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● *Original Contribution*

LOW-INTENSITY PULSED ULTRASOUND THERAPY STIMULATES CALLUS FORMATION BETWEEN HOST FEMUR AND CORTICAL ONLAY STRUT ALLOGRAFT

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Abstract—Cortical onlay strut allografting is a promising surgical option to reconstruct and reinforce the deficient femur in a hip arthroplasty. However, the union of the allograft to the host bone takes a long time. To accelerate the process of cortical onlay strut allograft healing, we studied the effects of low-intensity pulsed ultrasound (LIPUS) on callus formation. From 2 wk after the operation, LIPUS was given for 20 min/d at each end of the strut allograft. The LIPUS treatment group was assigned 14 allograft transplantations, while 21 control patients were treated without LIPUS. The LIPUS treatment group formed calluses and had complete bridging between the host femur and the allograft faster after operation (16.9 and 29.4 wk after operation, respectively) compared with the control group (40.7 and 82.0 wk after operation, respectively). Our findings showed that LIPUS stimulated bone bonding between the host femur and the cortical onlay strut allografts. (E-mail: hakiyama@kuhp.kyoto-u.ac.jp) © **2014 World Federation for Ultrasound in Medicine & Biology.**

Key Words: **Hip arthroplasty, Femur, Bone, Ultrasound stimulation, Strut bone allograft.**

INTRODUCTION

The treatment of patients with bone loss, fracture or nonunion of the femoral shaft after total hip arthroplasty (THA) remains challenging. Several surgical options, including megaprosthesis (Parvizi and Sim 2004), bone substitutes (De Long et al. 2007), impaction bone grafting (Gie et al. 1993) and onlay biological plate (Emerson et al. 1992) have been developed to reconstruct and reinforce the femoral shaft. Onlay biological plates have the biomechanical advantage of giving immediate structural support at the bone loss or fracture site. Onlay autografting using fibula bone augmented with autogenous cancellous bone grafts is considered a gold standard procedure to enhance new bone formation, restore bone stock and increase cortical strength by extracortical bridging. However, the size and amount of autogenous bones and the morbidity of the donor site limit the wider application of this technique. Therefore, the use of a strut allograft is an attractive alternative to an onlay bone autograft (Emerson et al. 1992).

Previous studies showed that cortical onlay strut allografting used in conjunction with hip arthroplasty achieved good clinical and radiologic results (Barden et al. 2001; Haddad et al. 2002). Cortical onlay strut allografts unite to the host bone through callus formation, which is expected to recapitulate a process of fracture healing (Emerson et al. 1992). However, the bone incorporation of allografts progresses slowly and it takes longer to complete the union to the host bone compared with fracture healing (Emerson et al. 1992; Enneking et al. 2001). In addition, cortical allografts are strong initially, but the repair process weakens them, leading to fatigue fractures (Enneking and Campanacci 1975). These findings result in the extension of the time of protected weight bearing for the patients, thus limiting daily activity. Therefore, improving allograft union and incorporation is important for achieving a successful reconstruction and a good clinical outcome.

Low-intensity pulsed ultrasound (LIPUS) is an exogenous biophysical stimulus to accelerate a process

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of fresh fracture healing through stimulation of osteogenesis, chondrogenesis, and angiogenesis. Duarte (1983) and Dyson (1983) were the first to apply LIPUS clinically for stimulation of bone formation. Pilla et al. (1990) showed that application of LIPUS for 20 min/d accelerated the recovery of torsional strength and stiffness significantly in rabbit fracture models. In a multi-center randomized double-blind placebo-controlled clinical trial, Heckman et al. (1994) demonstrated a significant reduction in the time of clinical and radiographic healings of tibia fractures.

Based on these findings, The Food and Drug Administration in the United States approved the use of LIPUS for the accelerated healing of fresh fractures in 1994 and for the treatment of established non-unions in 2000. Currently, a study has shown that daily LIPUS stimulation of the host-allograft junctions resulted in a 30% increase in reconstruction stiffness, paralleled by significant increases in callus maturity and periosteal bridging across the host-allograft interfaces in sheep (Santoni et al. 2008). However, there has been no evidence for the effectiveness of LIPUS on the healing of the cortical onlay strut allograft in humans. Therefore, we analyzed the potential of daily LIPUS stimulation of the host-allograft bone junctions in conjunction with hip arthroplasty.

MATERIALS AND METHODS

Between November 2000 and May 2011, we reviewed 35 patients (35 hips) retrospectively that had undergone primary or revision THA with cortical onlay strut allografts for femoral reconstruction (Table 1). The patients were assigned to the LIPUS treatment group ($n = 14$) or to the control group ($n = 21$). The LIPUS treatment group consisted of 2 men and 12 women, with a mean age at the time of operation of 63 y (range: 23–79 y), a height of 150.5 ± 7.0 cm and a weight of 49.8 ± 9.2 kg. The control group consisted of 4 men and 17 women, with a mean age at the time of operation of 65.8 y (range: 45–84 y), a height of 151.7 ± 9.0 cm and a weight of 52.5 ± 11 kg.

