A vascularized elbow allotransplantation model
in the rat

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Background: The aim of this research was to develop a rat model for vascularized composite allotransplantation (VCA) of the elbow.

Methods: We developed an animal model for VCA of the elbow in rats. Microvascular VCA was performed in 9 rats across a major histocompatibility barrier. Three different immunosuppressive regimens were provided. Joint mobility and weight-bearing capability were assessed throughout 90 days of life. Pedicle patency, bone blood flow, and histologic analyses were performed.

Results: In the cyclosporine group, forelimb activity was recovered during the postoperative 90 days. The extremity that was operated on was used in daily activities. There was minimal motion or use of the limb in the cyclosporine taper and control groups. The vascular pedicles were patent at the time of death in the cyclosporine-treated group but not in the remaining groups. Micro-computed tomography scan revealed union at the bone junctions, and the elbow joint appeared grossly normal on death in the cyclosporine treatment group only. Incomplete healing was observed in the other 2 groups, and the elbow joints were grossly destroyed. Histologic examination revealed normal cartilage and bone cells in the cyclosporine-treated group, whereas the nontreated groups demonstrated lymphocytic infiltration and loss of normal histologic features. Flow cytometry of blood samples obtained on days 14, 30, 60, and 90 showed no recipient cell chimerism in any of the groups.

Conclusions: We developed an animal model for elbow VCA. Immunosuppressed animals regained nearly normal function of forelimbs and maintained grossly normal elbow cartilage. Without cyclosporine treatment, the elbow transplants were rejected.

Level of evidence: Basic Science, In Vivo Animal Study.

Published by Elsevier Inc. on behalf of Journal of Shoulder and Elbow Surgery Board of Trustees.

Keywords: Vascularized; elbow; allotransplant; transplant; arthritis; rat

This study was approved by the University of Pennsylvania Institutional Animal Care and Use Committee: No. 804260. This study took place at the University of Pennsylvania, Philadelphia, PA, USA. One of the authors is an employee of the US Government and this work was prepared as part of his official duties. The views expressed in this article are those of the authors and do not necessarily reflect the official policy or position of the Department of the Navy, Department of the Army, Department of Defense, nor the U.S. Government. Nothing in the presentation implies any Federal/DOD/DON endorsement. None of the authors received financial support for this study.

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End-stage elbow arthritis in the young patient is a vexing problem. Potential surgical solutions include elbow joint débridement, resurfacing arthroplasty, nonvascularized elbow allografts, and total elbow replacement. None of these options, however, currently offers a realistic and long-term solution to alleviate pain and to maintain motion.\textsuperscript{1,3,6,7,9,10,15} Tissue-engineered joint replacements currently remain only a theoretical possibility.\textsuperscript{11} Whereas vascularized bone and joint autografts have proved successful for small joint reconstruction, expendable autograft sources are limited and do not provide viable donors for elbow reconstruction.\textsuperscript{2,8,22,24}

The only viable solution is often surgical arthrodesis or resection arthroplasty, leaving a patient with a minimally useful extremity with minimal or no motion.\textsuperscript{10,16} The ideal replacement for a destroyed elbow joint would be a living joint allogeneic transplant that precisely matches the dimensions and structural properties of the impaired joint. Despite the potential for this technology, few living vascularized transplantations of any large joint have been performed. There have been a few studies evaluating knee joint allotransplantation in rats, and there have even been vascularized allotransplantations in human knees, with only short-term success and then failure.\textsuperscript{4,5,14} Given the current immunosuppressive regimens, elective joint allotransplantation and the inherent side effects may not be widely accepted. The scale, however, could realistically be tipped in favor of performing elbow allotransplantation if the regimens could be improved by decreasing side effects of the medications.\textsuperscript{12,14}

An animal model is important to study this possibility further. The rat has been frequently used in vascularized composite allotransplantation (VCA) experimentation because of its easy handling, adequate size for microsurgery, and affordability. Multiple VCA models have previously been established in the rat.\textsuperscript{13,14,21,25,26} We are, however, unaware of a rat elbow joint allotransplantation model.

