Bilateral Hand Transplantation: Bone Healing Under Immunosuppression With Tacrolimus, Mycophenolate Mofetil, and Prednisolone

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Purpose: Little is known about bone healing after composite tissue transplantation that requires pharmacologic immunosuppression. Bone integration and callus development were assessed in bilateral hand transplantation.

Methods: In this study the course of callus development and callus maturation were assessed by color Doppler sonography and radiography in a double hand transplant and compared with forearm replantation.

Results: After hand transplantation, ingrowth of small vessels at the bone junction was observed at week 3, calcified callus became visible at month 4, and bone union was completed at month 11. A similar time course of bone integration was observed after replantation. Plating offered sufficient stability. A recipient periostal flap is thought to have improved blood supply and favored development and induction of callus.

Conclusions: Bone healing after hand transplantation under immunosuppression with tacrolimus, mycophenolate mofetil, and prednisolone is identical to that after forearm replantation. (J Hand Surg 2004;29A:1020–1027. Copyright © 2004 by the American Society for Surgery of the Hand.)

Key words: Bone healing, composite tissue transplantation, hand transplantation, immunosuppression.

The first human hand transplantation was performed in Ecuador in 1964. Immunosuppression with azathioprine and steroids, however, did not prevent irreversible rejection and thus loss of the graft. In a preclinical animal model cyclosporine was shown to prevent rejection of a limb transplant except for the skin. Nerves,[1] tendons,[2] muscles, bones, and joints[3] were transplanted successfully under the coverage of cyclosporin A or tacrolimus together with azathioprine and steroids. A regimen consisting of tacrolimus, mycophenolate mofetil, and prednisolone was then reported to prevent rejection of composite tissue allografts including the skin in a preclinical model.[4]

Since the first successful hand transplantation in 1998 a total of 5 double and 12 single hand transplantations and 1 double forearm transplantation have been performed worldwide.[5] Of these, 2 transplanted hands had to be removed for immunologic

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reasons. One of them was in the first recipient, who was not compliant with medication at all times.[6] Because various hand/forearm tissue components are believed to be differently antigenic, each component must be monitored separately.[6,7] Bone is known to have high antigenicity-inducing immunologic rejection when transplanted as an allograft. To reduce its antigenicity bone allografts have been successfully irradiated, frozen, freeze-dried, boiled, or treated with thimerosal (Merthiolate).[8] Unfortunately, all of these bone grafts had no viable structure left that contributed actively to the process of bone healing and were therefore associated with a high incidence of nonunion and fatigue fractures, and occasionally even with resorption.[9–11] Vascularized bone allografts under immunosuppression, on the other hand, showed superior biological and biomechanical behavior with higher rates of bone integration.[7,12–14]

In this study we report on our experience with a double hand transplantation with special emphasis on the time course of callus formation and bone healing compared with hand replantation.

Materials and Methods

Hand Transplantation

The hands of a 47-year-old policeman were traumatized severely by the explosion of a bomb that the man was trying to defuse. Both hands had to be amputated at the radiocarpal joint. Soft tissue coverage of the stumps was poor. Tendons and muscles of both forearms were retracted. Electrical stimulation was used to maintain active muscles for training of a possible myoelectric prosthesis. A double hand transplantation was performed on March 7, 2000.[15] The staged surgical procedure was performed according to the principles of macroreplantation including osteosynthesis, revascularization, musculotendinous and nerve reconstruction, and skin coverage. For bone reconstruction a proximally based flap of the interosseous membrane together with the periosteum was created at both recipient forearms proximal to the osteotomy site, which was located at the distal third of the forearm. Donor forearm bones, which had a diameter 2 mm greater than the recipient bones, were stabilized to the recipient bones with compression using 7- and 8-hole low-contact dynamic compression plates and 3.5-mm screws. No additional autologous bone grafts were used. The periosteal flap was positioned to cover the osteotomy sites before vascular reconstruction of the transplanted limb was completed. Total cold ischemia time was 150 minutes for the right hand and 170 minutes for the left hand. Forearms were splinted for 4 weeks to protect tendon healing.

