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Induced membrane technique using beta-tricalcium phosphate for reconstruction of femoral and tibial segmental bone loss due to infection: technical tips and preliminary clinical results

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Abstract

Purpose This study aimed to provide preliminary evidence regarding effectiveness of grafting beta-tricalcium phosphate (β-TCP) combined with a cancellous autograft for treating nonunion of long bones in the lower extremity due to infection by evaluating clinical and radiological outcomes.

Methods We retrospectively reviewed the clinical and radiological results in seven patients (six men, one woman; median age 39 years) treated by the induced membrane technique for nonunion of the femur or tibia due to infection. In the second stage of the procedure, the bony defect was filled with a combination of autologous cancellous bone and β-TCP, which were mixed in approximately the same proportions. The time interval between the second stage of the procedure and bone healing was investigated. Radiographic characteristics including maximum bone gap and radiographic apparent bone gap were evaluated.

Results The median follow-up period was 14 months. Bone healing was achieved in a median of six months after the second procedure. The median maximum bone gap and radiographic apparent bone gap were 55 mm and 34 mm, respectively.

Discussion Use of β-TCP, which has osteoconductive ability, with an autograft provided good clinical and radiological outcomes. The findings of this preliminary study suggest the potential of β-TCP as a useful bone substitute for autografts in the induced membrane technique.

Conclusions Our findings suggest that β-TCP may be an effective extender when using the induced membrane technique.

Introduction

Large segmental bone defects remain a major therapeutic problem for orthopaedic trauma surgeons because of prolonged healing periods, the need for several surgical interventions, and the necessity of amputation as the only definitive solution in the case of failure. Traditionally, bone transport distraction osteogenesis using the Ilizarov technique or a free vascularized bone graft are the surgical options in such cases. However, these techniques are associated with long healing periods and a relatively high complication rate [1, 2]. In 2010, Masquelet et al. developed the concept of the induced membrane technique for the treatment of large segmental bone defects due to nonunion or chronic osteomyelitis [3, 4]. Their technique consists of a two-stage reconstructive procedure. In the first stage, a bioactive membrane is created by placement of a temporary polymethylmethacrylate (PMMA) spacer, which protects against growth of fibrous tissue into the defect, and in the second stage, the PMMA spacer is removed and the membrane is filled with an autologous cancellous bone graft. Although several surgeons have reported good surgical outcome using this technique [5–8], the need to harvest a large autologous bone graft that can fill the large segmental bone defect is an important problem in the second stage of the procedure. To resolve this problem, the recent literature recommends use of the reamer-irrigator-aspirator (RIA), which allows the surgeon to harvest more than 50 mL of autologous bone graft on a regular basis [9–13]. Furthermore, cancellous allograft and demineralized bone matrix, which possess not only osteoconductive but also osteoinductive ability, can be used as extenders if their volume

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is less than 25% of the final graft composition [13]. Use of other materials has not yet been thoroughly investigated; however, if some types of artificial bone with only osteoconductive ability can be used as a graft material combined with autologous cancellous bone, a smaller amount of cancellous bone would be needed. Such a technique could be used to treat larger bone defects than currently possible.

Since 2014, we have grafted beta-tricalcium phosphate (β-TCP) mixed with autologous cancellous bone from the iliac crest to fill the bone defect in the second stage after creation of a membrane. In this article, we discuss our experience of using the induced membrane technique with β-TCP for reconstruction of femoral and tibial segmental bone loss caused by infection.

Patients and methods

We retrospectively reviewed the clinical and radiological results in seven patients treated by the induced membrane technique for nonunion of the femur or tibia due to infection between 2014 and 2015 at our institution. The Institutional Review Board at our institution approved the study and informed consent was obtained from all patients. The patients included six men and one woman with a median age of 39 (range 24–77) years. Two patients had sustained a femoral fracture (supracondylar fracture, 1; shaft-distal metaphyseal fracture, 1) and five patients had sustained a tibial fracture (proximal metaphyseal fracture, 2; shaft fracture, 3) as the initial injury. Table 1 shows whether each fracture was open or closed, and if open, its Gustilo classification. The cause of the initial injury was a fall from height in three patients, a road traffic accident in three patients (motorcycle, 2; bicycle, 1), and a fall while walking in one patient. Because nonunion occurred and the bone infection did not heal despite several sessions of surgical debridement, the patients were referred to our institution (Fig. 1). Diagnosis of an infected nonunion was made according to the following protocol: discharge of pus

