Published in final edited form as:

Microsurgery. 2012 February ; 32(2): 118–127. doi:10.1002/micr.20946.

Knee joint transplantation combined with surgical angiogenesis in rabbits – a new experimental model

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Summary

Purpose—We have previously described a means to maintain bone allotransplant viability, without long-term immune modulation, replacing allogenic bone vasculature with autogenous vessels. A rabbit model for whole knee joint transplantation was developed and tested using the same methodology, initially as an autotransplant.

Materials/Methods—Eight New Zealand White rabbit knee joints were elevated on a popliteal vessel pedicle to evaluate limb viability in a non-survival study. Ten additional joints were elevated and replaced orthotopically in a fashion identical to allotransplantation, obviating only microsurgical repairs and immunosuppression. A superficial inferior epigastric facial (SIEF) flap and a saphenous arteriovenous (AV) bundle were introduced into the femur and tibia respectively, generating a neoangiogenic bone circulation. In allogenic transplantation, this step maintains viability after cessation of immunosuppression. Sixteen weeks later, x-rays, microangiography, histology, histomorphometry and biomechanical analysis were performed.

Results—Limb viability was preserved in the initial 8 animals. Both soft tissue and bone healing occurred in 10 orthotopic transplants. Surgical angiogenesis from the SIEF flap and AV bundle was always present. Bone and joint viability was maintained, with demonstrable new bone formation. Bone strength was less than the opposite side. Arthrosis and joint contractures were frequent.

Conclusion—We have developed a rabbit knee joint model and evaluation methods suitable for subsequent studies of whole joint allotransplantation.

Introduction

Large osseous defects resulting from trauma, oncologic resection and infection challenge the reconstructive surgeon¹. This is especially true if joint reconstruction is required. Many treatment options available include high morbidity and failure rates. *Prosthetic reconstruction* is restricted by prosthetic loosening, infection, periprosthetic fractures and implant failure^{2, 3}. Knee *arthrodesis* severely impairs function and is thus a less preferable option⁴. The use of *bone transport*, *intercalary frozen (nonviable) allograft* or *vascularized bone autograft* preserves length but not motion. All have significant morbidity^{5–8}. Problems

include long treatment courses (bone transport), non-unions, infection and stress fracture (structural bone allografts,^{6, 9–11}). *Joint autotransplants* heal primarily and resist infection, but have considerable donor site morbidity with use restricted to transfer of small toe or hand joints into the hand¹². For larger joint reconstruction, *cryopreserved joint allografts* have proven disappointing^{13, 14}. A few cases of vascularized *joint allotransplantation* have been reported, with risk of both early infection and late failure.^{15, 16} Graft viability has been maintained only while long-term immunosuppression was applied with its attendant risks of malignancy, infection or graft versus host disease¹⁷. We believe these risks to be justifiable in life-critical applications such as organ allotransplantation. However, the risk benefit ratio in the non life critical situation of skeletal reconstruction is worse and the resulting ethical problems prevent the wide application of vascularized joint allotransplantation especially since alternatives such as prosthetic replacement exist.

Knee joint allotransplantation has been performed in rats^{18–21}, dogs^{22–25}, cats²⁶ and rabbits^{26–30}. All of these studies used long-term immunosuppression for graft survival. We have previously described the use of angiogenesis to replace the allogeneic circulation with autogenous vessels. This method allows long-term bone allotransplant survival in rat and rabbit models,^{31–33} and requires only short term immunosuppression. Here, immunosuppression is only used to maintain patency of the microsurgical nutrient pedicle and thereby allows bone survival for a short period. During this time neoangiogenesis arises from implanted vascularized host derived tissues and builds a new blood supply that takes over perfusion and allows further bone survival without immunosuppressive therapy since these host derived vessels are no target for the immune response. This method has never been used for composite tissue allotransplantation such as joint transplantation. In this preliminary study we describe a method to transplant the rabbit knee joint with simultaneous surgical angiogenesis, here with autogenous joints. This unique new model evaluates if knee joint transplantation with surgical angiogenesis is feasible and enables our planned future investigation of knee allotransplantation, and also provides information on vascularity, histologic and mechanical properties of vascularized joint autotransplantation.