The operations were performed with an anterolateral or a posterior approach. The acetabular component and the femoral component were replaced if necessary. Cortical onlay strut allografts produced from the tibia or the femur were processed as described previously (Hachiya et al. 1999) and were preserved aseptically in liquid nitrogen. Excessive debridement of the soft tissue was avoided to preserve periosteal circulation. The endosteal surface of the allograft strut was contoured to match the outer diameter of the host femur, and the interfaces were augmented with 'mashed' cancellous bone allograft generated from the resected femoral head by an acetabular reamer (Fig. 1a, b, and c). One to three struts were fixed to the host femur by metallic cables or cables with metallic plates and metallic mesh (Fig. 1d).

Patients in the LIPUS treatment group were provided with the Sonic Accelerated Fracture Healing System (Smith & Nephew, Memphis, TN, USA; Teijin Pharma., Tokyo, Japan) (Fig. 2). The ultrasound signal had a 1.5 MHz frequency; 1 kHz repetition rate; 200 μs pulse duration; and 30 mW/cm^2 spatial-average–temporal-average intensity (Azuma et al. 2001). The application of the LIPUS started 2 wk after the operation when the operative wound was cured. After both ends of the strut allograft were marked on the skin under an image intensifier, the ultrasound probe was applied with coupling gel on each end of the allograft from the surface of the femur. Treatment was given for 20 min/d at each site until complete bridging had been accomplished between the host femur and the allograft. The patients continued the LIPUS application after discharge from the hospital.

Standard radiographs of the anteroposterior and lateral views were taken after surgery at 2, 4, 6 and 8 wk, at 3, 6, 9 and 12 mo, and at 3 or 6 monthly intervals thereafter. Three surgeons (H.A., T.I. and H.O.) assessed the radiographs independently, and they noted the time of the appearance of the first bridging callus and of complete bridging. The presence of radiolucent lines at the cement-bone or cement-stem interface was recorded, as well as the presence of any femoral osteolysis, cortical hypertrophy, cement fractures or proximal femoral resorption (Engh et al. 1987). Loosening of the stem was defined according to the criteria of Harris and McGann (1986): Stem subsidence equal to or greater than 3 mm; cement fracture; a complete radiolucent line equal to or greater than 2 mm; or a radiolucent line in zone I equal to or greater than 2 mm. Hip function was evaluated using the Japan Orthopaedic Association hip scores.

This study was conducted with the approval of the Research and Ethics Committee of Kyoto University, Kyoto, Japan, and informed consent was obtained from all participants as per the WORLD Medical Association Declaration of Helsinki: Ethical Principles for Medical Research Involving Human Patients, 2008.

Statistical analysis

Statistical comparisons were performed using a t test (Aspin–Welch) with significance at $p < 0.05$.

RESULTS

The mean operative time and intra-operative blood loss were 239 min (range: 173–366 min) and 1226 g (range: 100–2092 g) in the LIPUS treatment group, and 327 min

Table 1. Summary of the patients

LIPUS (case)	Sex	Age (y)	Side	Diagnosis	Durations (wk)	Approach	Stem	Allograft (struts)	Wire	Plate/mesh
1	F	66	L	Stem loosening	32	PL	Exeter	2	DM	
2	F	57	R	High hip dislocation	24	PL	Biometric	2	DM	
3	M	38	R	Osteoarthritis	23	PL	Biometric	2	DM	
4	F	72	L	Stem loosening	23	PL	Exeter	2	DM	
5	F	59	R	Ppf	32	PL	Exeter 205	1	DM	
6	F	79	R	Infection	12	PL	Exeter 205	1	DM	
7	F	72	R	Stem loosening	28	PL	Exeter	1	DM	
8	F	58	L	Psuedoarthrosis	54	AL	KM type 7	2	Stainless	
9	M	23	L	Stem loosening	22	AL	KM type 7 long	1	Orthron	
10	F	79	R	Ppf	26	AL		1	Stainless	AO locking
11	F	79	L	Ppf	29	AL	KM type 7 long	2	DM	
12	F	70	L	Infection	27	AL	KM type 6 lomg	1	DM	
13	F	53	L	Ppf	40	AL	Hackstep	2	DM	
14	F	77	R	Ppf	40	AL	KM H3 long	2	DM	
Control										
1	F	57	R	Stem loosening		PL	Exeter	2	DM	Mesh
2	F	72	L	Stem loosening		PL	Exeter	2	DM	Mesh
3	F	53	L	Infection		PL	Exeter	2	DM	Mesh
4	F	77	R	Stem loosening		PL	Exeter	2	DM	
5	M	51	R	Infection		PL	Exeter	2	DM	
6	M	62	L	Ppf		PL	Exeter	2	DM	Mesh
7	M	71	L	Ppf		PL	Exeter	2	DM	
8	F	61	L	Ppf		PL	Exeter	2	DM	Mesh
9	F	82	R	Stem loosening		PL	Exeter	2	DM	Mesh
10	M	71	R	Stem loosening		PL	Exeter	2	DM	
11	F	45	L	Infection		PL	Exeter	2	DM	
12	F	65	L	Infection		PL	Exeter	2	DM	
13	F	67	R	Stem loosening		PL	Exeter	2	DM	
14	F	73	L	Ppf		PL	Exeter	3	DM	
15	F	60	L	High hip dislocation		PL	Exeter	2	DM	
16	F	77	L	Ppf		PL	Exeter	3	DM	
17	F	69	R	Stem loosening		PL	Exeter	2	DM	
18	F	62	R	Stem loosening		AL	KM type 6 long	1	Titanium	
19	F	64	R	Stem loosening		PL	Synergy	1	DM	
20	F	58	R	Stem loosening		PL	Exeter	2	DM	
21	F	84	R	Ppf		PL	Hackstep	2	DM	

F = female; M = male; R = right; L = left; Ppf = periprosthetic fracture; AL = anterolateral; PL = posterolateral; KM = Kyocera Medical Co. Op; DM = Dall-Miles Cable System.