from the skin incision or a fistula around the incision; no healing of the fracture evident on radiological evaluation 6 months after the initial osteosynthesis; and instability at the fracture site confirmed by stress radiography. The median time interval between the initial trauma and the first visit to our institution was nine (range 3–96) months. Pre-operatively, methicillin-resistant *Staphylococcus aureus* (MRSA) was identified as the causative organism in four patients and *Serratia marcescens* and Corynebacterium spp. in one patient; no causative organism was identified in the remaining two patients (Table 1). Five patients had no comorbidity and two patients (cases 4 and 5) had high blood pressure that needed medication; however, no patient had ischemic heart disease, hepatic or renal dysfunction, hematological disease, or a metabolic disorder such as diabetes mellitus. Reconstruction was performed by the two-stage induced membrane technique in all patients.

In the first stage of the reconstruction, radical debridement of all nonviable tissue was performed until bleeding and viable tissue was seen at the margin of the resection. After aggressive debridement, an antibiotic-impregnated PMMA spacer was inserted into the debrided bone defect. Next, 2 g of vancomycin powder was combined with 40 g of bone cement (Cemex RX, Tecres Corp, Verona, Italy). The PMMA spacer was placed and shaped to match the defect in situ and allowed to cure while in position. Our protocol for fixation in the first stage of the procedure is as follows. For a shaft fracture, cast immobilization without internal fixation is applied if a manual valgus-varus and extension-flexion stress test after sufficient press-fit insertion of the PMMA spacer shows almost no instability at the debrided site; if the manual stress test shows obvious instability, plating is indicated. Single or dual plating is applied if a fracture extends to the metaphyseal region. As a result, both the patients with femoral nonunion needed locking plate fixation (dual plate fixation, 1; lateral plate fixation, 1) to stabilize the bone defect after debridement. Among the patients with tibial nonunion, two with proximal metaphyseal nonunion needed locking plate fixation (dual

Table 1 Demographic and clinical patient characteristics

Patient	Age	Sex	Side	Bone	Site	Gustilo	Causative organism
1	35	Female	Right	Femur	Supracondylar	IIIA	MRSA
2	39	Male	Left	Femur	Shaft-distal metaphysis	Closed	MRSA
3	39	Male	Right	Tibia	Shaft	IIIA	Unknown
4	77	Male	Right	Tibia	Proximal metaphysis	II	*Serratia marcescens*, Corynebacterium spp.
5	61	Male	Left	Tibia	Shaft	Closed	Unknown
6	24	Male	Left	Tibia	Shaft	Closed	MRSA
7	25	Male	Left	Tibia	Proximal metaphysis	Closed	MRSA
Median	39						

