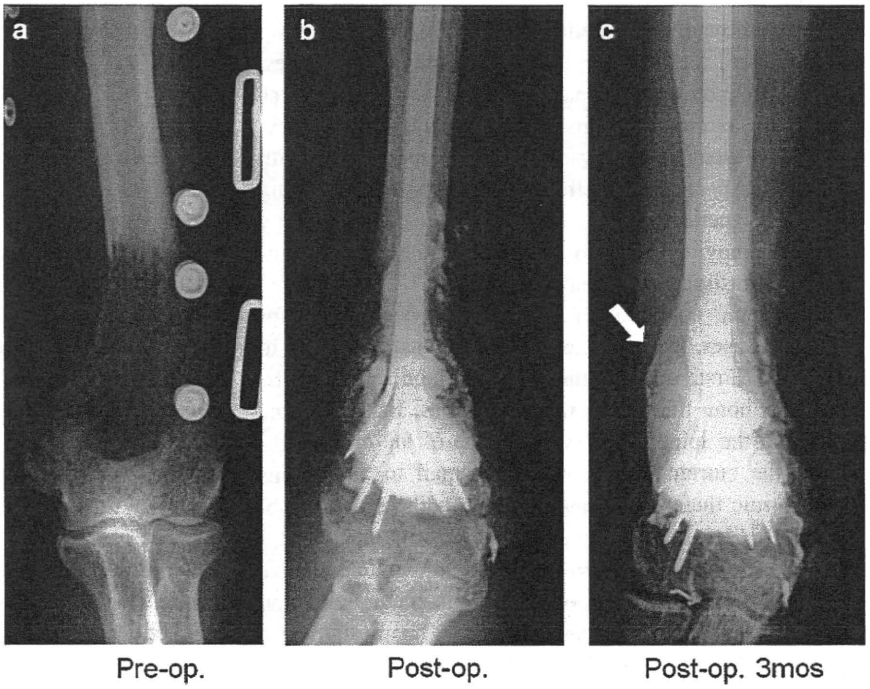

Fig. 5 A radiograph of a bladder cancer that had metastasized to the humerus (**a**). **b** After curettage of the lesion and reinforcement with wire, CPC containing magnetite was implanted into the cavity. **c** At 3 months after undergoing hyperthermia, massive new bone formation had become visible (*arrow*)

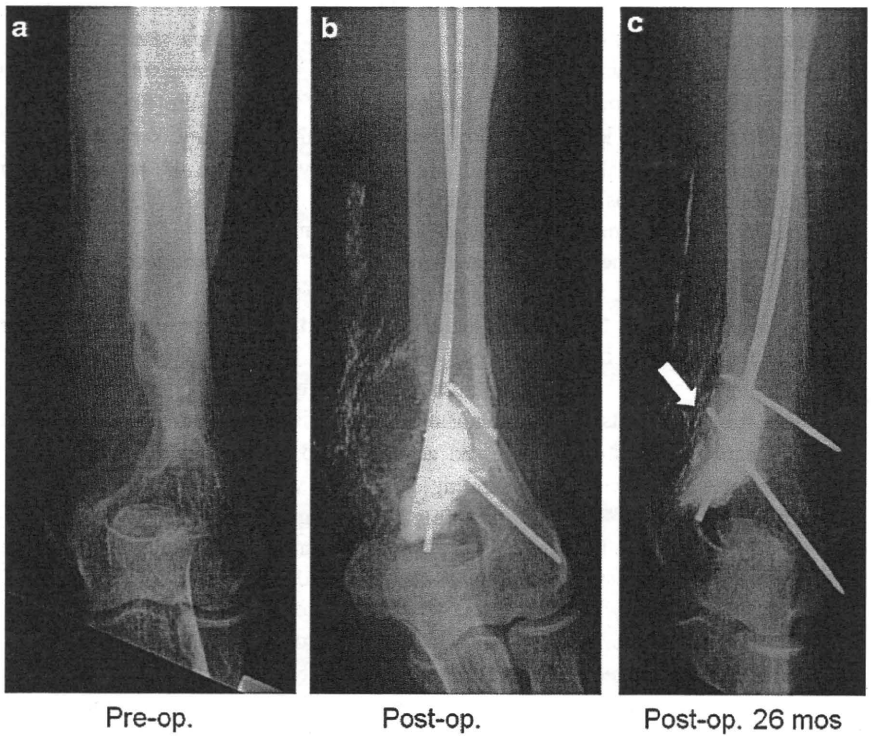

Fig. 6 A radiograph of a hepatocellular carcinoma that had metastasized to the humerus. After curettage of the lesion followed by reinforcement with wire, CPC containing magnetite was implanted into the cavity. At 26 months after undergoing hyperthermia, no recurrence was visible (*arrow*)

clinical evidence showing that heat induces tumor cell death by direct thermal toxicity and enhances the efficacy of some drugs, such as alkylating agents and platinum analogs [36, 37]. Recently micro- or nano-particles have attracted attention as a new heat source. Another new method for inducing interstitial hyperthermia is to inject a fluid containing magnetic nanoparticles intratumorally, and to apply alternating magnetic fields [23]. Kobayashi et al. [23] reported the use of magnetic cationic liposomes, where a group of cationic magnetic particles can be used as carriers to introduce magnetic nanoparticles into target cells, since their positively charged surface interacts with the negatively charged cell surface. Hyperthermia using cationic magnetic particles combined with chemotherapy or radiotherapy might also improve the therapeutic outcome of soft tissue sarcoma patients.

Despite the potential of therapeutic hyperthermia for bone metastases, there are some problems that still need to be solved. First, one of the most common sites of the metastatic bone tumors is the axial bones, including the pelvis and the spine. However, the size of the cylindrical coil of the current generator is too small to apply this hyperthermic therapy to these sites. To address this problem, we tried to make a magnetic field generator with a large coil. However, in our experiments, the increased size of the coil diminished the power of the magnetic field, and decreased the efficacy of heat induction. The design of a new and modified magnetic field generator is warranted. Second, thermal monitoring has recently evolved from the recording of the temperature at 1–8 fixed locations to real-time control based on high-density thermal profile mapping and/or non-invasive real-time characterization of temperature and distribution of physiological changes [38]. To assure its clinical effectiveness and to certify the safety of our hyperthermic treatment, effective real-time and non-invasive monitoring of the temperature is needed.