Induction therapy with antithymocyte globulin (Fresenius Medical Care, Bad Homburg, Germany) at a dosage of 2.5 mg/kg for 4 days was started during surgery and continued until day 3. Before revascularization, 500 mg of methylprednisolone was given intravenously. An additional 250 mg of methylprednisolone was given on day 1 and 125 mg on day 2. Steroids were then switched to oral prednisolone and tapered rapidly to 25 mg on day 8. Prednisolone was further reduced to 7.5 mg at 1 year. Tacrolimus (Fujisawa, Munich, Germany) was begun at a dose of 0.20mg/kg body weight in 2 oral doses and then adjusted to maintain levels of 15 ng/mL during the first month after surgery, 12 ng/mL between 2 and 6 months, and 10 ng/mL thereafter. In addition the patients were given 1 g of mycophenolate mofetil twice a day (Roche, Basel, Switzerland). No other agents influencing bone healing were administered. After rejection was shown by skin biopsy at week 8 it was treated successfully with 750 mg and 2 doses of 500 mg of methylprednisolone and topical treatment with tacrolimus and methylprednisolone.

Hand Replantation

A 56-year-old man cut off his left distal forearm with a motorsaw. His hand was replanted at the level of the distal third of the forearm after a total cold ischemia time of 4.5 hours on December 1, 2000. For bone reconstruction 7- and 8-hole low-contact dynamic compression plates with 3.5-mm screws were used and a proximally based periosteal flap was designed equivalent to the surgical technique used in the transplanted patient. No bone grafts were used. At the 1-year follow-up examination, supination measured 60°, pronation 70°, wrist extension 35°, and wrist flexion 20°. Pulp pinch was possible and palm–pulp distance was 1 cm. Grip was one third that of the noninvolved hand. There was a low level of protective sensibility and no evidence of reinnervation of the intrinsic muscles. This patient served as a control. No nonsteroidal anti-inflammatory medication or other agents influencing bone healing were administered.

Bone Healing

The biology of fracture repair can be described as a regenerative process staged in inflammation, soft callus formation, hard callus formation, and remodeling. In the first stages blood vessels arise from surrounding extraskeletal tissues. The soft callus consisting of car-

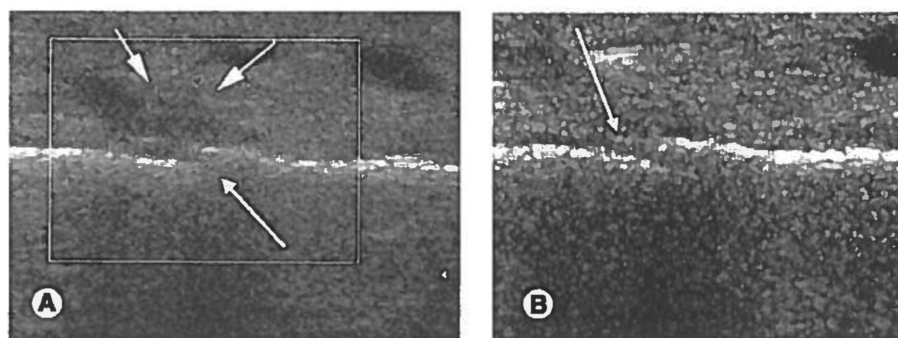

Figure 1. (A) Transplant patient, radius. Color Doppler ultrasound performed at week 3 shows vessels near the osteotomy site. Arrows: vessels, diameter approximately 1 mm. (B) Ultrasound B mode performed at week 3: longitudinal scan of the radius shows the osteotomy site (arrow).

tilage formation is marked by an increase of vascularity at the fracture site. During the continuing process of repair it is converted to calcified hard callus.[16] Because no bone biopsies were performed development of vascular neogenesis and of soft callus formation were assessed additionally by ultrasound. Development of calcified hard callus formation was assessed by radiographs. Onset and course of early blood vessel ingrowth and development of soft tissue callus were investigated at weekly intervals using color Doppler ultrasound and grayscale sonography at every osteotomy site until week 8.[17–19]

Grayscale sonography and color Doppler sonography examinations were performed with ultrasound equipment (HDI 5000, Advanced Technology Laboratories, Bothell, WA) using a broadband linear array working at 5 to 12 MHz. Color Doppler settings were adjusted for investigation of low-flow vessels. Pulse repetition frequency was set at 800 Hz, the wallfilter was set high, and color gain was at 70%. All sonographic examinations were performed by the same radiologist experienced in sonography. Development of late ossified callus formation was assessed with conventional radiographs on posteroanterior, lateral, and oblique projection at monthly intervals for 1 year.