Materials and Methods

The study was approved by the Institutional Animal Care and Use Committee (IACUC).

Perfusion of the lower leg

Eight New Zealand White rabbits were used in a preliminary study to simulate orthotopic transplantation of the knee as follows. The rabbits were euthanized with an overdose of 26% Sodium Pentobarbital (Sleepaway®, Fort Dodge Animal Health, Fort Dodge, IA). The right leg was opened through a longitudinal medial incision and the superficial femoral vessels were identified with the major side branches (saphenous artery, popliteal vessels, genicular arteries, anterior and posterior tibial vessels and peroneal vessels). To simulate knee joint transplantation with surgical angiogenesis, the anterior tibial, peroneal and saphenous vessels were ligated using vessel clips. Subsequently, the abdomen was opened through a longitudinal midline incision and the lower vena cava and the aorta were dissected for cannulation for microangiography (see below). After two hours the muscles of the anterior compartment (ant. tibial muscle, extensor hallucis muscle and extensor digitorum longus muscle) were evaluated for perfusion and compared to the contralateral side.

Whole knee autotransplantation

In 10 New Zealand White rabbits, the knee joint was elevated as below, with subsequent orthotopic replantation as a vascularized autograft. Additionally, we implanted a superficial inferior epigastric facial flap in the femur and a saphenous arteriovenous bundle in the tibia

(figure 1), to promote angiogenesis as we have described previously for bone allotransplantation^{31–34}. Anesthesia was induced with intramuscular injection of ketamine (Ketaset1, Fort Dodge Animal Health; 40 mg/kg IM), xylazine hydrochloride (VetTek, Bluesprings, MO; 7 mg/kg IM) and acepromazine maleate (Boehringer Ingelheim Vetmedica, Inc., St. Joseph, MO; 1 mg/kg IM). The rabbits were intubated and inhalation anesthesia was maintained with Isoflurane (Novaplus Hospira Inc., Lake Forest, IL) and 70% oxygen on spontaneous breathing. Venous access was gained through the marginal ear vein and used for fluid administration (Ringer's lactate, average 100ml/procedure I.V.). A heating pad prevented hypothermia and subsequent vasospasm.

Through a medial longitudinal incision, the superficial femoral artery was identified between the vastus medialis muscle and the adductor muscles. The whole musculature was removed from the graft leaving a 1 mm muscle cuff, the periosteum was not stripped. The quadriceps muscle was cut immediately proximal to the patella. Thereby, the knee vascular pedicle was exposed in the popliteal fossa. The popliteal and genicular vessels with multiple small metaphyseal and epiphyseal branches were identified and protected. Care was taken to preserve the posterior tibial vessels, since only these maintain the lower leg blood supply in recipient animals, whose saphenous vessels have been ligated and transposed into the bone for surgical angiogenesis. The anterior tibial artery and vein were ligated at this level, but preserved more distally to allow lower leg muscle perfusion by retrograde flow. The peroneal nerve was carefully dissected from the graft to prevent damage. Osteotomies were made, in the femur 2 cm proximal to the joint and in the tibia 5 mm distal to the tuberosity. Visible bleeding from the endosteum confirmed maintenance of the perfusion.

Three mm-wide holes were drilled spanning the proximal and distal bone osteosynthesis sites to allow passage of the SIEF-flap (femur) and saphenous A/V bundle (tibia). Osteosynthesis of the isolated knee graft was accomplished with 2 mm LCP-Locking Plates (Synthes, Monument, CO). Muscles were reattached using Vicryl sutures. The saphenous arteriovenous bundle was raised from the fascia of the lower leg, was ligated distally and was introduced into the tibial metaphysis to lie within its intramedullary canal. A superficial inferior epigastric fascial flap was harvested from the lower abdominal wall and placed within the femur as previously described³⁵ (figure 1). An adequate amount of fascia was included to fill the intramedullary canal for sufficient neoangiogenesis. Pedicle patency was confirmed by direct inspection. Fascial and layered skin closure was then performed.