(range: 220–432 min) and 1240 g (range: 190–4030 g) in the control group. From the Japan Orthopaedic Association hip scores, the points of pain (range: 0–40), range of motion (range: 0–20), walking (range: 0–20), and activity of daily living (range: 0–20) were 38 ± 2.5, 13 ± 1.9, 12 ± 6.0 and 16 ± 3.9 in the LIPUS treatment group and 39 ± 1.7, 16 ± 3.5, 13 ± 7.8 and 12 ± 5.0 in the control group.

An intra-operative complication was a femoral fracture in one patient in the LIPUS treatment group and in three patients in the control group. The post-operative complications were dislocation in one hip or a femoral fracture in one hip in the LIPUS treatment group with dislocation in two hips and femoral fracture in one hip in the control group. No patient had an infection. No femoral component was radiologically loose or revised for any reason, including aseptic loosening or osteolysis, after a mean follow-up of 29 mo (range: 9–72) in the LIPUS treatment group and 75 mo (range: 39–140) and the control group.

During the follow-up period, new appositional bone was observed at the ends of the strut allograft between the host femur and the allografts. No cases of nonunion were noted, and none of the allograft reconstructions failed. The initial radiographic finding of partial bridging was observed at 17 ± 8.4 wk and 41 ± 16 wk in the LIPUS treatment group (Fig. 3) and the control group (Fig. 4), respectively ($p < 0.001$). Complete bridging was accomplished at 29 ± 10 wk and 82 ± 45 wk in the LIPUS treatment group and the control group, respectively ($p < 0.001$).

DISCUSSION

Cortical onlay strut allografts are advantageous for restoring and reinforcing at the site of bone stock loss by biomechanical support. However, cortical allografts have slow graft incorporation and a high rate of delayed union or nonunion (Hamadouche et al. 2002). Therefore,

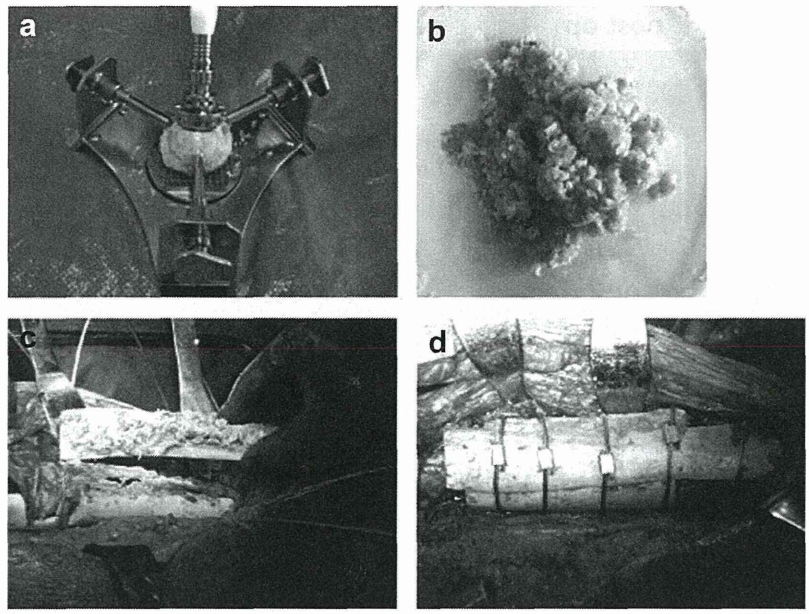

Fig. 1. Operative technique of cortical onlay strut allograft on the femur: (a) Production of 'mashed' cancellous bone allografts from resected femoral heads using acetabular reamer. (b) 'Mashed' cancellous bone allografts. (c) The interfaces between the cortical onlay strut allograft and the host femur are augmented with 'mashed' cancellous bone allograft. (d) Metallic cables fix the struts to the host femur.

in our study, we investigated whether LIPUS accelerates the union of the cortical onlay strut allografts on the host femur through extracortical bone bridging in conjunction with the femoral implant in THA.

In a previous study, Emerson et al. (1992) reported that the healing process of the cortical onlay strut allografts starts with edge round off, followed by partial and complete trabecular bridging. The average time of the onset of round off was 7 mo. The average time to partial and complete bridging between the cortical onlay strut allografts and the host femur was 8.3 mo and 12.5 mo, respectively. The rate of graft union was 96.6% (Emerson 2000). This evidence affects patient rehabilitation and extends the time of protected weight bearing, resulting in limitations in daily living and an increase in post-operative complications. Our study shows that the initial radiographic finding of partial bridging was observed at 17 wk in the LIPUS treatment group and at 41 wk in the control group. Complete bridging was accomplished at 29 wk in the LIPUS treatment group and at 82 wk in the control group. Thus, these results demonstrate that LIPUS stimulates bone healing of the cortical onlay strut allografts and accelerates the bone union between the host femur and the allograft.