Conclusion

About two decades ago, the median survival of patients with bone metastasis from advanced lung cancer was typically measured in months, while the median survival of the patients with bone metastases from prostate cancer or breast cancer was measurable in years [39, 40]. However, recent development of new chemotherapies, including targeted therapies, has dramatically improved these patients' prognoses. Paradoxically, the improvement of prognosis of these cancer patients will lead to an increase in the number of patients with pathological fracture or impending fractures due to bone metastases, even if systemic therapy for bone metastases makes great progress. The results of our first series of clinical hyperthermia using magnetic materials achieved good local control of metastatic bone lesions. However, further investigations are

needed before this technique can be employed as a standard therapy for bone metastases.

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Conflict of interest No author has any conflict of interest.

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Functional Recovery of the Donor Knee After Autologous Osteochondral Transplantation for Capitellar Osteochondritis Dissecans

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Functional Recovery of the Donor Knee After Autologous Osteochondral Transplantation for Capitellar Osteochondritis Dissecans

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Background: Osteochondral autograft transplantation has been advocated to treat severe osteochondritis dissecans of the humeral capitellum in throwing athletes to reproduce the normal hyaline cartilage and achieve long-term elbow function. Although some authors have reported good outcomes, the current authors are concerned about functional recovery of the donor knee after osteochondral grafts have been harvested.

Purpose: The present prospective study analyzed functional recovery of the donor knee after osteochondral graft harvest.

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

Methods: Twelve male patients (average age at surgery, 14.4 years) had severe osteochondritis dissecans of the humeral capitellum treated with osteochondral autograft transplantation from the contralateral knee joint. The donor knee of each patient was assessed for pain (visual analog scale), joint effusion, Lysholm score, radiographic findings, and muscle strength (60 and 180 deg/sec).

Results: At 3 months after surgery, 10 patients were pain-free (visual analog scale score, 0); none had knee joint effusion; and 10 gained 100 points in the Lysholm score. However, muscle power (60 deg/sec) of the knee extensor revealed 8 patients with reduced muscle strength at 3 months compared with the preoperative level, although 11 patients reached preoperative knee extensor muscle strength at 12 months. Radiographic findings at 24 months showed that none of the patients had knee osteoarthritis.

Conclusion: A time lag was evident in recovery between postoperative symptoms and muscle power at 3 months. However, harvesting osteochondral grafts did not exert adverse effects on donor knee function in young athletes at 2 years after undergoing osteochondral autograft transplantation for capitellar osteochondritis dissecans.

Keywords: elbow joint; osteochondritis dissecans; osteochondral autograft transplantation; muscle strength; knee joint

Osteochondritis dissecans (OCD) of the humeral capitellum (capitellar OCD) most often affects throwing athletes in their early teens. If diagnosed early, treatment includes nonoperative management such as avoidance of activities, bracing, and physical therapy. However, surgery is often required if the condition worsens. Current surgical options include joint debridement, removal of the loose bodies with or without drilling or curettage, abrasion chondroplasty, fixation of the fragment, and osteochondral transplantation (OCT).[11,21] The goal of treatment for capitellar OCD in adolescent throwing athletes is to prevent elbow osteoarthritis and to allow return to previous levels of throwing activities. Resurfacing osteochondral defects with hyaline cartilage is ideal, but articular cartilage has limited potential for self-repair. Hyaline articular defects can be repaired by innovative OCT in which single or multiple cylindrical osteochondral plugs harvested from areas of decreased weightbearing are transplanted into chondral defects.[6,10,20] Osteochondral defects in the knee and ankle joints have been successfully treated using this procedure.[6,7,14,19] Recent reports[11,22,25,26] have described the use of mosaicplasty to treat capitellar OCD as well as

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osteochondral defects in the knee joint. However, possible adverse effects of this procedure on donor sites must be considered, especially for young athletes. Although donor sites are left to heal and become filled in with bone and dense fibrous tissue,[1] the extent of knee function recovery and the timing of knee extensor and flexor muscle strength recovery are concerns.

To the best of our knowledge, only a few studies[12,16,23,24] have examined morbidity at graft harvest sites after OCT. Therefore, the effect on knee function after osteochondral graft harvests remains controversial. The present prospective study elucidates the outcomes of donor site knees after OCT for lesions of the elbow in young throwing athletes.

MATERIALS AND METHODS

Patients

The study comprised 12 male athletes involved in throwing sports (baseball, n = 10; softball, n = 1; javelin, n = 1) who had advanced capitellar OCD (12 elbows) and who underwent OCT during 2005; they were followed up for a minimum of 24 months (mean, 34.1). All elbows (9 right, 3 left) were on the throwing arms. The period between the start of elbow pain and surgery averaged 10.3 months (range, 2 to 48 months). The mean age of the patients at the time of surgery was 14.4 years (range, 12 to 17 years). None of them had a history of severe trauma or symptoms in the donor knee. The Committee for Ethics in Human Research at Mie University approved the study protocol, and all patients provided written informed consent before enrollment in the study.

Surgical Technique

All surgeries proceeded on supine patients under general anesthesia with a pneumotourniquet applied to the involved upper arm. Intra-articular lesions of the elbow were arthroscopically inspected through a proximal anteromedial portal. Free bodies were removed if present, and the proliferating synovium was debrided. The mean surface area of defects was 126.0 cm^2 (range, 65.9 to 238.6 cm^2). The open operation proceeded via the Kocher lateral approach between the anconeus muscle and the extensor carpi ulnaris muscles. After the defect was identified, its edges were debrided with curettes to healthy hyaline cartilage. The base of the lesion was abraded or curetted down to viable subchondral bone. Cylindrical osteochondral autografts were harvested and transferred with the Osteochondral Transfer System (Arthrex, Naples, Florida). The donor site was selected in the contralateral distal femur, depending on graft diameter. We consider that stance (pivoting) is important for throwing athletes. Yamauchi et al[27] found an annual increase in the knee extensor strength of the pivoting leg. Therefore, we harvested osteochondral grafts from the nonpivoting leg (contralateral knee). The size and number of osteochondral grafts were determined using sizer tamps for maximal replacement of OCD lesions. We obtained small cylindrical grafts from the lateral, less-weightbearing periphery of the femoral condyle proximal to the sulcus terminalis, using an appropriately sized tubular chisel. The lateral periphery bears less weight; thus, contact pressure is decreased.[5] The ideal depth of osteochondral grafts for rigid stability after transplantation was 15 mm.[7] The harvested grafts were transplanted into prepared osteochondral defects in the capitellum by press fitting them into a recipient socket without fixation materials. The mean total surface area of grafts was 60.7 mm^2 (range, 28.3 to 84.8 mm^2). We harvested 3 grafts (6.0 mm in diameter) from 3 patients, 2 (6.0 mm in diameter) from 7 patients, and 1 graft from 2 patients (6.0 mm and 8.0 mm in diameter). Each donor site was packed with a bone graft from the capitellum to prevent postoperative bleeding. The donor site was left unfilled to a depth of about 5 mm because less bone graft was harvested from the capitellum than from the donor site.

