Free Microvascular Epiphyseal-Plate Transplantation

An Experimental Study in Dogs

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Abstract: To evaluate the feasibility of transplanting vascularized epiphyseal plates while maintaining normal growth in the recipient site, twenty-two puppies from known, large breeds were divided into one control and three experimental groups of four animals each and one long-term group of six animals. The control group underwent insertion of a radiopaque marker in the fibular metaphysis bilaterally, and, in addition, a fibular osteotomy was performed on one side. In the experimental groups, a fibular switch was carried out, selecting one fibula as a vascularized graft and the other as a non-vascularized graft. Both the controls and the experimental groups were evaluated using serial roentgenograms, histological examination, fluorescent bone-labeling, and microangiography. One week, six weeks, three months, and seven months postoperatively, animals from each group were killed. Continuous growth was observed in the vascularized epiphyseal transplants and in the controls, with no statistical difference noted, whereas the non-vascularized transplants exhibited considerably less or no growth. Vascularized transplants demonstrated an average 21.2-millimeter increase in length while non-vascularized transplants showed a 6.6-millimeter increase. Histological examination, fluorescence bone-labeling, and microangiography confirmed the continued viability of the vascularized epiphyseal transplants in contrast to the non-vascularized transplants.

Clinical Relevance: This experiment has shown that it is possible to transplant a physis-containing vascularized graft from its normal site to the contralateral, mirror-image counterpart while maintaining normal growth capacity. The application of this technique clinically is minimum. Further investigations to determine how a transplanted growth plate reacts when transferred to a heterotopic anatomical site with altered stress loads are necessary before microvascular transfer of epiphyseal plates can be considered to be clinically applicable.

Recent developments in the field of microvascular surgery have stimulated new interest in surgical procedures that were previously considered but not technically feasible. One is the transplantation of skeletal growth plates. Previous non-vascularized attempts have given "variable and usually unsatisfactory results". In addition to descriptions of single clinical cases, several reports concerning experimental microvascular transfer of epiphyseal growth plates have been published in recent years. However, in only one of these series in which replantations were performed did normal growth occur in the transplant. This possibly was due to insufficient attention to the specific epiphyseal blood supply that provides the main nutrient flow to the epiphyseal plate. The purpose of the present study was to evaluate the feasibility of transplanting vascularized epiphyseal plates while maintaining normal growth in the recipient site.

Material and Methods

Twenty-eight puppies were selected from known, large breeds. Initially a pure-bred experimental model was attempted with English foxhounds, but as the source of these turned out to be insufficient, dogs of a pointer-foxhound cross were added. By controlling the breed, we intended to reduce the variability in growth; by selecting a large breed, we intended to maximize the expected growth.

We excluded from the study six dogs that, when they were killed, demonstrated evidence of failure due to surgical technique. Those six were among the first ten to be operated on and were found to have either non-patent veins or total lack of uptake in the vascularized graft of the fluorescent label that was administered on the first day postoperatively, or both. This study was an investigation of the results after successful vascular transfer; so these six dogs were not included. The remaining twenty-two dogs were divided into one control and three experimental groups of four animals each and one long-term experimental group of six animals. Each group included animals of both sexes from various litters.