Rabbits were allowed normal ambulation during the postoperative period. Pain therapy was applied with Buprenorphine (Buprenex, Reckit Benckiser, Richmond, VA, 0.12 mg IM) and Flunixin meglumine (5 mg IM) Trimethoprim/sulfamethoxazole provided infection prophylaxis (Tribrissen1, Five Star Compounding Pharmacy, Clive, IA; 0.2 ml SQ. daily for 3 days). Elizabethan collars were worn until suture removal after 14 days.

Radiographic evaluation

Plain radiographs were taken immediately postoperatively and 1, 2, 3, 4, 8, 12 and 16 weeks thereafter using a high-resolution mammography X-ray film (Min-R 2000 Film, Kodak, Rochester, NY) in two different oblique views (General Electric, Fluoroscope Model 46, Milwaukee, WI; 54 kV, 6 mA). Osseous healing was evaluated radiographically using a scoring system (no bone healing [0 points] to complete healing and remodeling [24 points]) based on that described by Taira et al and Giessler et al^{31, 36}. Additionally, arthrosis was evaluated using a score from 0 (no arthrosis) to 5 (complete joint destruction).

In addition, a computerized tomography (CT) scan (Inveon microCT, Siemens Medical Solutions, Inc., Knoxville, TN) of the knee allotransplant was performed to measure

moments of inertia of femur and tibia for biomechanical calculation purposes. The moment of inertia was calculated using the following formula:

$$\pi(D^4 - d^4)/64$$

With D being the outer diameter of the cortical bone and d being the corresponding inner diameter.

Sacrifice procedure and microangiography

At 16 weeks, additional x-rays were performed in maximum extension before sacrifice to measure the knee contracture angle in vivo. All animals were then euthanized with Sleepaway® (Fort Dodge Animal Health; 1 ml IV). The aorta and inferior vena cava were cannulated and flushed with saline (300ml). A colored polymer (Microfil®, FlowTech Inc., Carver, MA) was injected arterially under physiologic pressure (100–120 mmHg) until venous backflow of the polymer confirmed complete perfusion. Two hours later, the nutrient pedicle of the knee as well as the pedicle of the superficial inferior epigastric flap and the saphenous arteriovenous bundle was checked for patency as confirmed by colorization of the vessels.

After ex vivo measurements of contracture angles (see below) the knees were fixed in 10% formalin and decalcified in 14% EDTA in a laboratory microwave (Pelco Biowave 3450, Ted Pella, Redding, CA)^{33, 37} and subsequently cleared with the Spalteholz method³⁸. This enabled visualization of the blue Microfil filling the intraosseous vasculature, contrasted with the clear or slightly yellow bone. Two standardized orthogonal digital images were taken of each bone and the ratio of vessel area to total bone area was determined for both projections using imaging analysis software (Image-Pro Plus 7.0, Media Cybernetics Inc. Bethesda, MD, USA). An average of these ratios was calculated for later comparisons.

Biomechanical testing – passive range of motion

Knee contracture angles were measured ex vivo bilaterally. The femur was fixed in a custom made apparatus positioned on a fluoroscope (OEC Mini 6600, OEC Medical Systems Inc., Salt Lake City, UT). A force gauge (AccuT-4, Yamoto Corp., Colorado Springs, CO) was attached to the tibia 2.8 cm from the joint space and extension forces of 600g, 1200g and 1800g were applied perpendicular to the tibia axis. The knee extension lag was measured for each force applied.

Histology

After decalcification five micron-thick transverse diaphyseal sections of each femur and tibia were made and stained with hematoxylin/eosin. Bone viability was measured using osteocyte counts, expressed as the ratio of lacunae containing osteocytes to the sum of filled and empty lacunae using specialized image analysis software (Image-Pro Plus 7.0, Media Cybernetics Inc. Bethesda, MD, USA) at 20 × magnification.