The patients were permitted to walk on the first postoperative day after the operation, according to their pain levels. Only a few patients used the wheelchair for a few days. However, the harvest site was the patellofemoral joint (nonweightbearing area), so most patients did not require crutches or a stick. We did not direct the patients to perform any specific rehabilitation for the donor knee.

Postoperative Evaluation

We obtained a standardized history and conducted a physical examination at follow-up. Donor knee pain, the presence of joint effusion, scores on the Lysholm scale,[17] knee radiographs, and the strength of the donor knee flexors and extensors were assessed at follow-up. Joint effusion was confirmed by ballottement of the patella,[18] and the degree of donor knee pain was evaluated using a visual analog scale (0-10) at 1, 2, 3, 6, 12, and 24 months after surgery. Values according to the Lysholm score (0-100 points) were graded as excellent (>94 points), good (84-94 points), fair (60-83 points), or poor (<60 points) at 3, 6, 12, and 24 postoperative months. Anteroposterior, lateral, and skyline radiographic films taken 12 and 24 months after the surgery were assessed. The presence of osteophytes and joint space narrowing were evaluated on knee radiographs according to the Kellgren-Lawrence criteria.[13]

The strength of knee flexors and extensors of both lower extremities was assessed using an isokinetic dynamometer (Cybex, New York, New York) in seated patients with the hip flexed at 90°. The trunk and the thigh of the lower limb under assessment were tightly secured to a chair using Velcro® straps, and the mechanical lever arm was aligned to the axis of rotation of the knee. Testing proceeded at 60 and 180 deg/sec through the full range of motion of isokinetic contractions with the knee flexed at 90°. As a warm-up before data collection, knee extension and flexion repetitions were performed (5 to 10 submaximal and 5 maximal). Two minutes of rest prevented fatigue from impairing performance. Mean peak torque (measured in N·m) was established by averaging the maximal effort repetitions. Relative scores for quadriceps and hamstring

TABLE 1
Time Points of Recording Variables

		Months After Surgery					
	Preoperation	1	2	3	6	12	24
Donor knee pain[a]	×	×	×	×	×	×	×
Joint effusion	×	×	×	×	×	×	×
Lysholm scale				×	×	×	×
Knee radiographs	×					×	×
Muscle power of the knee	×	×	×	×	×	×	

[a]Visual analog scale.

peak torque were compared with those of the preoperative limb on the same side. Table 1 summarizes the time points at which variables were recorded.

Statistical Analysis

All data are expressed as means ± standard deviation. Differences among variables were determined by paired *t* tests using StatView software (Abacus Concepts, Berkeley, California). A P value of $< .05$ was considered statistically significant.

RESULTS

Appendix 1 (available in the online version of this article at http://ajs.sagepub.com/supplemental/) summarizes the postoperative results and patient data. The mean surgical duration was 76.8 minutes (range, 56 to 119 minutes). The size of the osteochondral grafts ranged from 6.0 to 8.0 mm; the average number of grafts was 2.1. The averages of postoperative pain in the donor knee (visual analog scale score) after 1, 2, 3, 6, 12, and 24 months were 0.8, 0.3, 0.2, 0, 0, and 0, respectively. Of the 12 patients, 10 were pain-free at 3 months after surgery. Knee joint effusion persisted in 7 of the 12 patients at 1 month, but none of the patients had effusion at 3 months. The average Lysholm scores of donor knees at 3, 6, 12, and 24 months were 96.3, 100, 100, and 100, respectively. Only 2 patients did not score 100 points at 3 months. One patient complained of donor-side knee pain and swelling after long-distance running, and another complained about donor-side knee pain after walking more than 2 km, swelling upon severe exertion, and slight impairment after climbing stairs and squatting. However, both these patients scored 100 points on the Lysholm scale at 6 months. Radiographic findings confirmed the absence of osteoarthritis in all patients after 12 and 24 months.

Appendix 2 shows relative torque (60 and 180 deg/sec) at 3, 6, and 12 months of follow-up. The relative isokinetic values of extensor strength at 60 deg/sec at these time points were 93.8%, 108.4%, and 128.1%, respectively; the recovery of flexor strength was greater, with relative strength of 113.1%, 123.6%, and 143.0%. The relative isokinetic values of extensor strength at 180 deg/sec at these time points were 105.4%, 118.6%, and 139.8%, respectively; the recovery of flexor strength was greater, with relative strength of 114.3%, 124.9%, and 140.3%. A few patients could not recover 80% of preoperative flexor strength at 3 months. However, the knee extensor strength at 60 deg/sec was reduced in 8 patients at 3 months; furthermore, 25.0% of patients (3 of 12) at 60 deg/sec and 16.7% of patients (2 of 12) at 180 deg/sec did not recover 80% of preoperative extensor strength at 3 months. Yet they all reached 80% of preoperative knee extensor muscle strength at 6 months, and almost all patients (11 of 12) exceeded preoperative strength at 1 year. All patients returned to the previous competitive level of their sport without any donor knee disturbances.

DISCUSSION

Because osteochondral defects can be resurfaced with hyaline cartilage, OCT is a useful option for treating severe capitellar OCD. However, small cylindrical osteochondral grafts for capitellar OCD must be harvested from an area of the knee joint that is less weightbearing. Therefore, one of the greatest disadvantages of this procedure is a possible adverse effect on the donor knee. Donor site morbidity is a concern, especially when transplanting grafts from a normal knee into a dysfunctional joint, such as that with capitellar, talar, or humeral head OCD. Our results revealed no adverse effects on donor knee function after OCT for capitellar OCD in young throwing athletes. However, the power of the knee extensors appeared to recover later than knee pain and joint effusion.

According to other experimental and clinical studies,[3,4,8,15] donor tunnels become covered with initial repair tissue within 6 weeks of graft harvesting, and fibrocartilage coverage develops within 8 to 12 weeks. Although clinical reports[4,7] indicate that the fibrocartilaginous surface can serve as appropriate coverage for areas of less weightbearing, excessive bleeding from donor tunnels seems to be an early postoperative complication. Hangody and Fules[7] reported that 34 of 652 patients treated with mosaicplasty developed painful hemarthroses after surgery and 4 of these developed further deep infections. These data suggest that excessive bleeding from empty donor holes represents a potential septic complication. Thus, an important technical consideration in harvesting osteochondral grafts is to prevent excessive bleeding from the donor site.