**Materials and methods**

All animal care complied with the guidelines of the Institutional Animal Care and Use Committee, the National Institutes of Health, and all national laws on the use of laboratory animals.

**Experimental design, part I**

In the first part of the experiment, inbred male brown Norway rats (genetic expression, RT1n) weighing 250 to 275 g were anesthetized and eventually euthanized with carbon dioxide; they were used to study the rat elbow and its vascular anatomy. Once it was adequately anesthetized, the rat was perfused with heparinized saline, followed by 30 mL of red latex or Microfil (17.1 mL contrast agent, 0.9 mL catalyst; Flow Tech, Inc., Carver, MA, USA). When the rat expired (termination of heartbeat and lung movement), it was placed in 4°C overnight to allow the latex to polymerize.

The following day, the skin of the rat was removed. The whole arm was exposed, and the neurovascular structures were closely dissected and studied.

**Experimental design, part II**

In the second part of the experiment, 9 vascularized elbow joints from brown Norway rats with residual biceps and triceps tendons and the median nerve were transplanted across a strong major histocompatibility complex barrier to Lewis rats. The animals were then randomly divided into 3 groups with different immunosuppressive regimens.

**Animals and anesthesia**

Brown Norway rats were also used as the donors in the second part of the experiment, and male Lewis rats (genetic expression, RT1l) weighing 250 to 275 g were used as the recipient animals. Both elbow joints of 1 donor rat were used for 2 transplantation procedures (N = 5). Donor rats were anesthetized with isoflurane inhalation at a dose of 3% for induction and 2% for maintenance by inhalation. After graft harvest, they were euthanized with an overdose of isoflurane (5%). Recipient rats were anesthetized with isoflurane as described for the donors. The rats were divided into 3 different immunosuppressive regimens.

**Group 1: cyclosporine treatment group**

Three of the 9 recipients were given cyclosporine immunosuppression at a dose of 16 mg/kg/day subcutaneously for the first week. The dose was then tapered down to a maintenance dose of 2 mg/kg/day thereafter.

**Group 2: temporary cyclosporine treatment group**

Three recipients were given cyclosporine at the dose of 16 mg/kg for 10 days, and then the immunosuppression was stopped.

**Group 3: control group**

Three recipients received no immunosuppression.

**Harvest of vascularized elbow joint allotransplant (donor)**

An anterior longitudinal incision was made over the entire arm extending to the chest wall. The axillary vessels were dissected all the way to the entrance of the thorax by elevating the pectoralis major. After ligation, all major branches from the pedicle to the axillary and brachial arteries were isolated and preserved. The median nerve was identified near the brachial artery. The ulnar nerve was identified at the midbrachial level and protected to preserve the ulnar collateral artery. The ulnar collateral artery traversing with the ulnar nerve was identified, and a muscle cuff was preserved to protect this vessel as it provides arterial supply to the joint. The major anterior muscle groups of the arm were identified. The biceps was transected at a distal level, leaving only a small tendinous portion. The flexors were identified and transected at the midforearm level. At this time, the median, radial, and ulnar arteries along with muscle perforators were identified, ligated, and cut. The ulnar recurrent vessel was identified, and a muscle cuff was saved to protect this vessel to the joint.
The elbow joint of the rat was flexed, and another 2.5-cm incision posteriorly over the brachium extending distally over the subcutaneous border of the ulna was made. The triceps was transected, leaving a small tendinous portion inserting into the ulna. The extensors were transected, and the posterior interosseous artery was identified between the ulna and the radius. The recurrent interosseous artery, a branch from the posterior interosseous artery, was identified and preserved as it went into the joint. The humerus, radius, and ulna were then further exposed in a subperiosteal fashion. The bones were then cut with a small power saw. The elbow allograft was taken just proximal to the deltoid tuberosity and 2 mm distal to the origin of the recurrent interosseous artery. The pedicle of the graft was then transected as proximal as possible at the level of the axilla.