Fig. 2. Sonic accelerated fracture healing system.

LIPUS has been used clinically to enhance bone repair in humans. Double-blind placebo-controlled clinical studies showed accelerated healing of Colles' and tibia diaphyseal fractures (Heckman et al. 1994; Kristiansen et al. 1997). Previous studies evaluated the possible mechanisms behind the faster healing process. Yang et al. (1996) reported that using a fracture model of rat femur chondrocytes or chondrocyte precursors might respond to LIPUS. LIPUS stimulates expression of the Aggrecan gene and the type II collagen gene and proteoglycan synthesis, resulting in enhancement of cartilage formation and maturation in the fracture callus. In addition, Rawool et al. (2003) showed increased vascularity around the fracture sites with the LIPUS treatment. Thus, LIPUS appears to increase periosteal callus size

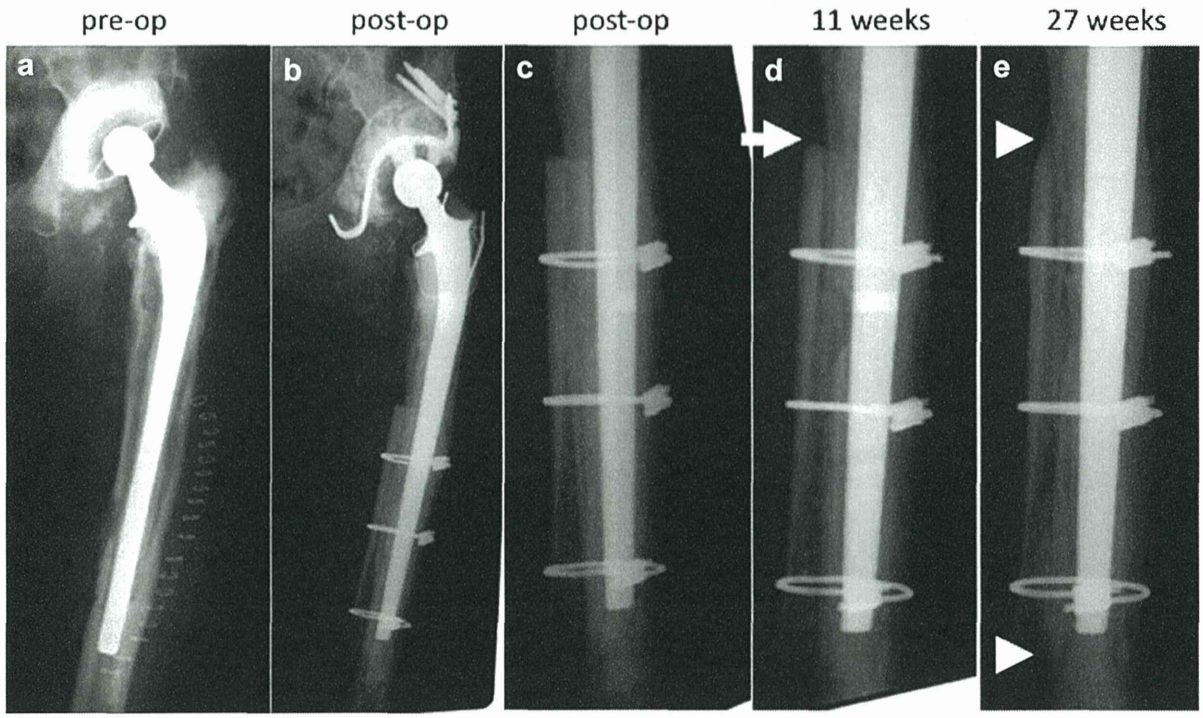

Fig. 3. Radiographs of a 70-y-old woman with an infection in her left hip in the LIPUS treatment group. (a) After application of an articulating cement spacer with antibiotics and before revision surgery. (b) At THA revision, using the Physio-Hip System type 6 long stem (Kyocera Medical Co., Osaka, Japan) with one cortical onlay strut allograft. (c) A high magnification image of the strut transplantation site at THA revision. (d) Eleven wk after operation, partial bridging callus (arrow) is obvious at the end of the strut. (e) Twenty-seven wk after operation, the bony callus has achieved complete bridging (arrowhead).

and to stimulate endochondral ossification by promoting maturation of a callus to a dense osseous.

As reported by Emerson et al. (1992) and Emerson (2000), cortical onlay strut allografts unite the host femur through a similar process to fracture repair in dogs. Eight wk after operation, the strut allografts were transformed substantially into a callus-like structure, with vascularized tissue and new bone formation at the interface, with recanalization of the strut allograft microscopically. At 12 wk, the strut allograft had been transformed completely into a callus-like structure. At 24 wk, the major portion of the strut allograft had remodeled to new cortical bone, although cancellous bone and dense mesenchymal tissue were still present.