MRSA, methicillin-resistant *Staphylococcus aureus*

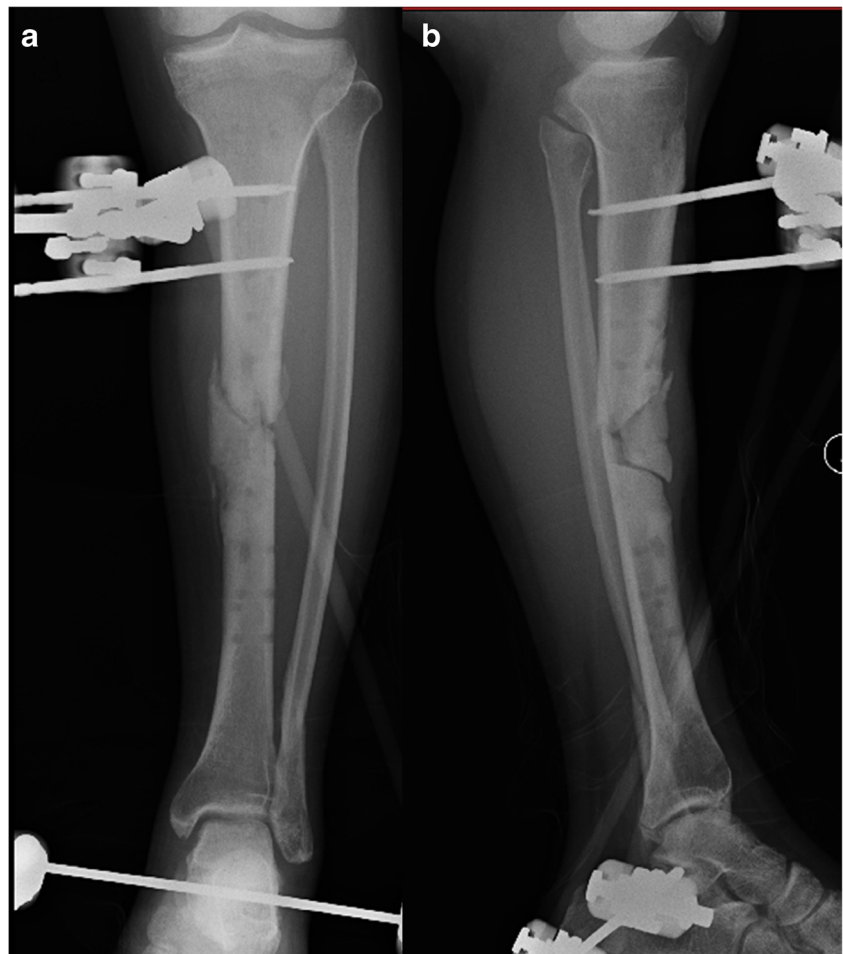**Fig. 1** Anteroposterior (a) and lateral (b) radiographs before the first-stage procedure in patient 6

plate fixation, 1; lateral plate fixation, 1) and three with non-union of the diaphysis needed below-knee cast immobilization instead of internal fixation (Fig. 2). No patient required soft tissue reconstruction due to extensive debridement of contaminated soft tissue, and primary wound closure providing an adequate soft tissue envelope over the remaining bone and spacer was possible in all cases. The tissue debrided in the first stage of the procedure was sent for culture in all cases. The causative organism was confirmed to be the same (MRSA) after debridement in four cases and remained unidentified in two; no culture was obtained in one patient in whom the causative organisms had been previously identified as *S. marcescens* and Corynebacterium spp. After completion of the first stage of the procedure, all patients received intravenous linezolid 600 mg twice daily for 3 days followed by intravenous daptomycin 8 mg/kg once daily until routine blood investigations indicated normalization of C-reactive protein, white cell count, and erythrocyte sedimentation rate. After confirmation of normalization of laboratory values, we perform the second stage of the procedure. However, if any laboratory value showed subsequent elevation, additional debridement was considered before performing the second

stage. Radiographic examination was performed in all patients to estimate the volume of the bone defect after aggressive debridement before the second stage. On anteroposterior and lateral radiographs, the volume of bone defect was defined as either cylindrical or conical on no bone contact or point bone contact, respectively, and the volume of each defect was estimated by calculation of the cylindrical or conical volume using a mathematical formula. Defects were defined as cylindrical or conical based on no bone contact or point bone contact according to the method reported by Stafford and Norris [11].

In the second stage of the procedure, the inserted PMMA spacer was accessed via the previous incision. The induced membrane was incised longitudinally, and the PMMA spacer was carefully removed to prevent damage to the self-induced membrane. Next, definitive internal fixation was performed. In femoral nonunion, additional medial locking plate fixation was performed in the case treated with lateral locking plate fixation in the first stage of the procedure. In tibial nonunion, additional medial locking plate fixation was performed in the case with metaphyseal nonunion treated with lateral locking plate fixation in the first stage of the procedure. In the three

Fig. 2 Anteroposterior (a) and lateral (b) radiographs after the first stage of the procedure in the same patient. The polymethylmethacrylate spacer was placed into the bone defect after aggressive debridement

remaining cases with tibial shaft nonunion, an intramedullary nail was inserted for definitive internal fixation (Table 2). After definitive fixation, the bone defect was filled with a combination of autologous cancellous bone graft from the iliac crest and β-TCP (OSferion, Olympus Terumo Biomaterials Corp, Tokyo, Japan) with a porosity of 75% (Fig. 3). An autologous cancellous bone graft was harvested from the anterior region of the iliac crest. A skin incision was made just posterior to the anterior superior iliac spine, and the

dissection was continued down to the periosteum of the iliac crest. The trap-door technique was then applied with continuity of the crest section and inner wall maintained, and cancellous bone was harvested from the medullary canal between the inner and outer wall. In all cases, we harvested a half volume of the preoperative estimated bone defect in autologous cancellous bone and applied a half volume of estimated defect in β-TCP for each case, and these were mixed in approximately the same proportions and then grafted to the defect. After the second stage