Iwasaki et al[12] reported that knee joint effusion (hemarthrosis) persisted for a mean of only 3 weeks postoperatively after being packed with bone wax. However, the effects of bone wax on the cartilage regeneration process remain unclear. Excessive bleeding from donor tunnels was lessened in our series by packing the tunnels with bone grafts from the recipient capitellum. Joint effusion that persisted at 1 month after surgery in over 58% of our patients (7 of 12) had disappeared by 3 months after surgery. Iwasaki et al[12] described magnetic resonance imaging (MRI) findings of the donor-side knees. They achieved 50% to 100% defect fill in 67% of their patients and normal or nearly normal signals at the donor harvest site in 44%. Cost restraints prevented us from performing MRI on the donor-side knee. However, no osteoarthritis change was recognized on radiographs of donor-side knees at 2 years after surgery.

This study is one of the few that have attempted to define potential donor site morbidity associated with this procedure. Hangody et al[9] described intermediate-term morbidity (average, 4.2 years) after talar mosaic-like implantation. They reported that knee complaints were resolved in 95% of patients with talar lesions within 6 weeks after surgery and in 98% of all their patients at 1 year. Al-Shaikh et al[2] described the results of 19 patients with talar OCD treated by osteochondral mosaicplasty over a mean follow-up of 16 months. The mean area of their harvested grafts was 76.4 mm. The mean postoperative Lysholm score was 96.8 (range, 82 to 100). Twelve patients were pain-free, 7 felt pain during severe exertion, and 1 experienced instability and swelling. They found that more advanced age correlated with a worse postoperative knee score, with performance being better among patients aged <30 years than >30 years. They also reported that knee scores were worse among patients with 2 harvested grafts than among those with 1 harvested graft. Reddy et al[24] described the morbidity of the osteochondral harvest sites of 11 patients with talar OCD over a mean follow-up of 47 months. Mean lesion size in their study was about 130 mm^2 with an average of 2.9 grafts. The mean postoperative Lysholm score was 81 (range, 49 to 100). They found that donor site morbidity after osteochondral harvest can be significant, and they recommended that surgeons consider and discuss these risks with their patients. Paul et al[23] reported the donor site morbidity of 200 patients with talar OCD over a mean follow-up of 36 months. In their study, the diameter of the harvested cylinders ranged from 7 to 32 mm. They found that higher body mass index and lower general satisfaction ratings negatively influence Lysholm scores. Our patients were younger than theirs; ours also had a lower body mass index and a smaller total harvested surface area. In previous studies, the knee on the same side as the talar OCD was the harvest site, whereas we harvested grafts from the contralateral extremity. These factors might have contributed to our more favorable outcomes. The functional results of the donor knees in young athletes with capitellar OCD in our study are similar to those of Iwasaki et al,[12] who concluded that donor knee function is not adversely affected after OCT for capitellar OCD in such athletes.

To our knowledge, the muscle strength of knee extensors and flexors after OCT has not been investigated. The relative isokinetic value of extensor strength at 60 deg/sec at 3 months after surgery was 93.8%; for this measure at this time, 66.7% of patients (8 of 12) did not achieve preoperative values, and 25.0% (3 of 12) did not recover 80% of preoperative extensor strength. Yet none of the patients had joint effusion, and almost all (10 of 12) were free of knee pain (visual analog scale score, 0) at 3 months. This discrepancy indicates that even if knee pain and joint effusion disappear, the power of the contralateral knee extensor cannot recover in over half of patients, and new traumas might occur during vigorous exercise. However, all patients reached 80% of preoperative knee extensor muscle strength at 60 deg/sec at 6 months after surgery, and almost all (11 of 12) exceeded the preoperative level at 1 year after surgery. We recommend starting extension training of the donor-side leg soon after surgery.

The shortcomings of this prospective study include a limited patient population and a short follow-up duration. We also did not examine donor-site joint cartilage by MRI or diagnostic arthroscopy. Long-term follow-up is needed to evaluate whether OCT will affect the onset of future knee osteoarthritis.

In conclusion, we identified a time lag in recovery between knee symptoms (knee pain, joint effusion, Lysholm scores) and knee extensor muscle strength. We believe that patients treated with an osteochondral graft from the knee are susceptible to new postoperative trauma during the early postoperative period. However, knee extensor muscle strength of almost all patients recovered within 6 to 12 months after surgery. We found that donor knee function was not adversely affected at 1 year after harvesting osteochondral grafts for mosaicplasty to treat capitellar OCD in young athletes.

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Thrombin-cleaved Osteopontin Levels in Synovial Fluid Correlate with Disease Severity of Knee Osteoarthritis

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Thrombin-cleaved Osteopontin Levels in Synovial Fluid Correlate with Disease Severity of Knee Osteoarthritis

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ABSTRACT. ***Objective.*** Although osteoarthritis (OA) is generally assessed using standard radiographic images in clinical practice, biochemical markers can be used to detect the disease and determine its severity. Osteopontin (OPN) is an extracellular matrix glycoprotein that is a potential inflammatory cytokine. Presence of the thrombin-cleaved form of OPN is well correlated with various inflammatory diseases. We examined whether thrombin-cleaved OPN in synovial fluid (SF) and synovium could be associated with the severity of knee OA.

Methods. SF samples were obtained from 139 knees with OA. Thrombin-cleaved OPN product was determined using Western blotting. Levels of thrombin-cleaved and full-length OPN in SF were determined by ELISA. Synovium samples were analyzed by immunohistochemistry using an antibody specific to the thrombin-cleaved form.

Results. Western blotting showed the presence of thrombin-cleaved OPN in SF from patients with advanced OA. Concentrations of OPN full-length in OA knees were not statistically different from those in controls ($p = 0.134$). In contrast, levels of OPN N-half were significantly higher in OA knees than in controls ($p = 0.042$). Statistically significant correlation was found between thrombin-cleaved OPN and disease severity by Kellgren-Lawrence grade 1, 2, 3, and 4 ($R = 0.274$, $p < 0.001$). Immunohistochemistry of the synovium showed stronger reactivity in samples from subjects with advanced OA.

Conclusion. Local generation of thrombin-cleaved OPN was increased with greater OA severity.
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Key Indexing Terms:
OSTEOARTHRITIS OSTEOPONTIN SYNOVIAL FLUID BIOMARKER THROMBIN

Although osteoarthritis (OA) is generally assessed using standard radiographic images in clinical practice[1], biochemical markers can be used to detect the disease and determine its severity[2]. However, no marker has gained complete acceptance in clinical practice for monitoring progression of OA. The etiology of OA remains poorly understood, and several biochemical factors are involved in the pathogenesis. Levels of serum cartilage oligomeric matrix proteins seem to parallel the radiographic progression of OA[3]. We previously reported that synovial fluid (SF) levels of tenascin-C[4], a matricellular protein, could be a biochemical marker of OA progression[5].