Hardware position allowed continuous investigation of the ulnar and radial cortexes of the radius and the radial and palmar cortexes of the ulna. Radiographically uniform bone structure at the former osteotomy site as seen in all projections was defined as homogeneous union of healed bone. Radiolucency at the osteotomy site without calcification was distinguished from a calcified filling defined as hard callus. Stability of the forearm bones was evaluated by radiologic signs of hardware loosening. The type of repair was classified on radiographs according to Burckardt[20] as follows: type I, bone healing identical to autografts with remodeling and incorporation of the graft and no fatigue failure; type II, chronic repair with delayed union or nonunion, peripheral resorption with loss of graft size, internal resorption, and decrease in mechanical strength; and type III, no healing and complete resorption of the graft.

Results

Bone Healing After Allotransplantation

Vascular invasion and early callus formation were detected by color Doppler sonography at week 3 in all 4 forearm bones of the limb transplant. The vessels were approaching the osteotomy sites from the median side and showed a high flow as compared with those in the replant patient (Fig. 1). At week 7 vascular signals had decreased and soft tissue callus formation was identified clearly by grayscale ultrasound at all 4 osteotomy sites (Fig. 2). On radiographs hard callus of the forearm bone on the right side and the left ulna appeared at month 4 and could be seen on the ulnar cortex of the left radius 2 months later (Fig. 3). Onset of osseous union was observed between month 7 and month 11. Solid union of the radial aspect of the right radius could be seen at month 7 followed by the ulnar side cortex of the ulna at month 7 and the radial side cortex of the ulna at month 9. On the left arm both cortexes of the ulna were united solidly at month 7 and both cortexes of the radius at month 11 after transplantation. At 1 year homogeneous osseous union of the 4 forearm bones was terminated (Fig. 4). All grafted bones were incorporated fully without any signs of chronic healing. No peripheral resorption was seen at either osteotomy site but some callus formation was visible. No decrease in graft size or internal resorption was observed because there was no loosening of the hardware devices in the transplanted bone (type I).

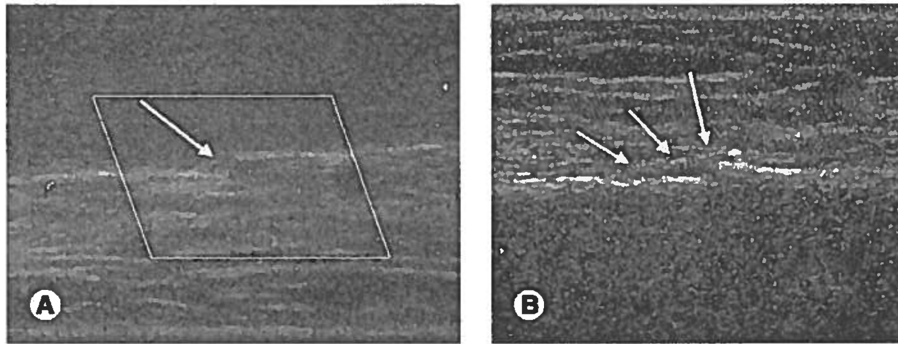

Figure 2. A) Transplant patient, radius at week 6. Color Doppler ultrasound shows no more vascularity close to the osteotomy site. (B) Ultrasound B mode performed at week 6. Longitudinal scan of the radius shows more delineated soft callus formation (arrows). (C) Conventional radiographs in anteroposterior and lateral projection at week 6 show no calcifications at the osteotomy site.

Bone Healing After Replantation

Vascular ingrowth became visible on color Doppler sonography as early as 3 weeks after replantation (Fig. 5). Soft callus was delineated on ultrasound at week 6 (Fig. 6). Hard callus appeared simultaneously at both forearm bones at month 6 (Fig. 7). First onset of osseous union was observed at the ulnar cortex of the radius at month 8 followed by the radial cortex at month 10 (Fig. 8). Healing of both cortexes of the ulna was completed at month 8 (Fig. 9).

Discussion

As of July 2003 a total of 12 single and 5 bilateral hand transplantations and 1 double forearm transplantation have been reported.[5] Still, little is known about the healing of the bones of these composite tissue grafts. In the setting of an allograft the biology of bone healing can be influenced by drugs used for immunosuppression but also by immunologic reactions such as acute and chronic rejection. Therefore, bone healing may proceed normally, be delayed, or end in resorption[3,20] and can be assessed by radiography, Doppler ultrasound, or 3-phase bone scans.[17–19,21,22]

According to the available literature various strategies were applied to optimize bone union. They were based first on maximal stability at the osteotomy site using various types and sizes of hardware and on measures that are believed to favor bone healing.