In humans, at 20 mo, there is bridging between the host and the strut allograft with partial vascular invasion of the allograft and at 32 mo, the graft is re-vascularized completely and united to the host femur (Emerson 2000). Therefore, it could be possible that LIPUS stimulates the healing process of the strut allografts. Indeed, in our study, LIPUS induced a much shorter period of strut allograft union. Santoni et al. (2008) showed LIPUS therapy improved allograft incorporation in ewes. Daily LIPUS treatment of the proximal and distal host-allograft junctions increased torque to failure and torsional stiffness. Therefore, these findings suggest that the biologic effects of LIPUS on promoting strut allograft union are similar to the mechanisms of fracture healing.

Several procedures are reported for better union of the strut allografts. Multi-perforations of the cortical strut allograft bone have been advocated as a means to increase the interface between the living soft tissues of the host and the allograft and to achieve higher new bone formation (Delloye et al. 2002). However, perforation of the cortical strut allograft weakens the biomechanical properties and increases the risk of fracture.

Some researchers used autogenous cancellous grafting at host-allograft junctions, improving the allograft union rate (Barden et al. 2003; Gross et al. 2003). However, this did not accelerate the healing procedure. Wu et al. (2007) reported that the recombinant human bone morphogenetic protein (BMP)-2/gelatin device placed at the host-allograft junctions improves the quality, quantity and time required for new bone formation and graft healing. Unfortunately, BMP-2 is not available clinically in all countries, including Japan. In contrast, daily LIPUS administration is non-invasive, and furthermore, patients can lease a LIPUS device, as the Japanese

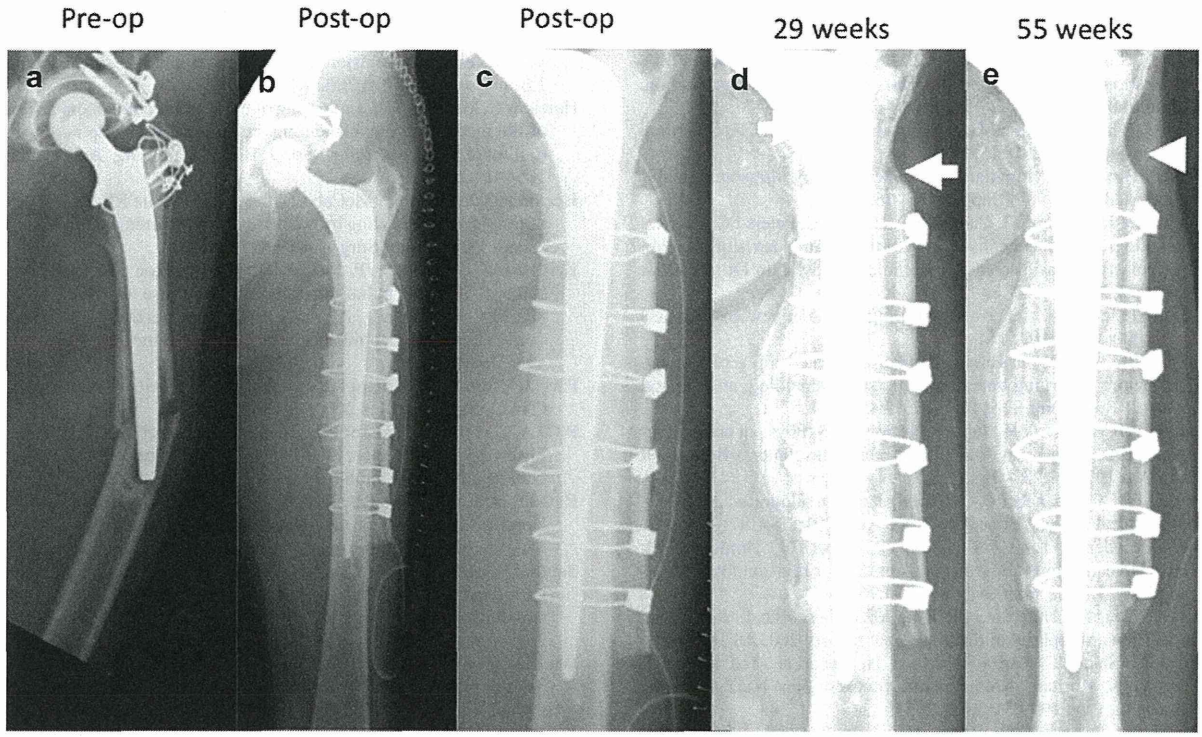

Fig. 4. Radiographs of a 73-y-old woman with periprosthetic femoral fracture in her left hip in the control group. (a) Before revision surgery. (b) At THA revision using the Exeter long stem (Stryker International, Mahwah, NJ, USA) with two cortical onlay strut allografts. (c) A high magnification image of the strut transplantation site at THA revision. (d) Twenty-nine wk after operation, partial bridging callus (arrow) is obvious at the end of the strut. (e) Fifty-five wk after operation, the bony callus has achieved complete bridging (arrowhead).

insurance system covers its cost. Moreover, Azuma et al. (2001) reported that even short-term treatment with LIPUS is effective at any stage of femoral fracture healing in rats. Therefore, LIPUS treatment is a simple and patient-friendly procedure to accelerate strut allograft union.