The tibia plateau cartilage and subchondral bone was similarly prepared. We measured cartilage thickness, and assessed cartilage quality using a previously described histology score (0; normal, 10; complete destruction)³⁹.

Quantitative Histomorphometry

Two different fluorochromes were administered, at 14 days (calcein; 20mg/kg SC) and 4days (tetracycline; 20 mg/kg I.M.) prior to sacrifice. The resultant fluorescent bands visible with UV microscopy of unstained five micron sections were analyzed for Quantitative

histomorphometry with a computerized semiautomatic image analysis system (Osteomeasure, Osteometrics, Atlanta, GA). We measured bone area (BAr) and bone perimeter at a 4× magnification. And single label perimeters (sL.Pm) of Calcein [bright green] or Tetracycline [yellow], double label perimeters (dL.Pm, Calcein and Tetracycline present) and the average distance of the double labels (Ir.L.Wi) using 20× magnification⁴⁰. This data was used to calculate the double label area (DLAr), bone formation (BFR) and mineral apposition rates (MAR).

All measurements were performed separately for femurs and tibias as well as for endosteal and periosteal layers.

Biomechanical testing – cantilever bending

Both the operated and the contralateral knees were explanted and exarticulated after range of motion measurements. The femur was fixed proximally in bone cement and a bending moment in anterior posterior direction was applied exactly 16 mm distal to the graft-host junction. The tibia was fixed in bone cement distally and measurements were similarly obtained with the bending force applied 16 mm proximal to the graft-host junction. A hydraulic material testing machine (MTS, Minneapolis, MN) was used for force application with the testing rate prescribed as 1 mm/min. The ultimate stress was calculated at two different sites (directly above the osseous fixation and at the point where failure actually occurred) using the following equation:

$$\sigma = F(L - L_0)D/I$$

(σ ultimate stress; F , maximal force; L distance root to applied force; L_0 distance to fracture; D outer diameter of the bone; I moment of inertia as calculated)

Biomechanical testing – Young's modulus of cartilage

Tibia plateaus were fixed on a Wood's metal base. A perpendicular force was applied using a 1 mm diameter indenter attached to a hydraulic material testing machine (EnduraTEC Elf 3220, Minnetonka, MN). The Force (N) and Displacement (mm) were measured using a 4.46 kg load cell (MOB-10 Load Cell 10LBS, Transducer Techniques, Temecula, CA). The Young's modulus of the cartilage was calculated with the following equation:

$$E = \frac{(1 - \gamma^2)\pi a}{2kh} S$$

(E = elastic modulus (Mpa); γ = Poisson's ratio (0.5); a = radius of the indenter (0.5 mm); k = factor dependent on the ratio a/h according to Hayes et al, h = cartilage thickness (mm))

Statistics

Statistical analysis was performed using SPSS software (SPSS Inc., Chicago, IL, USA). Results of bone healing scores, arthrosis scores and histologic quality assessment of tibia plateau cartilage were analyzed using a Chi-Square test. The results are expressed as medians.

Capillary density, contracture angles, bone viability, cartilage thickness, histomorpho-metric measurements, and biomechanical results of cartilage and bone were first analyzed for normal distribution using a Kolmogorov-Smirnov test. Results on the surgery side were

compared to the contralateral side using an independent samples t-test. Differences were considered significant at $P < 0.05$. Results in the manuscript are expressed as means $\pm$ SEM.

Results

Lower leg perfusion in 8 preliminary rabbits

Colorization of the anterior compartment muscles as a measure of lower leg perfusion was confirmed by direct inspection. Colorization was uniformly reduced on the study side when compared to the contralateral leg. However, all study legs showed at least some capillary filling judged to be sufficient for muscle survival.

Lower leg perfusion after autologous knee replantation

Corresponding with these results from preliminary perfusion studies, ten rabbits after knee joint replantation combined with surgical angiogenesis showed viable anterior compartment muscles at sacrifice. Foot dorsal extension was possible in all rabbits during the survival period.