In their first patient the Lyon group[23] used 7-hole plates and 4.5-mm screws for fixation of both forearm bones. Autologous cancellous bone chips from the iliac crest were placed around osteotomy sites to support bone healing. Radiographs at 3 months confirmed solid callus formation and disappearance of the cleft between donor and recipient bones at month 6 but also showed some porosity. The patient had 1 mild cutaneous rejection 8 weeks after transplantation, which was treated successfully with an increase in prednisone dosage from 20 mg/day to 40 mg/day and topical application of immunosuppressive creams (tacrolimus). Bones of their second patient, who had double hand transplantation, were fixed in the same way as in the first patient.[24] Despite 2 rejection episodes bone healing was reported to be normal.[25] The forearm bones of the first Louisville patient were stabilized with 3.5-mm metal plates.[26] Two rejection episodes 6 and 20 weeks after surgery

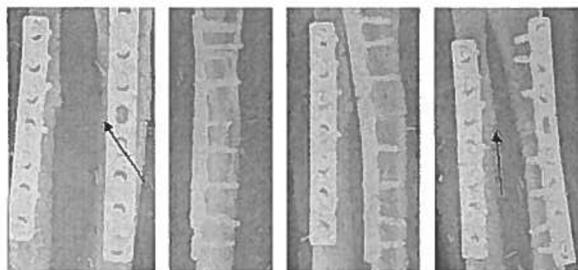

Figure 3. Transplant patient. Calcified bone formation at the osteotomy site of the transplanted forearm 4 months after surgery. Arrow: osteotomy site.

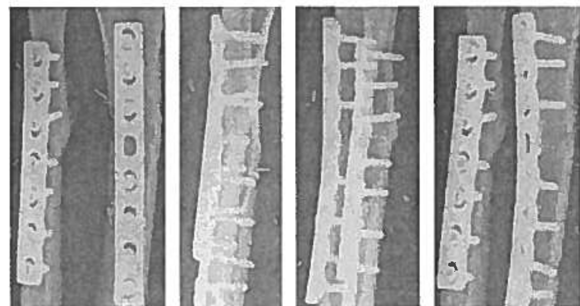

Figure 4. Transplant patient. Homogenous union of the forearm bones in the transplanted arm at month 11.

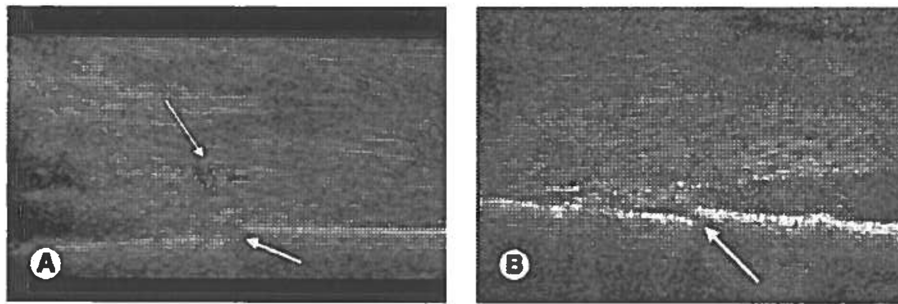

Figure 5. A) Replant patient. Color Doppler ultrasound performed at week 3 with vessels close osteotomy site. Short arrow, osteotomy site; arrow, vessels. (B) Replant patient. Ultrasound B mode at week 3. Arrows, osteotomy site, soft callus.

apparently did not have any impact on bone healing in this patient.

In our patient we used 7- and 8-hole plates and 3.5-mm screws for bone stabilization. To improve bone healing a vascularized periosteal flap was created and all osteotomy sites were covered with it.[27]

The group from Guangshen[28] removed mechanically the bone marrow from both forearm bones of both donor hands and 1 of the 2 hands also was irradiated to reduce the risk of graft-versus-host reaction, which has indeed never been observed after composite tissue transplantation. Immunosuppression was tacrolimus-based in all instances and included steroids and MMF. Despite their known effect on bone metabolism, glucocorticoids seem not to delay bone healing compared with the time course in replantation.