Preparation of recipient bed and transfer of vascularized elbow joint

In the recipient Lewis rat, an anterior incision, from the distal third of the forelimb to the proximal third of the brachium, was made over the elbow joint. The brachial artery and the median and ulnar nerves were identified. The ulnar nerve was isolated from the cubital tunnel and was transposed anteriorly. The radial nerve was identified, and special care was paid to isolate the radial nerve at the radiocapitellar joint where the nerve lies close and is easily damaged during the removal of the recipient elbow. Four muscle groups, biceps, triceps, flexors, and extensors, were identified and cut off at their insertions. Then, the humerus, radius, and ulna were exposed and isolated from the soft tissue subperiosteally from the middle arm to the middle forearm. The recipient elbow joint was removed by cutting the brachial bone at the deltoid tuberosity level and the radius and ulna 2 mm distal to the insertion of the biceps. The donor and recipient elbow joints were then held side by side, and any adjustments to the length of the bones was performed to create a proper fit of the allograft (Fig. 1).

Intramedullary fixation and 90/90 wiring were used. Early in our preliminary study design, 90/90 wiring failed to produce reliable union, and intramedullary fixation was selected for stabilization with improved results (Fig. 2). A 20-gauge needle and two 27-gauge needles were used as intramedullary rods for the humerus and the ulna and radius, respectively, after reaming with a slightly smaller needle. All recipient muscle groups (intact) were then attached to their corresponding donor parts with nonabsorbable 7-0 monofilament suture. The median nerve was coapted to the recipient median nerve in an end-to-side pattern to improve proprioception to the joint. A 2-cm incision was then made at the cervical region of the recipient rat. The external jugular vein was exposed and prepared as the recipient vein. The internal carotid artery was found just medial to the sternocleidomastoid muscle and prepared as the recipient artery. The recipient artery (internal carotid) and the recipient vein (external jugular) were ligated and transected as distal as possible to leave long recipient vessels for anastomosis to the donor vessels. The donor pedicle was then tunneled to the neck region, and the end-to-end anastomosis was performed with 11-0 nylon sutures under a surgical microscope. Finally, the wounds were closed with absorbable sutures.

In vivo assessment

The arms that were operated on were assessed daily for signs of rejection, such as color changes, edema, and decreased motion. Other complications, such as bleeding, wound healing, and signs of infection, were also assessed daily for the first 10 days, then every other day.

Assessment of pedicle patency, graft blood flow, and joint architecture

After 90 days of survival, patency of the pedicle was evaluated in a second nonsurvival anesthetic procedure. The transplant was exposed under the operating microscope, and the pedicles were carefully tested for patency by downstream occlusion and release. Blood flow was then measured by performing the previously discussed contraction agent perfusion (Microfil),
followed by gross inspection and micro-computed tomography (micro-CT) scanning. Micro-CT scanning was performed both before and after the blood supply Microfil test using the in vivo micro-CT 40 (Scanco Medical AG, Brütisellen, Switzerland) at 10-μm resolution. Three-dimensional angiograms were then reconstructed. The bone morphologic changes were then compared with a normal rat elbow joint.

**Histologic assessment of viability and inflammation**

The transplanted elbows were then sectioned for histologic assessment of the elbow joint and the cortical bone. Assessments of cartilage structure were made by looking for any signs of chondrocyte and matrix degeneration.

**Recipient chimerism evaluation**

A chimerism analysis was performed on 1 × 10^4 cells using FACScan (BD Biosciences, San Diego, CA, USA).

**Results**

**Part I: vascular anatomy of the rat elbow**

In the anatomic study, we found that 4 arterial branches constitute the periarticular network. These branches were consistently identified in the 3 rats studied. We identified (1) the recurrent interosseous artery, (2) the recurrent ulnar artery, (3) the ulnar collateral artery, and (4) the collateral radial artery (Fig. 3).