This study has several limitations. First, this was not a randomized controlled study. The surgeons and the patients determined the LIPUS usage. Second, further limitations are a small study cohort and that the pre-operative diagnosis for the revision THA was miscellaneous. The bony condition of the host femur may affect the healing process of the cortical onlay strut allograft. Third, in the present study, LIPUS was administered on a small area at the ends of the strut allografts. The treatment of LIPUS to larger areas in host-allograft junctions using multiple transducers is needed clinically to expand its stimulatory effects, leading to faster fixation of the cortical onlay strut allografts. In addition, in this study we used LIPUS of 30 mW/cm^2 only and did not analyze the effect of LIPUS of a higher acoustic intensity, *i.e.,* 100 mW/cm^2. Further studies may be needed to clarify these points. Finally, we implanted several geometrically different stem designs: A polished double tapered stem; a double tapered stem with a smooth-surfaced finish and a collar; a long stem or a regular stem, even though the stem survivorship was 100%. The difference in stem design may affect the post-operative stability of the reconstructed femur, especially in cases of periprosthetic femoral fractures.

In conclusion, this study demonstrated the potentiated therapeutic advantage of LIPUS in improving the bone union between the host bone and the cortical onlay strut allograft.

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Peripheral nerve regeneration through a silicone chamber implanted with negative carbon ions: Possibility to clinical application

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ABSTRACT

We investigated whether a tube with its inner surface implanted with negative-charged carbon ions (C^- ions) would enable axons to extend over a distance greater than 10 mm. The tube was found to support nerves regenerating across a 15-mm-long inter-stump gap. We also investigated whether a C^- ion-implanted tube pretreated with basic fibroblast growth factor (bFGF) promotes peripheral nerve regeneration. The C^- ion implanted tube accelerated nerve regeneration, and this effect was enhanced by bFGF. Silicone treated with C^- ions showed increased hydrophilic properties and cellular affinity, and axon regeneration was promoted with this increased biocompatibility.

1. Introduction

In peripheral nerve reconstruction, autologous nerve graft is gold standard for nerve injuries with nerve defects. However, if the patient has a large nerve deficit, it is impossible to reconstruct with nerve autograft since the source of donor nerve is limited. Artificial nerve graft would be a useful alternative to conventional nerve autografts, because of the donor site morbidity and nerve source limitations [1,2].

Nerve axons will regenerate within an interneural stump gap through a tube of artificial material: a process termed tubulation, and many researchers have studied this process in attempts to use it clinically as an alternative to autogenous nerve grafting [1–3]. In the rat sciatic nerve, the maximum distance across which axons can regenerate is about 10 mm, and no neural structures are formed with a greater inter-stump distance through an unmodified silicone rubber tube [4]. In tubulation, neural cells migrate from both proximal and distal nerve stumps when they are joined by a tube, and axons extend from the proximal nerve stump. Thus, a neural structure is formed between the nerve stumps and neural connection occurs through the tube.

We investigated whether a tube with its inner surface implanted with negative-charged carbon ions (C^- ions) would enable axons to extend over a distance greater than 10 mm. In addition, we also investigated whether a C^- ion-implanted tube pretreated with basic fibroblast growth factor (bFGF) promotes peripheral nerve regeneration.

2. Materials and methods

We prepared the silicone tube whose inner surface was implanted with C^- ions at an ion energy of 10 keV at a dose of 3×10^{15} ions/mm^2. Briefly, An 18-mm-long section was opened longitudinally and four 5-0-gauge nylon sutures were placed along either edge of the opened tube. The tube was fixed on a pedestal using the sutures so that its inner surface was exposed. An inner area was implanted with C^- at an ion energy of 10 keV at a dose of 3×10^{15} ions/mm^2. The sutures fixing the tubing to the pedestal were then removed. The tubing was allowed to return to its original shape, and the slit was sealed with a small amount of liquid silicone rubber. Male Sprague–Dawley rats (10–12 weeks old, weighing 200–240 g) were used. All experiments were performed in

Abbreviations: C^- ions, negative-charged carbon ions; bFGF, basic fibroblast growth factor.

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accordance with the guidelines of the Animal Research Committee, Graduate School of Medicine, Kyoto University. The rats were anesthetized by an intraperitoneal injection of sodium pentobarbital (Nembutal: 40 mg/kg body weight) and ketamine chloride (Ketalar: 40 mg/kg body weight).

2.1. Experiment 1

The right sciatic nerve was subjected to surgery, for which the rats were divided into two equal groups: a control and a C^- group. The right sciatic nerve was exposed and a 10-mm-long nerve segment was removed in the middle thigh. In the control group the epineuria of the proximal and distal nerve stumps were sutured to either end of an 18-mm-long segment of untreated silicone rubber tube using 10-0 monofilament nylon sutures, leaving a 15-mm-long inter-stump gap. In the C^- group the rats were treated in the same way as the control group, except that an 18-mm-long C^- ion-impregnated silicone rubber tube was used. Twelve and 24 weeks after tubulation, electrophysiological studies were performed. The experimentally treated right sciatic nerve was exposed and stimulated just distally to the piriformis muscle and at the popliteal fossa using a bipolar silver electrode. Two pairs of needle electrodes were inserted into the pedal adductor muscle to check for the presence of an evoked action potential. After the electrophysiological study, the right sciatic nerve was transected just distal to the piriformis muscle at the popliteal fossa. The transected nerve segments were fixed in 2.5% glutaraldehyde, postfixed with 2% osmic acid, and embedded in epoxy resin. Transverse sections (1–2 mm thick) were taken from the most proximal, the middle, and the most distal region of a nerve that had regenerated within the tube. Each section was stained with 0.5% (w/v) Toluidine blue solution and examined by light microscopy.