All operations were performed when the animals were

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four months old. Preoperatively, roentgenograms were made to establish that normal epiphyseal plates were present. Then the animal was anesthetized with pentobarbital (Nembutal, Abbott) and intubated. Under aseptic conditions, a seven-centimeter segment of the proximal part of the fibula, including the head, with the proximal epiphysis, was isolated on the popliteal artery and vein as previously described. After the extremity had been shaved and prepared, a lazy-s incision was made on the lateral side of the hind leg, from a point proximal to the knee joint and extending distally to the dorsal branch of the lateral saphenous vein. The cutaneous fascia was divided, and the peroneal nerve was identified as it passed around the fibular metaphysis. The nerve was followed distally, and the plane between the fibularis longus and extensor digitorum lateralis was developed. The nerve was reflected cranially toward the long fibular muscle. Deep to the nerve, the cranial tibial artery and vein were identified, and the vessels were isolated and ligated twice with silk at the level of the mid-point of the fibular diaphysis. The proximal ligature was sutured to the extensor digitorum lateralis, and the vessels were divided. They were then freed from the cranial and tibial sides, and the branches were divided in these directions. The vessels were pushed toward the fibula, working from the distal end toward the fibular head. After the vessels had been dissected as far proximally as possible, the fibula was isolated by sectioning the surrounding muscle and periostium at the mid-fibular level. An osteotomy was performed at this level and then the interosseous membrane was divided in a proximal direction, carefully protecting the cranial tibial vessels. The nutrient artery of the tibia was identified and ligated. The peroneal nerve was dissected free from the fibular metaphysis, and the fibular origin of the fibularis longus was divided at a distance five to ten millimeters from the bone. The proximal tibiofibular joint was opened, and the deep ligaments were cut. After dislocation of the fibular head proximally and division of the muscular branches to the fibularis longus and the extensor digitorum longus, the flexor hallucis was divided proximally from the osteotomy, leaving a thin cuff of muscle on the fibula. The vascular pedicle, which consisted of the popliteal artery and vein, was isolated as far proximally as was possible, and a vessel loupé was placed around it. Any remaining attachments were then divided, leaving the graft perfused through the isolated vascular pedicle.

This isolated segment corresponded to approximately one-half the length of the entire fibula. The same isolating technique was used bilaterally. A fibular switch was then carried out, selecting one fibula as a vascular graft and the other as a conventional, non-vascular graft. The isolated vascular pedicle, consisting of the popliteal artery and vein supplying the proximal fibular epiphysis, was ligated as far proximally as possible in order to have a long vascular pedicle for anastomosis to the recipient popliteal artery and vein on the other side (Fig. 1). Vascular anastomoses of the artery and the singular vein were performed using a magnification factor of sixteen with an operating microscope (Zeiss OpMi7) and 9-0 nylon sutures (Ethilon, Ethicon) on a BV 100-4 needle. Patency of the anastomoses was determined by the quality of the distal pulsations and with two jeweler's forceps, placing one of them one millimeter from the anastomosis on the side distal to the direction of blood flow and the other one, just distal to the first. The vessel was gently clamped with the proximal forceps, and the distal forceps was then closed and moved over a three-millimeter segment of the vessel. The proximal forceps was released, whereupon blood rapidly filled the empty segment, indicating patency of the anastomosis. The non-vascular grafts were stripped of all soft tissue except periostium. Both grafts were secured distally with an osteosuture of number-4 monofilament stainless-steel wire through previously hand-drilled holes and were secured proximally with interrupted 3-0 Dexon sutures. No attempt was made to reconstruct the lateral ligaments of the knee, as the knees were stable to manual testing. During the operation, a radiopaque marker was inserted in the metaphysis of each each fibula at some distance from the physis. The wounds were closed with continuous 3-0 Dexon sutures in the fascia and subcuticular 3-0 Dexon sutures in the skin. The average duration of ischemia was one hour and fifty-four minutes, and during this period of time the graft was stored in an ice-chilled saline bath.

The control group consisted of puppies that underwent insertion of a radiopaque marker in the fibular metaphysis bilaterally and, on one side, a fibular osteotomy at approximately the same level as in the experimental animals. This control osteotomy was also stabilized with an osteosuture, as previously described.
All animals were given antibiotic prophylaxis with one million units of penicillin G-procaine injected intramuscularly one-half hour before the start of the operation. Intraoperatively, they received an intravenous infusion of 500 milliliters of lactated Ringer solution. Postoperatively, full weight-bearing without any immobilization was allowed.

The dogs were followed with serial roentgenograms, and fluorescent bone-labels were administered in a tetra-

![Fig. 2-A](image1)

**Fig. 2-A**

Figs. 2-A and 2-B: Anteroposterior roentgenograms of an experimental animal with the vascularized graft on the right and the non-vascularized graft on the left.

Fig. 2-A: Postoperative roentgenogram. Radiopaque markers are visible in the fibular metaphyses.