**Part II: rat elbow allotransplantation**

The mean surgical duration for the donor elbow harvest was 2 hours, and the mean time for the recipient transplantation was 4.5 hours. Warm ischemia time was 30 minutes. Three rats failed to recover from anesthesia and subsequently died. The remaining animals returned to their normal activities the second day after transplantation.

**In vivo assessment**

**Group 1: cyclosporine treatment group**

In the cyclosporine-treated group, postoperative edema was observed in the first 5 days after the operation. No elbow joint rigidity was noted throughout the experiment, and the shoulder joint regained normal activity and range of motion within 10 days after operation. The elbow joint motion as well as the motion of the wrist and fingers continuously improved in the 3 months after the operation. The rats used the forelimb that was operated on in daily life for holding food and scratching their faces. These animals also ambulated with normal quadrupedal ambulation. No signs of rejection were observed (Fig. 4).

**Group 2: short-term cyclosporine treatment group; and group 3: control group, no immunosuppression**

In the short-term cyclosporine-treated group and in the control group receiving no immunosuppression, similar postoperative edema was noted for about 5 days. No obvious signs of rejection were demonstrated after the operation; however, the grafted side showed less shoulder and elbow range of motion compared with the contralateral side and compared with the cyclosporine treatment group. Throughout the 3-month survival, these elbows became progressively stiffer until they were rigid by the time of sacrifice.

**Assessment of pedicle patency, graft blood flow, bone union, and joint architecture**

The transplant was exposed under the operating microscope, and the pedicles were carefully tested for patency by downstream occlusion and release. Blood flow was then measured by performing the previously discussed contraction agent perfusion (Microfil), followed by gross inspection and micro-CT scanning.

**Group 1: cyclosporine treatment group**

The pedicles remained grossly open on visual inspection as well as with the patency test. Radiologic evaluations after
Microfil perfusion demonstrated the patency of the pedicle and the circulation of the graft (Fig. 5). In the cyclosporine-treated group, plain radiographs and CT scans of the graft showed good position and good fixation of the transplanted elbow 1 week after the operation (Fig. 6). Bone union was demonstrated at 90 days postoperatively. Histologic samples revealed viable bone without signs of ischemic necrosis or rejection in the cyclosporine-treated group at post-transplantation day 90. Viable bone marrow was also observed in the bone samples.

Groups 2 and 3: short-term cyclosporine group and control group
The pedicles were grossly constricted and did not have flow with the patency test. Microfil perfusion showed vascular sprouting around the graft in a highly abnormal pattern with no evidence of the main pedicle (Fig. 7). No bone healing was seen 3 months after the operation, and micro-CT showed obvious degeneration, absorption, and fractures of the joint (Fig. 8). Histologic sections revealed little or no viable bone marrow and minimal viable cartilage at the elbow joint. There was a large amount of lymphocyte infiltration visible in both of these groups (Fig. 9).

Recipient-donor chimerism evaluation
No obvious chimerism was evidenced on day 10, day 30, and day 60 after transplantation.

Discussion
Previous studies in a rat population have shown that surgical neoangiogenesis has allowed short-term bone allotransplants to survive without immunosuppression.\textsuperscript{14} Whereas a great deal of research is currently under way in promoting tolerance of VCA, the allotransplant of an elbow joint may differ significantly. As in our model, the skin is not necessary to transplant the joint, and thus the antigenicity of the transplant may differ significantly from a transplant that contains highly antigenic skin.

During the past decades, osteoarticular elbow allograft replacement has become a potential option for elbow reconstruction in the severely damaged elbow.\textsuperscript{2,3,6-11,14-16,22,24} The initial postsurgical results were often satisfactory, yet most cases inevitably resulted in severe and obvious degenerative joint changes in the midterm follow-up.\textsuperscript{10} With the rapid advances of VCA, vascularized joint allograft
transplantation might become the solution for end-stage elbow arthritis if immunosuppression can be minimized. Diefenbeck et al have published their results of knee allotransplantation, which have unfortunately all been lost because of graft vasculopathy.