Osteopontin (OPN), an extracellular matrix glycoprotein, is a potential inflammatory cytokine that modulates a variety of pathological conditions. OPN is a matricellular protein and is expressed in synovial tissue and cartilage from patients with rheumatoid arthritis (RA) and OA, suggesting a similar involvement in the pathogenesis of inflammatory arthritis as tenascin-C[6,7,8,9]. Expression of OPN was shown to be upregulated in OA cartilage and synovium. OPN could be involved in synovial cell attachment to chondrocytes or cartilage matrix, in addition to its involvement in destruction of cartilage matrix by the production of collagenases in articular chondrocytes[7,9,10,11]. Proteolytic modification of OPN by thrombin cleavage reveals cryptic binding sites for α9ß1 and α4ß1 integrins, preferentially expressed by neutrophils and by monocytes and lymphocytes, respectively[12,13,14,15]. The presence of the thrombin-cleaved form of OPN is well correlated with various inflammatory disease activities[16]. We measured SF levels of the N-terminal half of thrombin-cleaved osteopontin (OPN N-half) in RA directly, and demonstrated that OPN N-half levels were elevated in RA compared with OA[17].

This cross-sectional study determined levels of OPN N-half and non-thrombin-cleaved osteopontin (OPN full-length) in SF from patients with OA. OPN N-half expression in synovium was also evaluated. We hypothesized that OPN N-half in SF and synovium may be associated with the severity of knee OA. To prove this hypothesis, we examined the SF and synovium levels of OPN N-half

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and evaluated a possible correlation between OPN N-half levels and radiographic grading of knee OA.

MATERIALS AND METHODS

Patients and samples. SF samples (n = 139) were obtained from 99 women and 40 men with primary OA of the knee at the time of surgery or before intraarticular injection of hyaluronic acid. All OA patients fulfilled the American College of Rheumatology clinical and radiological diagnostic criteria for OA[18]. Knee radiographs were evaluated according to the Kellgren and Lawrence (K-L) classification[1] as follows: grade 1, doubtful narrowing of joint space and possible osteophytic lipping; grade 2, definite osteophytes and possible narrowing of joint space; grade 3, moderate multiple osteophytes, definite narrowing of joint space, some sclerosis, and possible deformity of bone contour; and grade 4, large osteophytes, marked narrowing of joint space, severe sclerosis, and definite deformity of bone contour. According to the K-L grading scale, 15 knees were K-L grade 1, 42 K-L grade 2, 41 K-L grade 3, and 41 K-L grade 4. Radiographic OA was considered present if K-L grade was more than 2[19]. Knees with K-L grade 1 were defined as controls. All patients had serum C-reactive protein concentrations within the normal range for healthy adults. Subject characteristics are shown in Table 1. There was no difference between OA patients and controls in terms of age, sex, and body mass index (BMI). Synovium samples were obtained at the time of surgery in 38 patients with OA of the knee: 6 knees were K-L grade 2, 12 were K-L grade 3, and 20 were K-L grade 4.

All patients gave informed consent, and this study was approved by the local ethics committee.

Western blot analysis. Immunoblotting of SF was done to examine the presence of OPN N-half. From each SF sample, 0.15 ml was diluted twice with phosphate-buffered saline (PBS), added to 20 μl of DEAE Sepharose Fast Flow (GE Healthcare UK Ltd., Buckinghamshire, UK), mixed for 30 min at room temperature, washed 5 times with PBS, and then eluted with 1.0 ml of 0.7 M NaCl in PBS. The eluates were diluted twice with 2× sodium dodecyl sulfate (SDS) buffer (4% SDS, 20% glycerol, 125 mM Tris HCl, pH 6.8, 10% 2-mercaptoethanol), boiled, and applied on Western blotting with horseradish peroxidase-labeled anti-human OPN (O-17) rabbit IgG Fab' (IBL, Gunma, Japan). Antibody (O-17) is raised against synthetic peptides corresponding to the internal sequence of mouse OPN (^{17}LPVKVTDSGSSEEKLY^{32}) and can bind both OPN full-length and OPN N-half[20].

Enzyme-linked immunosorbent assay (ELISA). SF was centrifuged at 15,000 g for 15 min, and the supernatants were stored at –80°C until analyzed. ELISA was applied to quantify the levels of OPN full-length and OPN N-half as described[15]. For the OPN N-half ELISA, Immuno Module Plates (Nalge Nunc, Rochester, NY, USA) were coated with anti-OPN N-half (34E3) mouse monoclonal antibody (in 0.1 M carbonate buffer, pH 9.5) at 4°C overnight, then blocked with 1% bovine serum albumin (BSA) in PBS containing 0.05% NaN_3 at 4°C overnight. The mouse monoclonal antibody (34E3) specifically reacts to SLAYGLR and SVVYGLR, exposed by thrombin cleavage of mouse and human OPN, respectively. The OPN N-half ELISA system does not recognize full-length OPN and detects OPN after thrombin cleavage[20]. The specificity of the antibody (34E3) and ELISA was as described[20]. Sample and standard proteins were diluted with 1% BSA, 0.05% Tween 20 in PBS, added to each well, and incubated at 37°C for 1 h. After 7 washes with washing buffer (0.05% Tween 20 in phosphate buffer), 100 μl of horseradish peroxidase-labeled anti-human OPN (O-17) rabbit polyclonal antibody was added to each well and incubated 30 min at 4°C. After 9 washes with washing buffer, 100 μl tetramethyl benzidine buffer as a substrate was added to each well and incubated for 30 min at room temperature in the dark. Color development was stopped by addition of 100 μl stop solution (1 N H_2SO_4). The optical density of each sample was measured at 450 nm. To quantify the levels of non-thrombin-cleaved osteopontin (OPN full-length), a human osteopontin assay kit (IBL) was used with 2 antibodies (O-17 and 10A16). A mouse monoclonal antibody (10A16) is raised against synthetic peptides corresponding to the internal sequence of human OPN (^{155}KSRSFQVSDEQYPDATDE^{172}) and can bind to OPN full-length but not OPN N-half. The percentage of OPN N-half (% N-half) was then determined [OPN N-half divided by all OPN (OPN N-half plus OPN full-length)][17].