![Fig. 2-B](image2)

**Fig. 2-B**

Roentgenogram made six months postoperatively. The increased distance of the radiopaque marker on the right shows growth of the vascularized transplant as compared with the left, where the marker has become displaced and no growth has occurred.
chrome pattern in dosages according to the method of Rahn. Oxytetracycline was given on the first day after operation (thirty-five milligrams per kilogram of body weight); alizarin complexone, five weeks after operation (thirty milligrams per kilogram of body weight); DCAF, twelve weeks after operation (twenty milligrams per kilogram of body weight); and xylenol orange, twenty-five weeks after operation (ninety milligrams per kilogram of body weight).
body weight). Roentgenograms were made with the animal in a standardized position and with a constant film-to-tube distance of eighty-four centimeters within one week after the operation and five, twelve, twenty-five, and twenty-nine weeks postoperatively. The distance between the implanted marker and the proximal tip of the fibula was measured with a ruler to the nearest 0.5 millimeter on each roentgenogram. At one week, six weeks, three months, six months, and seven months postoperatively, one group of animals was killed. The animals in the control group were killed seven months postoperatively, at which time growth had ceased.

Perfusion of the hind limbs was performed with barium sulphate suspension (Micropaque, Picker) through catheters inserted in the femoral artery close to the inguinal ligament, at a pressure of 120 to 130 millimeters of mercury. The anastomoses were dissected out, and a visual attempt to establish patency was made.

The entire fibula was harvested for histological preparation and was carefully sectioned longitudinally, preserving both sides of the epiphyseal growth plate in order to obtain maximum information. Half of the specimens were fixed in 10 per cent buffered formalin, decalcified in formic acid, dehydrated in alcohol, embedded in paraffin, sectioned at five micrometers, and stained with hematoxylin and eosin. The remaining specimens were fixed in absolute alcohol, embedded (undecalcified) in methylmethacrylate, sectioned with a diamond-blade saw, and ground between glass plates to 100 micrometers for fluorescent microscopy.

For microangiography, 1.5-millimeter sections were cut from the paraffin blocks containing the decalcified fibular heads. The sections were placed on two by two-inch (5.1 by 5.1-centimeter) HRP plates (Kodak) and exposed for ten minutes at thirty-five kilovolts and five milliamperes. The plates were developed for five minutes in HRP developer, fixed, and washed for one hour. The plates could then be examined by slide projection or under a microscope.

**Results**

The postoperative course was uneventful except for some toe-extensor weakness, which always subsided rapidly. No infections or major wound complications occurred. Continuous body growth ensued, and there was an increase

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**Figure 4**

Graph of growth measurements taken from roentgenograms at each of the given time-periods, comparing the control fibulae and the vascularized and non-vascularized transplanted fibulae.

**Figure 5**

Graph of measurements taken from the roentgenograms of the control animals, showing cessation of growth between twenty-five and twenty-nine weeks postoperatively.
in weight from an average of 10.8 kilograms at operation to 21.3 kilograms in the animals that were killed six months later.

The roentgenograms demonstrated continuous growth in the successful vascularized transplants and in the normal control dogs, whereas the non-vascularized transplants exhibited considerably less or no growth (Figs. 2-A through 3-B). The registered growth, measured as the total increase in length, is presented in Figure 4. The marker-only controls demonstrated an increase in length of 20.6 ± 1.0 millimeters while the osteotomized controls and the vascularized transplants demonstrated increases of 21.5 ± 0.6 and 21.2 ± 1.7 millimeters, respectively. The non-vascularized transplants grew only 6.6 ± 4.4 millimeters. As is evident from Figure 5, no growth occurred during the seventh month of this study in either the experimental groups or the control group. For statistical evaluation, measurements from six-month roentgenograms were used. The observed total growth was evaluated with Student's t test. The difference between marker-only controls and osteotomized controls was significant (paired test, one-tailed, p < 0.02). No difference could be verified between the osteotomized controls and the vascularized transplants (one-tailed, p > 0.30), whereas the difference between the vascularized and non-vascularized transplants was highly significant (one-tailed, p < 0.001).