Bone Healing

Rigid fixation with locking plates and self tapping screws allowed uneventful bone healing in all animals. Initial callus formation was observed as early as 2 weeks post surgery (average score 3). The maximum score of 24 points was reached by all animals after 8 weeks (figure 2). None of the grafts showed non-unions or graft fractures.

Arthrosis

Final assessment revealed a risk of moderate to severe degenerative arthritic changes in the transplanted knee. During the first 4 weeks only minor radiologic changes were observed (median score 1 (range 1–4 points). At final evaluation (16 weeks) 7 animals maintained joint status with a mean score of 2. However, 3 rabbits developed advanced degenerative changes with complete destruction of the knee joint (5 points, figure 3).

Microangiography

Patency of intramedullary SIEF-flaps (femur) and arteriovenous saphenous bundles (tibia) was observed in all animals after perfusion with Microfil[®] (Figure 4).

Neoangiogenic vessels arising from the implanted intramedullary tissue were observed in all bones. The average capillary density of the femur on the surgical side was 19.09 ± 0.03 %, and 14.40 ± 0.03 % in the contralateral control. These differences were not significant ($p=0.27$). Similarly, a trend for higher capillary density in operated tibiae was seen (17.85 ± 0.03 % and 16.34 ± 0.02 %, ($p=0.72$) for surgical and control bones, respectively).

Contracture

The contracture angle (knee position at maximum passive extension) evaluated on x-rays immediately prior to sacrifice was $78.0^\circ \pm 10.41^\circ$ in the surgical leg and $14.0^\circ \pm 3.7^\circ$ in the contralateral leg. These differences were statistically significant ($p<0.0001$).

Similarly, the average contracture angle measured *ex vivo* using fluoroscopy was significantly higher in surgical legs when compared to the contralateral side in all animals (mean knee extension at 600g: $8.0^\circ \pm 1.4^\circ$ [control] vs. $81.9^\circ \pm 17.6^\circ$ [transplant, $p=0.001$]; at 1200 g: $1.5^\circ \pm 1.2^\circ$ [control] vs. $71.5^\circ \pm 18.1^\circ$ [transplant, $p=0.001$] and at 1800g: $1.3^\circ \pm 1.2^\circ$ [control] vs $61.1^\circ \pm 19.3^\circ$ [transplant, $p=0.006$]).

Histology

The ratio of filled lacunae to the sum of filled and empty was $78.21 \pm 0.02\%$ in the transplanted femora and $80.26 \pm 0.01\%$ contralaterally ($p=0.38$). The tibias showed ratios of $72.23 \pm 0.03\%$ and $77.36 \pm 0.01\%$ on the surgical and contralateral sides, respectively; $p=0.16$.

The median *cartilage quality score* on the surgical side was 3.5, compared to a score of 0 in control knees ($p<0.001$). The average *cartilage thickness* in control knees was $489 \pm 57 \mu\text{m}$ and $368 \pm 84 \mu\text{m}$ in transplanted knees ($p = 0.25$).

Histomorphometry

Histomorphometry results are presented separately for femur and tibia and summarized in table 1.

Average endosteal single label perimeters (sL.Pm) in the surgical femurs were significantly higher when compared to the contralateral side ($p=0.049$), whereas periosteal sL.Pm were not significantly different ($p=0.09$). Likewise, endosteal double label perimeters were significantly increased on the surgical side ($p=0.003$). Endosteal bone formation rates (BFR) were significantly greater in transplanted bones ($p=0.006$), demonstrating considerable new bone production in areas supplied by the neoangiogenic autogenous circulation. Neither endosteal ($p=0.64$) nor periosteal ($p=0.93$) mineral apposition rates (MAR) were different when measurements on the surgical femur were compared to contralateral values.