The amputated hand of the first Lyon patient showed histologic signs of rejection of the skin but not of other

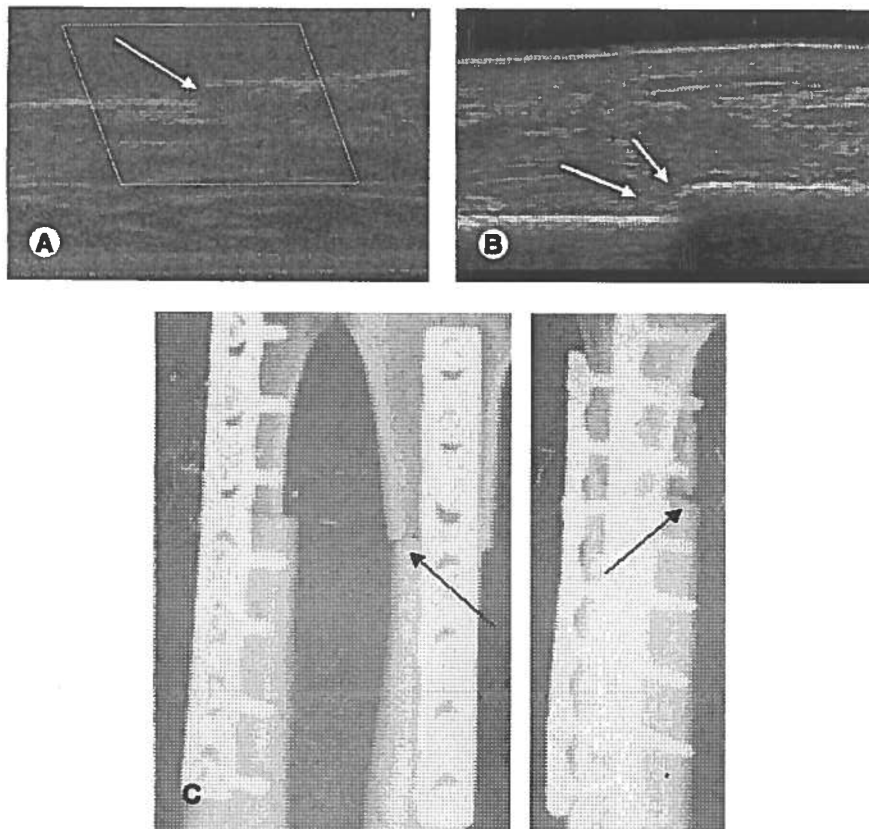

Figure 6. (A) Replant patient. Color Doppler ultrasound performed at week 6: no vessels. (B) Replant patient ultrasound B-mode: soft callus (arrows) was delineated at week 6. (C) Radiographs in anteroposterior and lateral projection show no calcifications at the osteotomy site (arrow).

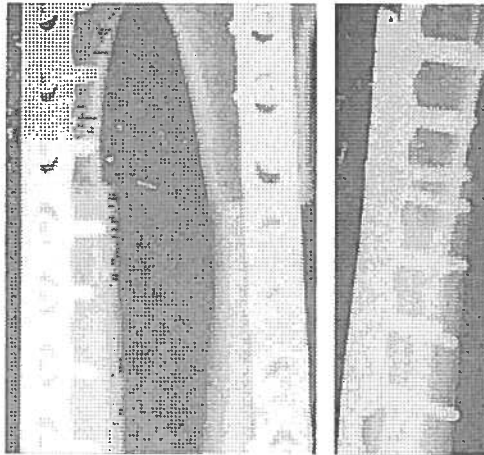

Figure 7. Replant patient. Calcified callus formation at month 6.

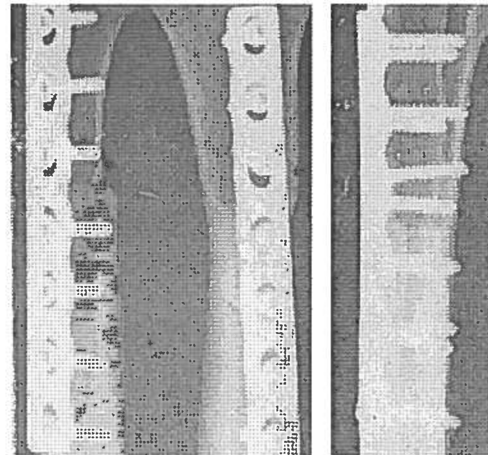

Figure 9. Replant patient. Bone union at month 10.