Table 2 Surgical dates for the seven patients

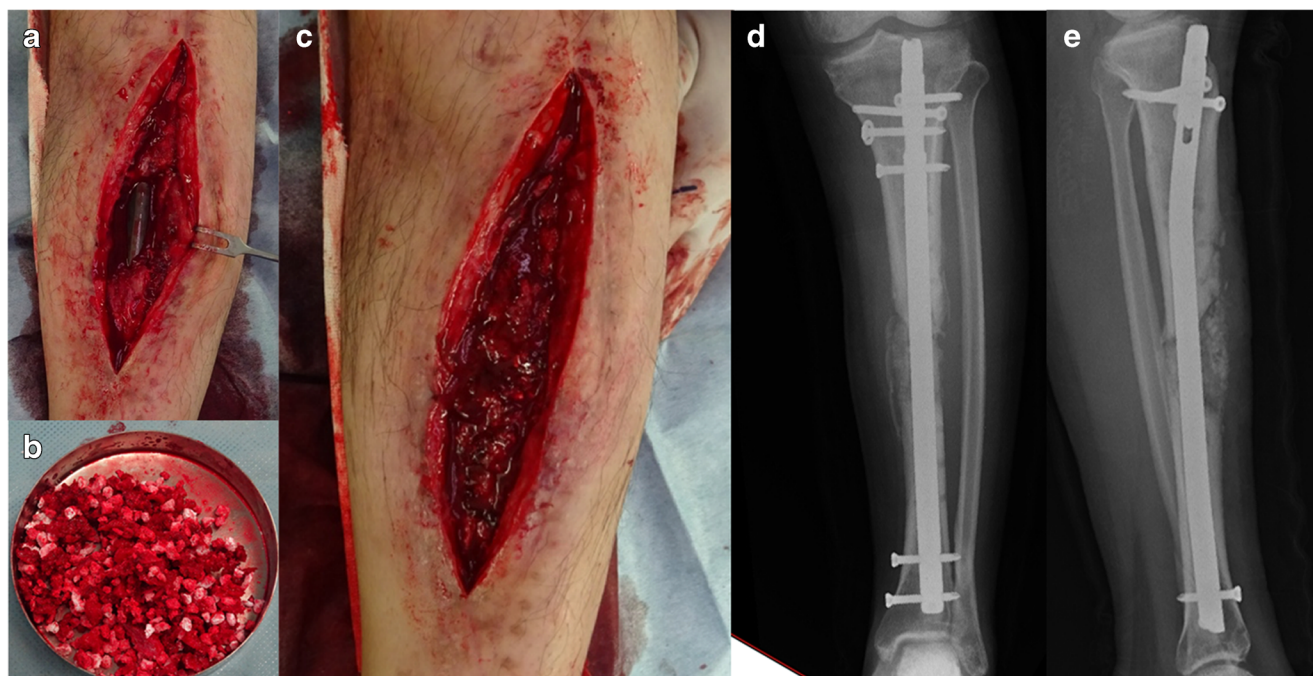| Patient	Interval between injury and first stage (months)	Interval between first and second stage (weeks)	Fixation at first stage	Definitive fixation
1	8	28	Dual plate	Dual plate
2	14	14	Lateral plate	Dual plate
3	23	6	Cast	Dual plate
4	23	11	Cast	Dual plate
5	10	11	Cast	Nail
6	6	6	Lateral plate	Nail
7	8	13	Dual plate	Nail
Median	10	11		