Histomorphometric measurements for the surgical tibias showed similar differences. Endosteal single label ($p=0.043$) and double label perimeters ($p=0.001$) were significantly increased on the surgical side. No differences were observed for periosteal measurements. However, no statistical differences were observed for endosteal and periosteal BFR and MAR in comparison to the contralateral leg (table 1)

Cantilever Bending

In most animals, cantilever bending resulted in fracture at a site remote from the osteosynthesis. Failure at the transplant-tibia junction occurred in two rabbits and at both femur and tibia junctions in one additional rabbit. In all other tested specimens, failure occurred proximal to the femoral and distal to the tibial osteosynthesis.

We found the ultimate stress at the area of bone fixation to be significantly lower in surgical femora when compared to contralateral values ($100.59 \pm 9.57 \text{ MPa}$ vs. $204.95 \pm 36.69 \text{ MPa}$; $p=0.013$). Similar calculations for the tibiae were not significant ($189.38 \pm 16.76 \text{ MPa}$ vs $244.36 \pm 22.01 \text{ MPa}$; $p= 0.062$). The ultimate stress at the fracture site of the femurs was $88.84 \pm 13.49 \text{ MPa}$ on the surgical and $198.86 \pm 33.50 \text{ MPa}$ on the contralateral side ($p=0.007$), whereas the results for the tibiae were $116.68 \pm 27 \text{ MPa}$ (surgical leg) and $244.36 \pm 22.01 \text{ MPa}$ (contralateral knee). Differences were significant ($p= 0.002$, figure 5)

Young's modulus of cartilage

The Young's elastic modulus of the cartilage was $44.67 \pm 6.54 \text{ MPa}$ and $53.78 \pm 9.72 \text{ MPa}$ on the surgical and contralateral side, respectively. Differences were not significant ($p=0.43$).

Discussion

Segmental loss of a joint represents a considerable problem in reconstructive surgery. Since most reconstructive methods available still comprise important drawbacks, further research is required to improve patients' outcome.

Vascularized joint allotransplantation would be an ideal reconstructive method if problems of immunity could be overcome. Transplantation of a whole joint from a donor, similar to any organ transplant, obviates donor site morbidity for the patient, and might permit better healing at the bone junction sites than allograft bone while providing useful joint function. Reported results of a handful of cases to date have been poor^{15-17, 41}. Concerns regarding long-term immune modulation by tolerance induction or drug therapy make such non-life critical composite tissue transplants controversial. It should be noted that experimental work on whole joint transplantation, systematically studying the many parameters influencing results, remains deficient.

We have previously reported a novel method to maintain viability of bone allotransplants without long-term immunosuppression, using neoangiogenesis from autogenous tissue implanted within the bone at the time of transplantation and microvascular repair of the allogeneic nutrient circulation³¹⁻³⁴. A short two-week period of postoperative immunosuppression maintains viability of the allograft through the nutrient pedicle. Thereafter, new neoangiogenic blood supply from implanted recipient-derived tissue takes over graft perfusion, allows further bone survival and provides a source of pluripotential cells contributing to bone remodeling and healing. The creation of a neoangiogenic circulation is termed 'surgical angiogenesis'. Its application to transplant an entire joint could be an important advance in some types of composite tissue allotransplantation.

Since no model of joint allotransplantation with surgical angiogenesis exists to date we have developed a new model allowing knee joint allotransplantation for the future. To develop this new model we used autogenous joint transplantation in this preliminary study to answer if the application of surgical angiogenesis to composite tissue allotransplantation is feasible. Contact of bone to the vascularized host derived tissues in the intramedullary canal seems to be important to optimize ingrowth of neoangiogenic vessels in the bone. Therefore, we included a sufficient amount of fascia in the SIEF-flap and the saphenous arteriovenous bundle to fill the intramedullary canal of femur and tibia, respectively. One of the main questions was if neoangiogenesis occurs from these tissues in joints similar to previous studies that successfully applied these techniques to segmental diaphyseal bone³¹⁻³³.