In a beautifully performed study, Wavreille et al published their anatomic research for a potential vascularized elbow joint transplant in humans in 2006. Although this would be the ultimate goal (vascularized elbow allotransplantation), more basic science research is clearly needed before this endeavor. An animal model is needed to further progress this potential surgical treatment. Our research aimed to develop a vascularized elbow allograft model in the rat across a major histocompatibility barrier.

**Part I: vascularity of the rat elbow**

We determined, as previously noted, that 4 main branches vascularize the elbow joint. In addition, we noted that there was a large branch from the median nerve to the elbow joint. We thought that this was likely a major proprioceptive component to the elbow joint and thus performed an end-to-side coaptation to the recipient’s median nerve. It was our intent that this coaptation will help the proprioceptive function of the joint and prevent neuropathic degeneration.

**Part II: rat elbow allotransplantation**

This study demonstrates that a vascularized elbow allograft transplantation can be performed in the rat across a major histocompatibility complex barrier. We have additionally confirmed that short-term immunosuppression and no immunosuppression lead to rejection of the allograft transplant. The in vivo assessments demonstrated a large difference between group 1 and groups 2 and 3. We were not able to distinguish groups 2 and 3 either clinically or in the laboratory, and therefore the results of both have been presented together. The clinical differences between the cyclosporine group 1 and the other 2 groups were obvious. The immunosuppressed rats’ forelimb use increased over time, whereas the other 2 groups’ limbs became stiff and were used minimally.

The patency of the pedicle was verified surgically as well as with angiography using Microfil and then by micro-CT scanning of the elbow joints. The main pedicle of the brachial artery was seen in group 1, whereas in the other groups, the main pedicle was no longer visualized patent crossing the joint. In the other groups, we found an abundance of neovascular tissue surrounding the joint, which appeared to be an attempt to revascularize the elbow joint unsuccessfully. The use of the micro-CT scanner allowed us to verify that there was indeed union at our osseous junctions in the group 1 rats treated with cyclosporine, whereas nonunions were obvious in the other 2 groups. In addition, the joint destruction was seen when micro-CT images of the nonimmunosuppressed elbows were performed. The histologic slides of the joint graphically demonstrate the preservation of the joint structure in the immunosuppressed group and progressive destruction in the other 2 groups.

Chimerism has been demonstrated after bone marrow transplantation in rat models; however, no chimerism has been reported in humans to date. We did not detect any donor-specific chimerism in our animal model, and this result was not unexpected.

We did note some problems in our experimentation worthy of note. Early on, we used 90/90 wiring and noted that the rats had a difficult time healing the transplant bone junctions, so we switched to intramedullary fixation with
needles. During the postoperative 90 days, there were no severe complications necessitating graft removal. There were superficial wound infections in 2 animals that healed after débridement.

**Conclusion**

Despite the low number of transplants performed, this study confirms the surgical feasibility and survival of a vascularized elbow allograft transplant in a rat model when it is properly immunosuppressed. Short-term follow-up demonstrated no obvious degenerative joint changes, and the animals appeared to be using their limbs normally. Despite the feasibility of this procedure, more transplants need to be performed, and a more quantitative approach to results measurements would improve the validity of this study in the future.

**Disclaimer**

The authors, their immediate families, and any research foundation with which they are affiliated have not received any financial payments or other benefits from any commercial entity related to the subject of this article.

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Figure 9 The *upper left image* is a control elbow that did not have surgery. The *upper right* is a transplanted elbow that received cyclosporine; note the relatively preserved joint architecture. The *lower left* is a destroyed elbow joint in the short-term cyclosporine group. The *lower right* is the control group that was transplanted without immunosuppression.