2.2. Experiment 2

We prepared the C^- ion-implanted silicone tube described in Experiment 1. The C^- ion-implanted tubes were soaked in a solution of 10 μg/mL bFGF for 2 h and then used for the adsorption and tubulation studies. The adsorption study was performed using ^{125}I-bFGF to measure the adsorption of bFGF to the tubes. The normal silicone tubes and C^- ion-implanted tubes were immersed in the ^{125}I-bFGF solution and rinsed with pure water to remove excess nonadherent ^{125}I-bFGF. Growth factors were radiolabeled with Na^{125}I using the conventional chloramines-T method. The normal silicone tubes and C^- implanted tubes were soaked in a 10 μg/mL ^{125}I-bFGF solution and rinsed with pure water, and the radioactivity on the tubes was counted using a gamma counter. Male Sprague–Dawley rats, 10–12 weeks old and weighing 200–240 g, were randomly divided into three equal groups: an FGF group, a C^- group, and a C^--FGF group. In the FGF group, the epineuria of the proximal and distal nerve stumps were sutured using 10-0 monofilament nylon sutures to either end of an 18-mm-long segment of the normal silicone rubber tube that had been pretreated by immersion in bFGF solution, leaving a 15-mm-long inter-stump gap same as Experiment 1. The rats in the other groups were treated in the same way except that, in the C^- group, an 18 mm-long silicone rubber tube that had been implanted with C^- ions was applied without pretreating with bFGF, and in the C^--FGF group, an 18-mm-long C^--ion-implanted silicone rubber tube that had been immersed in bFGF solution before implantation was used. At 12 and 24 weeks after the surgery, electrophysiological and histological studies were performed in the same method described in Experiment 1.

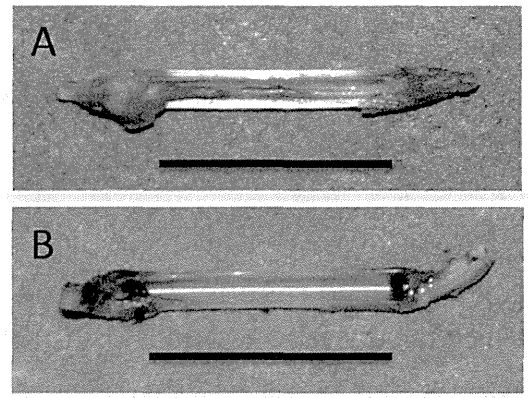

Fig. 1. Macroscopic appearance of the nerves that regenerated inside the silicone tube 24 weeks after tubulation. (A) Regenerated neural structures were found in the C^- treated tube group. (B) No neural tissue was observed in the control group. Bar = 15 mm.

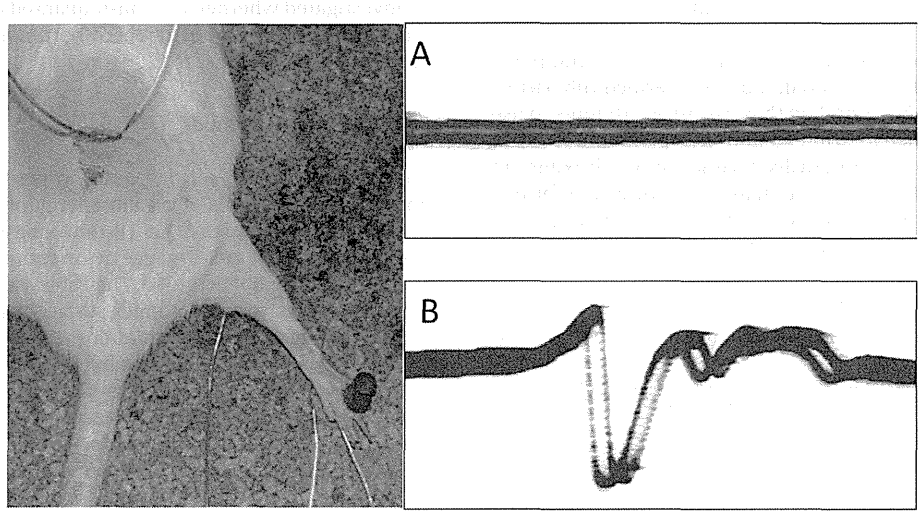

Fig. 2. Electrophysiological study in the C^- treated tube group at 12 and 24 weeks after tubulation. (A) No evoked action potentials are apparent in the pedal adductor muscles at 12 weeks. (B) All rats in the C^- treated tube group evoked action potentials at 24 weeks.