Immunohistochemistry. Expression of thrombin-cleaved OPN in synovium was determined by immunohistochemistry using the avidin-biotin complex method with anti-OPN N-half (34E3) mouse monoclonal antibody. Synovium samples were fixed in 10% buffered formalin, embedded in paraffin, and cut into 4-μm sections. After deparaffinization with xylene and rehydration through a series of graded ethanol solutions, the sections were pretreated in 10 mM citrate buffer, pH 6.0, in a microwave oven for 5 min for antigen retrieval. Sections were treated with superblock solution (Scytek Laboratories, Logan, UT, USA). After washing, sections were incubated in 0.3% H_2O_2 in methanol for 15 min to block endogenous peroxidase activity, blocked with 2% bovine serum albumin, and incubated with the primary antibody (34E3) overnight at 4°C. Sections were washed with PBS and reacted with the secondary antibody (biotinylated goat anti-mouse IgG antibody) for 1 h at room temperature. After washing, they were incubated with avidin-peroxidase complex for 30 min. Sections were then developed with diaminobenzidine tetrahydrochloride substrate solution and counterstained with hematoxylin. The results of immunoreactivity for synoviocytes and subintimal tissues were identified using the point system of Salter[21], as follows. For synoviocytes, no staining = 0 point; staining of < 25% of synoviocytes = 1 point; staining of 25%–75% of synoviocytes = 2 points; and staining of > 75% of synoviocytes = 3 points. For subintimal tissues: no staining = 0 point; focal weak staining of subintimal tissues = 1 point; focal strong staining of subintimal tissues = 2 points; and extensive strong staining of subintimal connective tissue = 3 points.

Statistical analyses were performed using StatView for Windows version 5.0 (SAS Institute, Cary, NC, USA). The Mann-Whitney U-test was used to determine the differences between SF levels of OPN full-length, OPN N-half, and % N-half in OA patients and controls. Correlation analyses were performed for SF levels and disease severity using Spearman's rank correlation test, and a multiple regression test was also used. Correlation between the immunohistochemical scores of synovium and disease severity were also estimated. P values < 0.05 were considered significant.

Table 1. Characteristics of the patients. Values are mean ± standard deviation except for sex.

	Controls		Osteoarthritis			p
K-L grade	1	2	3	4	2, 3, 4	
No.	15	42	41	41	124	
Age, yrs	69.7 ± 11.5	72.3 ± 12.3	73.8 ± 6.9	74.5 ± 7.0	73.5 ± 9.1	0.1571
Sex female, %	47	60	88	76	74	0.0543
BMI, kg/m^2	23.1 ± 3.0	23.1 ± 2.3	24.1 ± 2.8	25.9 ± 5.1	24.4 ± 3.8	0.3419

K-L: Kellgren and Lawrence grade; BMI: body mass index.

RESULTS

Expression of thrombin-cleaved OPN protein. In SF from patients with OA, the antibody O-17 reacted with full-length and N-half OPN with molecular weights of 48 kDa and 30 kDa, respectively (Figure 1). These results showed that samples of OA SF contained full-length OPN, and that the level of N-half OPN was considerably elevated in patients with advanced OA (Figure 1, right lane), whereas a faint band was rarely detected in the early OA sample (Figure 1, left lane).

ELISA. SF levels of OPN full-length, OPN N-half, and % N-half are shown in Figure 2. The levels of OPN full-length in OA knees were not statistically different from those in controls ($p = 0.134$; Figure 2A). In contrast, the levels of OPN N-half were significantly higher in OA knees than in controls ($p = 0.042$; Figure 2B). The % N-half increased significantly in OA knees compared to controls ($p = 0.003$; Figure 2C). In rank correlation tests, the SF levels of OPN full-length showed no difference ($R = 0.111$, $p = 0.191$; Figure 3A); however, the levels of OPN N-half and % N-half correlated with disease severity ($R = 0.400$, $p < 0.001$; Figure 3B, and $R = 0.416$, $p < 0.001$; Figure 3C, respectively). Apart from one clear outlier in K-L grade 4, showing extremely high concentration of OPN N-half (7.8 nM), OPN N-half levels correlated with disease severity ($R = 0.282$, $p < 0.001$). In addition, statistically significant correlation was found between OPN N-half and disease severity by K-L grade 1, 2, 3, and 4 ($R = 0.274$, $p < 0.001$). After adjusting the levels of OPN N-half as well as % N-half for sex and age, the correlation between % N-half and the radiographic grading levels remained statistically significant ($R^2 = 0.053$, $p = 0.015$); however, the levels of OPN N-half showed no difference ($R^2 = 0.036$, $p = 0.089$).

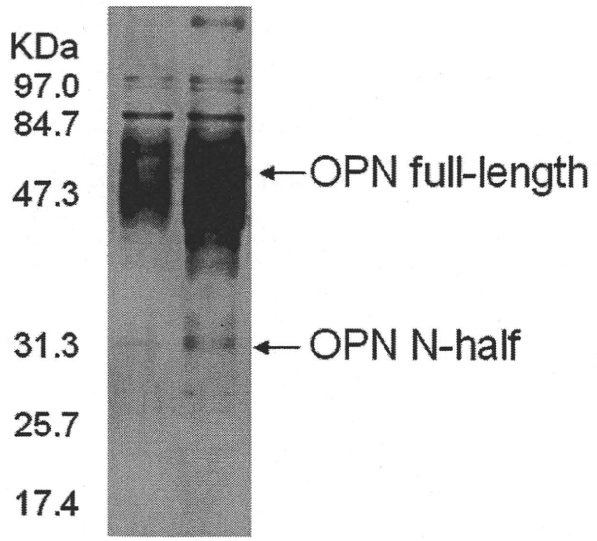


Figure 1. Representative Western blot analysis of synovial fluid. The antibody O-17 reacted considerably with bands around 48 kDa and 30 kDa in advanced OA (right lane). A faint band around 30 kDa was rarely detected in early OA (left lane).