Evaluation of the histological specimens obtained when the animals were killed showed that all vascularized transplants retained a normal epiphyseal growth plate with typical, orderly cartilage-cell columns in addition to a vascular network that contained Micropaque (Fig. 6). The non-vascularized grafts, on the other hand, demonstrated fragmentation of the epiphyseal plate (Fig. 7). One week postoperatively, the cartilage cells were pyknotic and there was partial disappearance of the cell nuclei. At six weeks, contorted regenerative efforts and partial replacement by fibrous scar were seen (Fig. 7). By three months postoperatively, the physes had totally disappeared except for a thin subperiosteal remnant. Revascularization and creeping substitution in the non-vascularized grafts was rapid, and by six weeks well defined, Micropaque-filled vessels reappeared in the sections. Similarly, the widespread occurrence of empty lacunae indicating osteocyte death, which was observed at one week, had been normalized. Fracture-healing

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**Fig. 6** Photomicrograph of the epiphyseal plate from a vascularized fibular graft, one week postoperatively (hematoxylin and eosin, × 100). The cell columns are orderly, with dark-stained nuclei, and are representative of all vascularized growth-plate transplants in this study.

**Fig. 7** Photomicrograph of the epiphyseal plate from a non-vascularized fibular graft, six weeks postoperatively (hematoxylin and eosin, × 100). The growth cartilage is fragmented, and no orderly cartilage-cell columns are noted.
at the distal end of the transplant was rapid in both vascularized and non-vascularized grafts, and no difference was detectable in histological sections.

In the longest-surviving groups, alterations indicating the impending normal physiological closure of the growth plate were seen in the vascularized grafts. At six months, secondary spongiosa extended into the cartilage columns that had replaced the primary spongiosa normally found in growing bone. At seven months, histological evidence of complete plate closure was present in some specimens, whereas others retained characteristics similar to those seen at six months. No difference was detectable between the vascularized grafts and the specimens from the control group.

![Fluorochrome labeling in a vascularized epiphyseal plate, three months postoperatively. The dense white band is DCAF, which has been incorporated into the calcification front (arrows). The metaphysis is to the right.](image)

**Fig. 8-A**

![Fluorochrome labeling in a non-vascularized tibular graft sectioned through the vicinity of the fragmented epiphyseal plate, three months postoperatively. DCAF is incorporated in the bone due to revascularization by creeping substitution. The growth plate is fragmented (arrows), and there is no well defined zone of calcification as in Fig. 8-A.](image)

**Fig. 8-B**
In the undecalcified sections that were examined for fluorochrome uptake, all four fluorochromes were present in the successful vascularized grafts. The distribution was strikingly similar to that seen in the controls. In the nonvascularized grafts, on the other hand, the oxytetracycline that had been administered on the first postoperative day was never found whereas the fluorochromes given from the fifth week onward were visible, providing further proof of the rapid remodeling and revascularization. The distinctive pattern of fluorescence with incorporation of DCAF in the calcification front was present near the growth plates in the controls and the vascularized grafts but was completely absent in the non-vascularized grafts (Figs. 8-A and 8-B).

Microangiograms demonstrated a normal vessel pattern in the vascularized transplants (Fig. 9), whereas the non-vascularized transplants exhibited a total lack of interosseous vessels one week postoperatively (Fig. 10). By six weeks postoperatively, the non-vascularized transplants demonstrated increasing vascularity as a result of creeping substitution; however, this was less than that observed in the vascularized transplants at the same time-period. At six months, almost total dissolution of the non-vascularized transplants was observed.

Discussion

Skeletal growth is a dynamic process that is influenced by several factors. The epiphyseal growth plate is the dominant site of longitudinal growth of the diaphysis in long bones. Both the time of cessation of growth and the rate at which it occurs determine the final length of a bone.

In this study, the growth rate decreased at six months postoperatively, corresponding to a canine age of ten months. Roentgenograms made one month later — seven months postoperatively — showed that no growth had occurred during the previous month. These findings were essentially identical in the experimental and control groups and are in accord with previous findings. Cessation of growth, which has not been evaluated in previous studies dealing with vascularized epiphyseal transplants, normally precedes actual physeal closure and occurred normally after vascular epiphyseal transfer in our experiment.