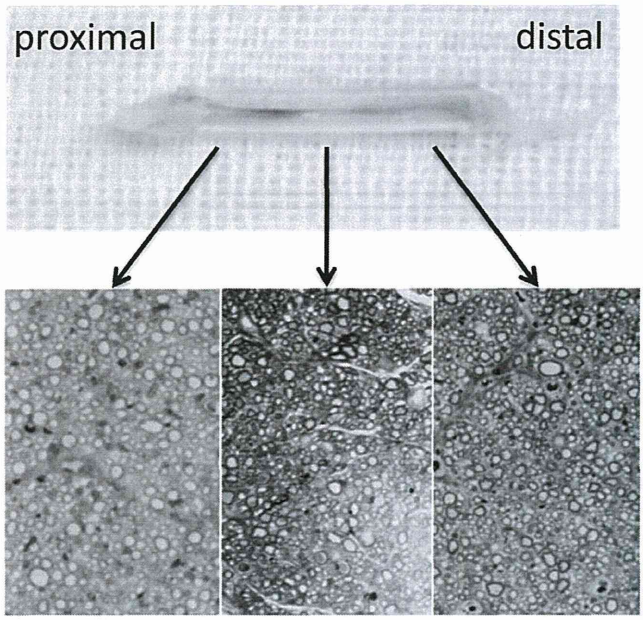

Fig. 3. Histology of the regenerated nerves in the C^- treated tube group at 12 weeks after tubulation. Regenerated axons were found in proximal, middle and distal part of the neural structures in the C^- group.

3. Results

3.1. Experiment 1

All rats in the C^- tube developed neural tissue within the tubes (Fig. 1A). However, in the control group none of the rats had developed neural tissue at either time point (Fig. 1B). In the control group, no rats showed evoked action potentials in the pedal adductor muscle at 12 or 24 weeks. However, in the C^- tube group, all rats evoked action potentials in the pedal adductor muscles at 24 weeks, although none were apparent at 12 weeks (Fig. 2). Myelinated axons were observed in the proximal, middle, and distal sections of the regenerated nerves in all rats in the C^- tube group at 12 and 24 weeks (Fig. 3).

3.2. Experiment 2

To measure the affinity properties of bFGF on the surface, the normal silicone and C^--ion implanted silicone plates were immersed in the ^{125}I-bFGF solution, and the radioactivity was measured by a gamma counter to detect ^{125}I-bFGF attached to the surface of the tubes. The mean amount of attached bFGF was 0.748 ± 0.071 μg in normal plates and 1.649 ± 0.113 μg in C^--ion-implanted tubes (Fig. 4). All rats in the C^- and C^--FGF groups developed neural tissue within the tubes (Fig. 5). However, none of the rats in the FGF group developed neural tissue or showed evoked action potentials in the pedal adductor muscle at 12 or 24 weeks. None of the rats in the C^- group exhibited evoked action potentials in the pedal adductor muscles at 12 weeks, but all exhibited evoked action potentials at 24 weeks. In contrast, all rats in the C^--FGF group exhibited evoked action potentials in the pedal adductor muscles at 12 weeks. Myelinated axons were observed in the proximal, middle, and distal sections of the regenerated nerves in all rats in the C^- and C^--FGF groups at 12 and 24 weeks (Fig. 6). In each section, the vascular network was more developed in the C^--FGF group than in the C^- group.

4. Discussion

Tubulation in the rat sciatic nerve is a well-established experimental model for studying mechanisms regulating axonal regeneration [4]. Rat sciatic nerve axons will regenerate over a 10-mm gap through an unmodified silicone rubber tube [4]. However, as the inter-stump distance increases, the thickness of the neural structure formed in a tube becomes thinner. No neural structure is formed in an unmodified silicone rubber tube with a 15-mm inter-stump gap [4]. In Experiment 1, nerves successfully regenerated over a 15-mm gap through tubes implanted with C^- ions. Myelinated axons were observed at all sections of the regenerated nerve at 12 weeks, and electrical stimulation of the nerve proximal to the tube evoked action potentials in the pedal adductor muscle by 24 weeks. Thus, nerves that regenerated through the tube by 12 weeks reached the pedal adductor muscle by 24 weeks. The silicone rubber surface showed increased hydrophilic properties after the implantation of negative carbon ions [5,6]. Such a treated surface can also bind fibronectins and laminins, resulting in increased cellular affinity and biocompatibility [5]. It is known that the

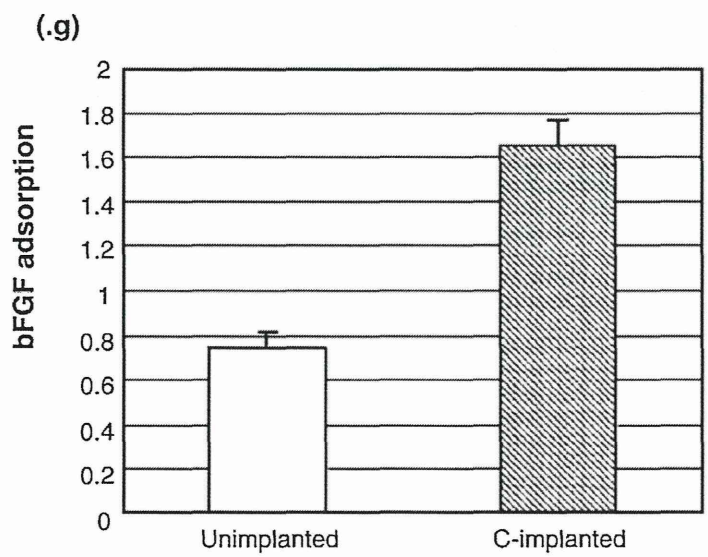

Fig. 4. Affinity properties of bFGF on the silicone rubber surface. More bFGF was detected in the C^- ion implanted silicone rubber tubes than normal silicone tubes.