Fig. 3 Intra-operative photographs showing (a) definitive fixation by means of intramedullary nailing before grafting, (b) mixed autologous cancellous bone graft from the iliac crest and beta-tricalcium phosphate, and (c) the filled bone defect after grafting. (d) Anteroposterior and (e) lateral radiographs immediately after the second stage of the procedure in the same patient

of the procedure, the same intravenous antibiotic treatment protocol as after the first stage of the procedure was used in all patients. Active range of motion (ROM) exercises of the ipsilateral knee and ankle joint were allowed a few days after surgery, and all patients were allowed one-quarter partial weight bearing eight weeks after surgery. Thereafter, weight bearing was permitted if the patient had not experienced pain on partial weight bearing. Full weight bearing was allowed at four months after surgery. However, if the patient felt anxious about full weight bearing without a crutch, they continued partial weight bearing with a single crutch until they felt confident about full weight bearing. Weight bearing status and radiographic healing of the nonunion were evaluated during postoperative clinical follow-up. Radiographic healing was defined as the presence of a bridging callus on three of four cortices; if radiographic healing was in doubt, computed tomography was used to clarify the bone healing in detail.

In all patients, we examined whether bone healing on radiological assessment was achieved, and if so, the elapsed time after the second of the stage procedure. Local findings (including swelling, heat, redness, pain on palpation at the site of injury, and the condition of the healed wound) were documented and blood investigations were performed at routine outpatient clinic appointments for all patients to exclude recurrence of infection. The radiographic characteristics of all patients, including the maximum bone gap and radiographic apparent bone gap (RABG), were also evaluated [14]. The maximum bone gap was defined as the maximum length of the medial, lateral, anterior, and posterior gaps as measured on anteroposterior and lateral radiographs. RABG was defined as a mean value of the lengths of these four gaps.

Results

The median follow-up period for the seven patients was 14 (range 12–19) months. Bone infection was controlled in all patients until the most recent follow-up. Furthermore, all patients achieved bone healing, and the median time interval between the second stage of the procedure and bone healing was six (range 4–9) months (Fig. 4). The duration of intravenous antibiotic treatment was no more than 3 weeks after the first stage of the procedure in six patients (1 week, two patients; 2 weeks, three patients; 3 weeks, one patient). The remaining patient underwent intravenous antibiotic treatment for two weeks, at which time normalization of laboratory values was confirmed; however, daptomycin was re-administered intravenously four weeks after the first stage of the procedure because of re-elevation of the CRP level. This patient needed additional debridement at nine weeks after the first stage of the procedure and the same protocol of intravenous antibiotic treatment was administered for five weeks thereafter. She underwent the second stage of the procedure 28 weeks after the first stage. The median estimated defect volume was 13.6 (range 5.5–26.6) cm^3.
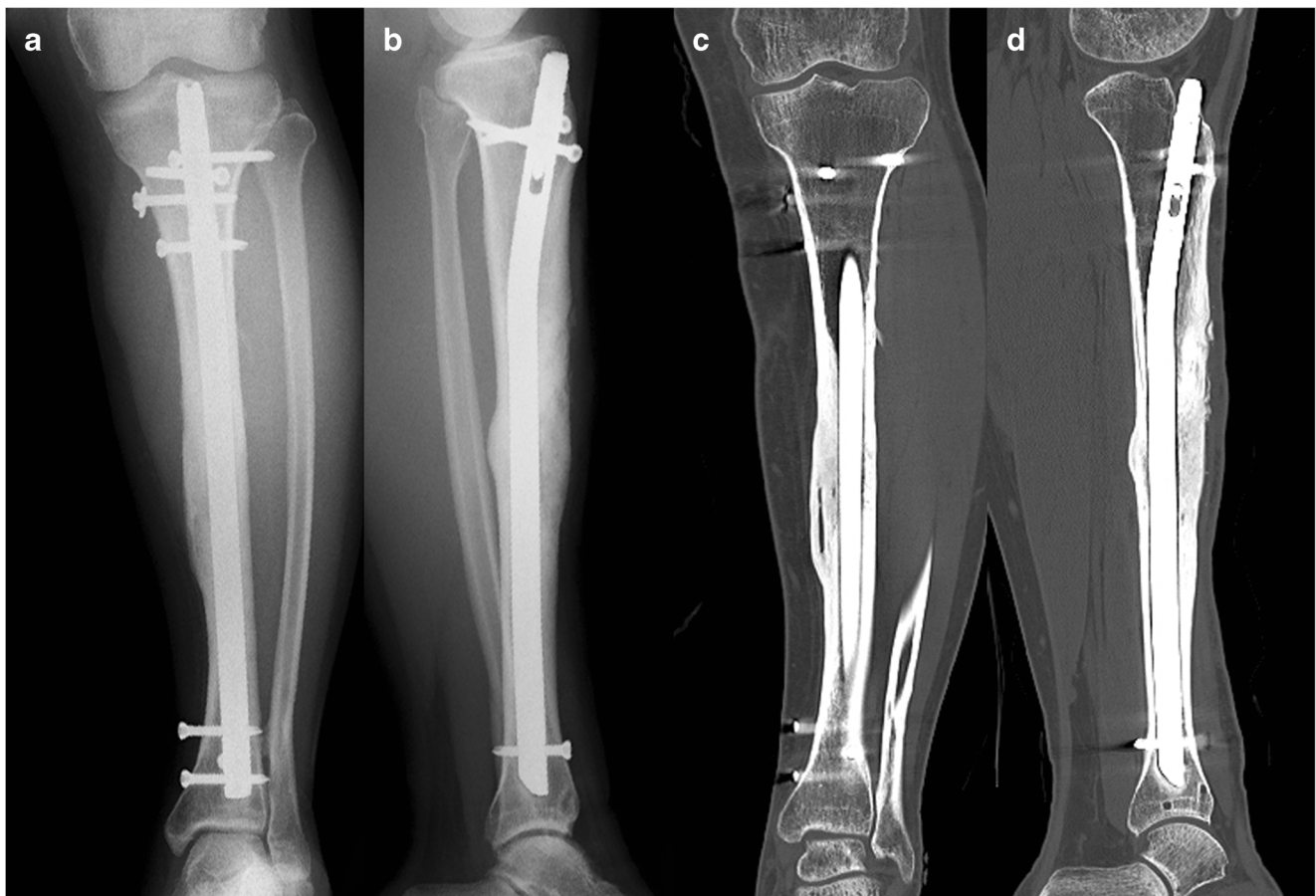
Fig. 4 (a) Anteroposterior and (b) lateral radiographs at 4 months after the second stage of the procedure, and (c) coronal and (d) sagittal computed tomography at 6 months after the second stage of the procedure in the same patient