The amount of growth obtained in the transplanted,
vascularized grafts did not differ statistically from that observed in the controls. The growth rate, therefore, was also normal. This finding is in agreement with the results of studies of vascularized limb replantation\textsuperscript{13-18} but is in conflict with the results of studies in which vascularized epiphyseal-plate transplantation was performed and growth was reduced to 65 per cent\textsuperscript{11,12} and 82 per cent\textsuperscript{1} of normal. This difference is probably due to the preservation of the delicate vessels entering the epiphysis of the graft in the present model. Téot reported that only 30 per cent of normal blood flow remains when the periosteal-perichondrial anastomoses alone supply the epiphysis\textsuperscript{30}. The reduced blood supply to the epiphysis in experimental models reported previously was probably insufficient to maintain normal growth. The exact demands of the growth plate for nutrients are unknown, but it has been demonstrated that tissue Po\textsubscript{2} is low in normal situations\textsuperscript{4} and that the growth rate is altered by changing the oxygen tension\textsuperscript{51}. We have demonstrated, in microsphere experiments, that the isolation technique employed in our model does not carry the risk of reducing blood flow to the zones surrounding the epiphyseal growth plate\textsuperscript{24}. Thus, the graft in this experiment was not subjected to circulatory disturbance.

The method that we chose for growth registration — measuring roentgenograms — has well known limitations. However, by measuring the same distance on roentgenograms made independently of one another and in the same animal on one occasion, it was possible to estimate the random error to be less than one millimeter, which we considered to be acceptable. The systematic error from the magnification was constant during the experiment and therefore was negligible.

Experimental conditions were carefully controlled in this study. Careful selection of animals resulted in a very small variation in the amount of growth, which allowed a reliable comparison between the experimental and control animals. The animals in the control group had one side with and one side without an osteotomy. The osteotomized side demonstrated a small but statistically significant overgrowth, which is in agreement with prior reports describing overgrowth after fractures and osteotomies in growing individuals\textsuperscript{1,3,9,17}. Extensive dissection of the fibula in the controls was considered unnecessary, since it has been shown that trauma to surrounding muscle does not provide additional growth stimulus to that elicited by an osteotomy\textsuperscript{9}. Extensive dissection also carries the risk of injuring the minute vascular branches supplying the epiphysis, a possibility that could explain the failure to obtain normal growth in control specimens that were elevated on a vascular pedicle and then replaced\textsuperscript{7}.

In this experiment, in addition to the vascularized epiphyseal graft, the dogs had a non-vascularized conventional graft on the opposite side. This permitted direct comparison between the two types. In only two of the six long-term dogs did any significant growth occur in the conventional graft. This growth was, however, considerably less than that on the vascularized side in both dogs and amounted to 45 per cent in one and 76 per cent in the other. This finding is in accord with the results of previous studies\textsuperscript{1,6,16,18,19,25,28,35,36} in which isolated instances of predominantly subnormal growth occurred in transplanted non-vascularized plates. This growth is too unpredictable to be clinically useful\textsuperscript{6,26,36}.

This experimental model, although successful, has its limitations. All of the animals had well developed secondary ossification centers in the transplanted epiphysis and thus were not extremely young when surgery was performed. On formation of the ossification center the vascular network changes drastically in the epiphysis — from an end-arterialized, territorially restricted circulation to a freely anastomosing network. This probably means that very early transplantation would require the preservation of all circulatory sources — a task that, at least for the fibula, seems technically impossible. This experiment also shows only that it is possible to transplant a physisc-containing vascularized graft from its normal bed to the contralateral, mirror-image counterpart while maintaining the normal growth capacity. The clinical applicability for this is slight, and the study should be considered as a first step in assessing the feasibility of vascularized epiphyseal transfers. Further investigations to determine how transplanted growth plates react when transferred to more disparate anatomical sites with altered stress-loads, both longitudinally and transversely, and perhaps with different normal growth rates are necessary before microvascular transfer of epiphyseal plates can be included in the clinical armamentarium.

References