components of the graft.[29] Because no bone biopsies have been performed in any of the patients knowledge about the histomorphology of bone rejection is derived from animal experiments. There is much evidence that bone is immunogenic. The marrow contained in bone, endosteal, and periosteal cell surface antigens and in the bone matrix may be responsible for immunogenicity.[20]

In theoretical studies with vascularized bone marrow transplantation, stromal and marrow cells act early after transplantation, circulate to the lymphopoietic system of the recipient, and are reported to generate tolerance in long-term survival.[29] Bone marrow seems to be not so important in allograft rejection, but it plays an important role in allograft survival, which seems to depend on the persistence of donor marrow progenitor cells in the bone marrow compartment.[30,31]

Cell-mediated immunity plays a minor role in the rejection of composite tissue allografts and of bone alone as compared with antibody-mediated response. There is some evidence that cytotoxic antibodies directed against bone allografts do indeed appear and may coincide with cellular immunity although they seem not to be involved directly in the rejection process. In contrast to avascular allografts primary vascularization of limb tissue allograft is reported to change the pattern of rejection into considerable humeral response early after transplantation. The various components interact with the host immune system in a complex pattern eliciting less immune response than an individual tissue allograft.[7] No chimerism has been reported in any of the hand transplants.

Because the skin seems to be one of the most immunogenic parts of a hand allograft, in clinical practice monitoring of the bone for acute rejection is not necessary. The time course of bone healing was not affected by the single rejection episode in our patient. Even though the onset of antirejection treatment was delayed a few days because of misinterpretation of skin histology, clinical and histologic signs of rejection disap-

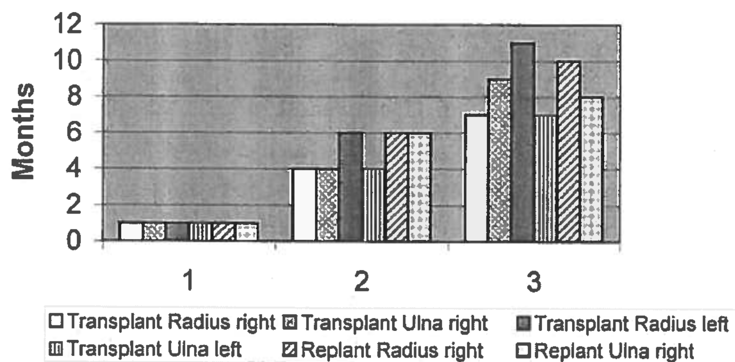

Figure 8. Callus maturation.

peared completely within 2 weeks. Even repeated acute rejections, as have been reported by other centers, seem not to have a relevant negative impact on bone healing when treated adequately.

Tacrolimus is reported to induce increase of alkaline phosphatase, a marker of osteoblast activity, and also to enhance osteoblastic differentiations induced by bone morphogenic protein–4.[32,33] The use of tacrolimus also is beneficial in lowering the necessary dose of glucocorticoids and thereby influencing bone mass evolution positively.[34] A high dose of tacrolimus, however, showed adverse effects on bone metabolism with alteration of cortical and trabecular bone.[35]

Because the early stage of callus formation is characterized by vessels approaching the osteotomy sites from the median aspect of the forearm, it can be assessed clinically by color Doppler sonography. With proceeding callus maturation, vascularization decreases and development to more dense collagen and osteochondral tissue is observed, which can be investigated by grayscale ultrasound.[17–21] In the presented double hand transplantation vascular ingrowth during early callus formation appeared at the same time as in a patient who had had replantation. The fact that the onset of callus formation with first signs of vascular ingrowth occurred at the medial aspect of the forearm, where the local periosteal flap was positioned, may be evidence that this strategy was helpful. Early callus formation and early revascularization imply that immunosuppression had no adverse effect on the vascular ingrowth.

Not only the initial phase of the bone healing process but also the disappearance of vascular ingrowth and callus maturation to more collagen and ossified structures were comparable to bone healing after replantation. Thus, immunosuppressive drugs used so far after hand transplantation seem not to impair cellular biology during maturation of soft tissue callus to chondral and ossified callus.

Compared with fracture healing union between an allograft and host bone proceeds at a slower rate. Therefore maximal stability for a prolonged period at the allograft–host junction is crucial to permit physical exercise as soon as possible until bone healing is completed. After accurate osteotomy bone stabilization with 3.5-mm plates an additional local periosteal flap, as used in our patient, should be sufficient to enable bone union in forearms without the use of autogenous bone graft.

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