The median maximum bone gap was 55 (range 25–80) mm and the median RABG was 34 (range 24–65) mm (Table 3). There were no complications that were thought to be related to application of β-TCP in any of the seven patients.

Discussion

Masquelet et al. developed a two-stage induced membrane technique comprising formation of a biologic membrane followed by delayed massive autologous cancellous bone grafting for treating segmental bony defects [3, 4]. Traditionally, the autologous cancellous bone grafted into the bone defect is harvested from the anterior or posterior iliac crest in the second stage of the procedure. However, such harvesting from the iliac crest is accompanied by a risk of donor site pain and morbidity. Furthermore, the average volume of cancellous bone that can be harvested from the posterior iliac crest on one side is reported to be only 38 mL [11]. Femoral bone grafting using RIA has become a popular way of overcoming the disadvantages of autograft harvest from the iliac crest [9–13]. Although autograft harvest using RIA is not

a new technique, it is now commonly used in conjunction with the induced membrane technique, which requires a large amount of autograft bone. Recent studies have compared these techniques and demonstrated the advantages of the RIA technique over autograft harvest from the iliac crest, including less pain and morbidity at the donor site, a shorter time required for harvest, and a higher volume of harvested autograft [9–13]. However, RIA is also associated with major complications, such as fracture

Table 3   Surgical results in seven patients

Patient	Bone healing (months)	Estimated defect volume (cm3)	Maximum bone gap (mm)	RABG (mm)
1	4	22.6	45	39
2	6	16.2	80	65
3	6	5.5	55	26
4	9	10.7	25	24
5	5	26.6	60	34
6	4	13.6	60	41
7	6	8.9	45	31
Median	6	13.6	55	34

RABG, radiographic apparent bone gap

of the femur at the RIA donor site [12]. Donor site morbidity is thus an important problem associated with the induced membrane technique, and a large volume of harvested autograft can be expected to increase the risk of donor site morbidity.