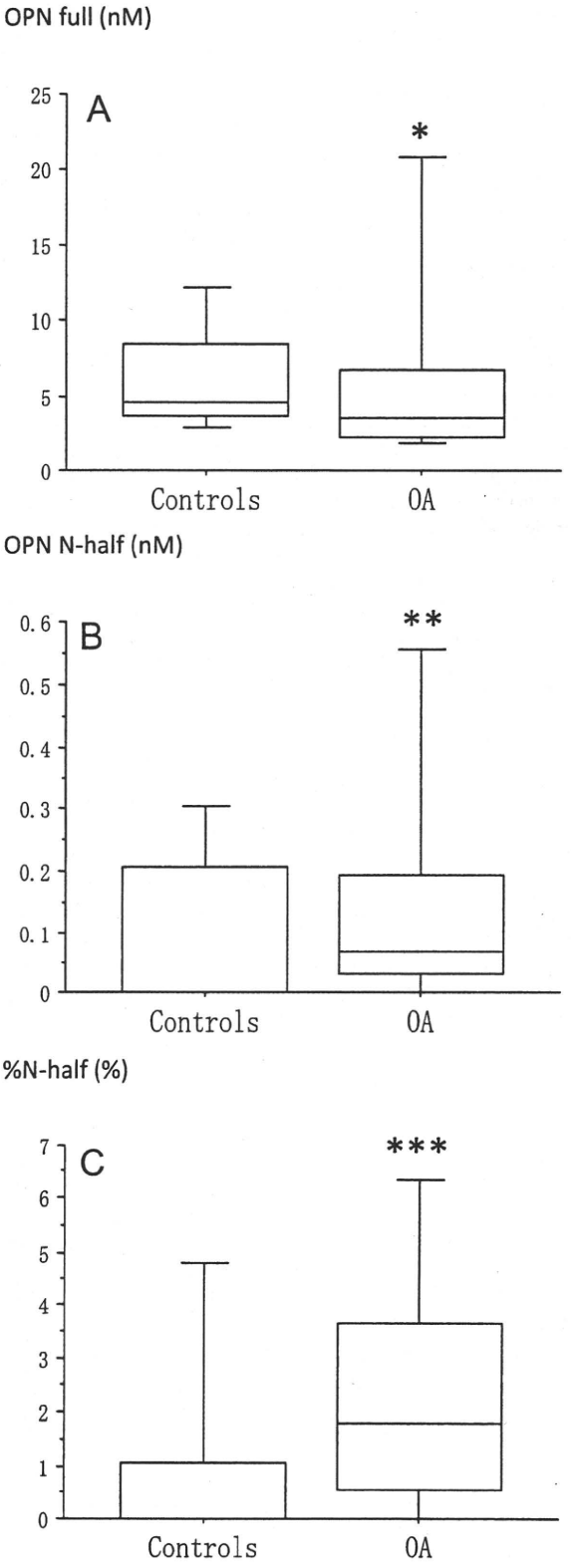


Figure 2. Levels of OPN full-length (A), OPN N-half (B), and % N-half (C) in synovial fluid from subjects with OA and controls. Lower and upper lines in the box represent 25th and 75th percentiles, and the median is shown. Bars show the range of 10th and 90th percentiles. *p = 0.134; **p = 0.042; ***p = 0.003.

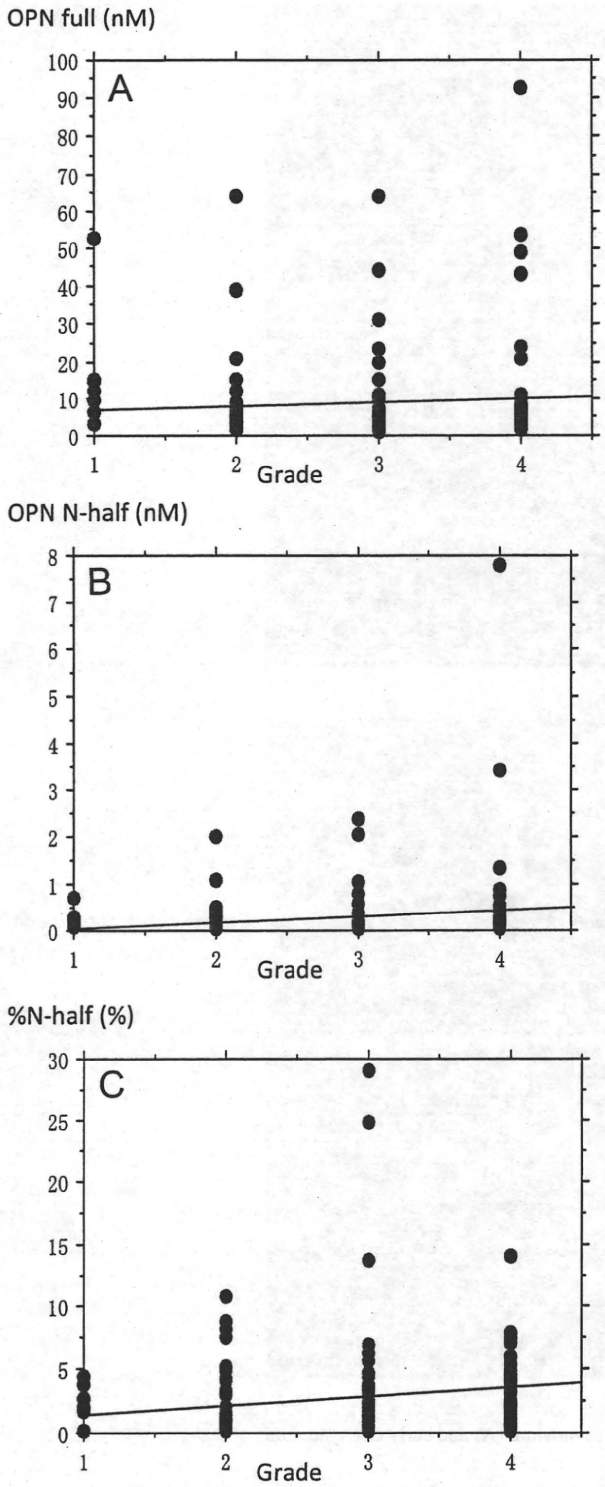


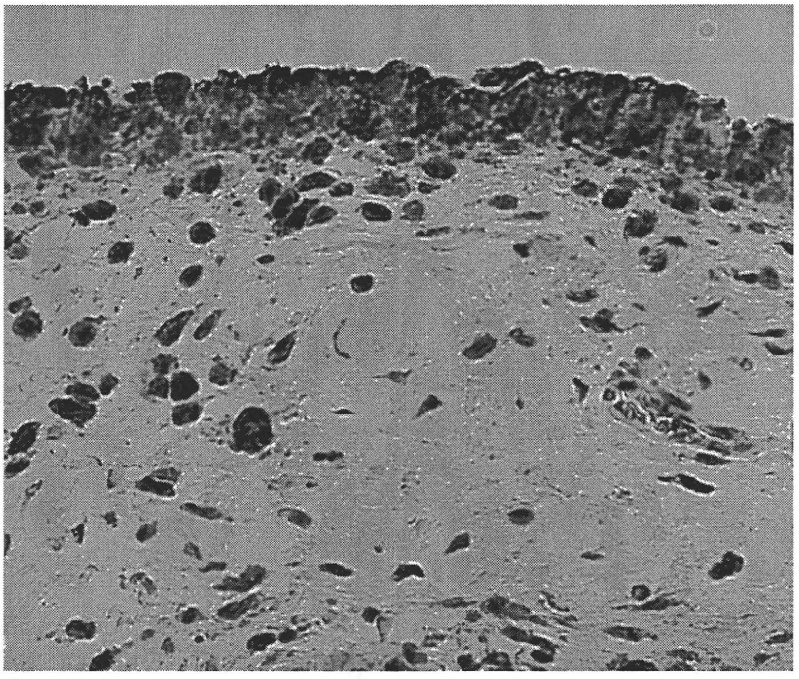
Figure 3. Correlation of disease severity with synovial fluid levels of OPN full-length (A), OPN N-half (B), and % N-half (C).