There have been reports of extenders, such as cancellous allograft or demineralized bone matrix, being used in patients with an insufficient volume of harvested autograft [4, 15]. In these reports, an extender volume < 25% of the final graft composition was used with no increase in complication or nonunion rates. However, use of allografts is sometimes accompanied by an increased risk of disease transmission and infection as well as incomplete incorporation. Bone morphogenetic protein 7 has been used as an osteoinductive material in the induced membrane technique, but increased rates of delayed deformation and bone graft resorption have been reported [15].

At present, β-TCP is the most widely used bone substitute in the orthopaedic field, with favorable characteristics such as easy resorption, high osteoconductivity, and high replanting ability, and its usefulness has been reported by several surgeons [16–19]. In spinal surgery, Dai and Jiang compared the usefulness of β-TCP and local autografts with that of conventional iliac crest bone grafts in lumbar spine fusion [17]. They concluded that β-TCP combined with a local bone graft provided the same rate of radiographic fusion as that of conventional grafts and comparable clinical outcomes. In fracture surgery, Jiang et al. achieved good outcomes using β-TCP as a bone graft substitute for osteosynthesis of calcaneal fractures [16], and Shen et al. reported good surgical outcomes in patients with tibial plateau fractures treated by open reduction and rigid internal fixation combined with application of β-TCP [19]. In the present case series, β-TCP could be used as an extender by mixing it with autograft bone in the second stage of the procedure, with good clinical and radiological outcomes. The median maximum bone gap and RABG were 55 mm and 34 mm, respectively, on radiographic evaluation. This was especially remarkable because the lengths of the post-operative bone defects were almost equal to those reported in previous studies [7, 20, 21] even though we used β-TCP, which has osteoconductive ability but no osteoinductive ability. If grafted β-TCP is surrounded by soft tissue, interposition of the soft tissue could prevent formation of bone. However, in the induced membrane technique, a bioactive membrane is created by placement of a temporary PMMA spacer, which protects against growth of fibrous tissue into the bone defect. We consider that this effect may avoid the interposition of fibrous tissue in the bone defect and prevent resorption of β-TCP in the early healing phase.

An important consideration when using β-TCP as an extender by mixing it with autograft bone is reduction of osteogenic capacity because autograft bone has both osteoconductive and osteoinductive ability whereas β-TCP has only osteoconductive ability. However, despite this potential risk, the seven patients in this series achieved bone healing at a median of six months after surgery by grafting of autologous cancellous bone and β-TCP mixed in approximately the same proportions. This finding suggests that patients without major comorbidity can achieve bone healing even if half of the grafted material is β-TCP that does not have osteoinductive ability.

In the induced membrane technique, an external fixator is typically used in the first stage of the procedure in patients with diaphyseal defects [22]. In contrast, in the present study, cast fixation was applied in three cases with diaphyseal defects because the press-fit insertion of the PMMA spacer conferred adequate stability. Avoidance of an external fixator for diaphyseal defects appears to offer important advantages, such as protection against pin site infection; subsequently, definitive fixation using an intramedullary nail can be performed safely in the second stage of the procedure.

The present study has several limitations. This was a retrospective preliminary case series with a limited number of patients. The average follow-up period was 14 months, and at least two years of follow-up would be needed to clarify fully the usefulness of β-TCP as an extender. This is because of several reports of delayed stress fractures that have occurred as late as two years after reconstruction. However, an increased incidence of this complication is associated with use of external fixation for definitive stabilization as compared with fixation using nail or plate and screw constructs, as used in the present case series [4, 23]. Next, this study did not include a control group that underwent grafting of autologous bone from the iliac crest or RIA autografting for comparison with the present technique. Furthermore, the reconstructed defect was restricted to long bones of the lower extremity. In the future, it will be necessary to perform a randomized controlled trial involving a large sample that compares the current procedure with other methods accompanied by long-term follow-up examinations and accurate, objective post-operative evaluation, including radiological examination. Moreover, it would be important to identify the optimal ratio of autograft and β-TCP that would provide reliable bone healing with lowered volumes of harvested autograft as compared with conventional techniques. Although our preliminary data are limited, further evaluation in a large series may clarify the usefulness of β-TCP as a bone substitute in the induced membrane technique.

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