Immunohistochemistry. Synovium samples showed positive labeling of OPN N-half in synovial lining cells and subintimal tissues in synovial samples from patients with OA (Figure 4). The mean scores of OPN N-half for synoviocytes were 0.67 ± 0.51 for grade 2 disease, 1.83 ± 0.71 for grade 3 disease, and 1.90 ± 0.72 for grade 4 disease. The mean scores for subintimal tissues for grade 2, 3, and 4 disease were 0.50 ± 0.55, 1.33 ± 0.49, and 1.35 ± 0.49, respectively. The scores of OPN N-half for synoviocytes and subintimal tissues correlated weakly with disease severity (R = 0.383, p = 0.020 and R = 0.358, p = 0.030, respectively).

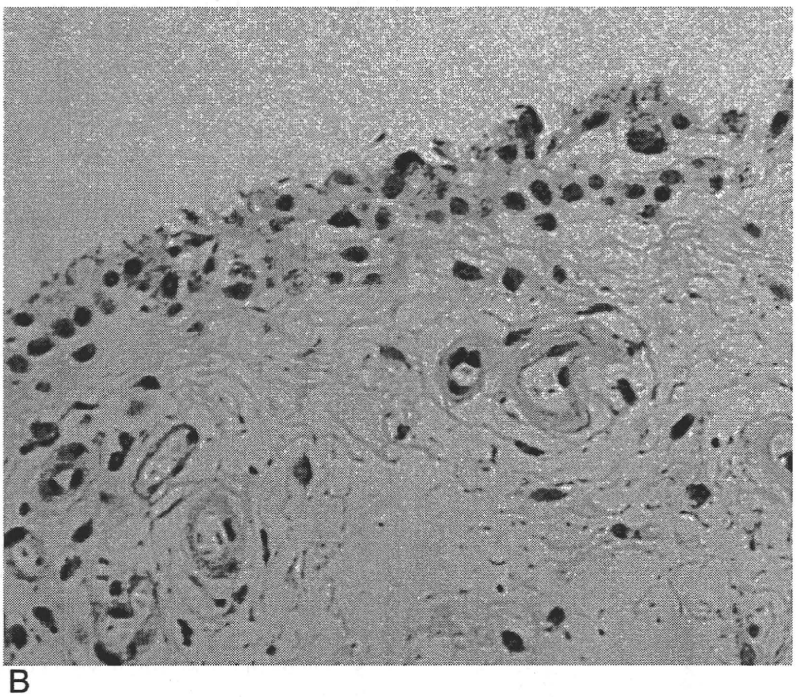
DISCUSSION

We have demonstrated that SF concentrations of OPN N-half correlated with radiographic severity of OA. Elevated levels of OPN in the SF were possibly caused by either the release of OPN residing in the local tissues, including the synovium, articular cartilage, and bone, or the increase in its production, or both[11,22]. It is suggested that cell adhesion, migration, or inflammation could be involved in the release of OPN[23]. It is likely that the excessive production of cytokines and growth factors by the inflamed synovium and activated chondrocytes plays an important role in the pathophysiology of OA. Proinflammatory cytokines have been demonstrated to play a pivotal role in the development of the disease process. Tumor necrosis factor-α (TNF-α) and interleukin 1ß (IL-1ß) can stimulate their own production and induce chondrocytes and synovial cells to produce other cytokines and chemokines, including IL-6, IL-8, and matrix metalloprotease[24,25,26]. Previous studies have demonstrated that overexpression of OPN induces proinflammatory cytokines and chemokines, including IL-1ß, IL-6, IL-8, TNF-α, CXCL1, and CCL2[9,27]. Sharif, *et al*[28] reported that elevated levels of thrombin-cleaved OPN correlated with increased levels of multiple proinflammatory cytokines including TNF-α, IL-6, and IL-12p40, and chemokines IP-10 and eotaxin, whereas levels of OPN full-length did not correlate with levels of proinflammatory cytokines other than IL-6. Elevation of thrombin-cleaved OPN levels may play some role in a specific inflammatory response, including the pathogenesis of OA.

Our study demonstrated the immunohistochemical expression of OPN in the synovium and that synovium levels of OPN correlated with disease severity. Gao, *et al*[11] reported OPN immunohistochemical staining in articular cartilage of knee OA, and the articular cartilage levels of OPN correlated with disease severity. Our study showed the SF levels of OPN N-half were correlated with disease severity, but the levels of OPN full-length were not. Two previous studies[11,22] also showed that SF OPN levels significantly correlated with OA severity. The study by Honsawek, *et al*[22] used the same commercial ELISA kit (IBL) that we used, and Gao, *et al*[11] used another ELISA kit (Uscn Life Science & Technology Company, Wuhan, China). No description was given in either study of whether they used the kit for OPN full-length or OPN N-half. If the studies used the kit for OPN full-length, one possible reason for the difference in these results might be the molecular fragility of



A



B

Figure 4. Expression of OPN N-half in advanced OA synovium (A) and early OA synovium (B) (original magnification ×200).

OPN. OPN can easily be cleaved into 2 fragments by thrombin, because the thrombin cleavage site is present in the OPN molecule.

In a previous study, we revealed that cartilage repair in tenascin-C knockout mice was significantly slower than that in WT mice and that the deficiency of tenascin-C progressed during cartilage degeneration[29]. In contrast, human SF levels of tenascin-C were shown to be elevated in OA in the advanced stage[5]. Matsui, *et al*[30] demonstrated that OPN could be a critical intrinsic regulator of cartilage degradation and that OPN deficiency resulted in accelerated development of OA-like tenascin-C deficiency. These findings and our present results suggest remodeling of cartilage and synovium could play some role in the increased expression of OPN.