Similar to previous results in segmental bone transplantation in rats and rabbits we found neoangiogenesis arising from implanted vascularized tissues in the transplants and since capillary density measurements on transplanted knees were generally increased when compared to the contralateral side we believe that neoangiogenesis from the implanted tissues improved the vessel density even in our autotransplants. Since the previous studies showed that these neoangiogenic vessels sufficiently allow bone survival in rats as well as rabbit allotransplantation³¹⁻³³, we believe that bone survival in allogenic knee joint transplantation is likely. Further knee allotransplantation studies with surgical angiogenesis and only short term immunosuppression are required to evaluate if the composite tissues of a complete joint allotransplant can survive only with a new neoangiogenic circulation arising from implanted host derived tissues, since the nutrient pedicle of allotransplants will be rejected and subsequently occluded after withdrawal of immunosuppression. Survival and material properties will be compared to allotransplants with long term immunosuppression and therefore patent nutrient pedicle. If viability of a knee joint allotransplant can be sufficiently maintained only by surgical angiogenesis, this may allow joint

allotransplantation without long term immunosuppression thereby significantly improving the risk benefit ratio of allogenic joint transfers.

Orthotopic knee joint allotransplantation is possible in rats^{19, 42, 43}. Previous investigators have isolated the knee joint on a single vascular pedicle in rabbits, cats and dogs decades ago. This included feasibility studies with knee isolation and autologous replantation^{23, 26–28} and allotransplants with immunosuppression^{23, 26–28}. However, high failure rates and infectious complications with less than 50% graft survival were consistently observed^{26, 27, 30, 44}. These high complication rates may be partly attributed to technical problems and insufficient immunosuppression.

Immunosuppression is not the only issue facing investigation of bone and joint transplantation. Skeletal fixation is a major problem in the rabbit model, due too their strong hind limb musculature and relatively poor skeletal strength^{23, 27}. The use of intramedullary rods, described by several authors^{30, 31, 34, 45} have high rates of non-union (20%) and incomplete remodeling (70%)^{31, 34}. Given the need to place vascularized tissue within the intramedullary space using our methods, this experimental model uses locking plates and screws. This technique provides stable fixation and resulting high union rates^{22–25, 44, 45}.

Another potential problem is lower limb ischemia. Knee removal, required prior to transplantation requires ligation of peroneal and anterior tibial vessels in the rabbit. This leaves the posterior tibial and saphenous artery intact. Preservation of the saphenous vessels has been reported as crucial to maintain perfusion of the hind limb in both rabbits and dogs^{27, 44}.

The need to place a saphenous arteriovenous bundle within the tibia for surgical angiogenesis in this model refutes this assertion, as our animals' demonstrated sufficient distal perfusion, including the anterior compartment muscles. None of our ten study animals showed perfusion problems, infection or signs of anterior tibial muscle dysfunction (e.g. drop foot) during follow up and all anterior tibial muscles were vital at sacrifice. In contrast to previous descriptions^{27, 44} we therefore showed that retrograde flow in the anterior tibial vessels through connections to the posterior tibial and peroneal vessels sufficiently maintain blood supply of the rabbit's hind limb including the anterior compartment. In the present study the transplant was autogenous, designed to test the surgical methods before embarking on allotransplantation in the future. In these studies the posterior tibial and the peroneal vessels must be maintained to ensure distal perfusion in the recipient animal since these are not subject to an immune response. The effect of knee joint transplantation on bone material properties has not been reported previously. However, Giessler et al found a significant decrease in average ultimate bone strength values in segmental bone autotransplantation in rabbits when compared to matching contralateral femoral segments³⁴. Additionally, several factors have been identified that reduce bone material properties in rabbits. Meffert et al. found the amount of soft tissue damage to impair bone regeneration and Park and colleagues observed a delay of regeneration of bone biomechanical properties after open osteotomies when compared to closed fractures^{46, 47}. Protection from biomechanical stress was shown to decrease bone strength in rabbits, since plate fixation of tibial osteotomies for 12 weeks lead to weaker bone than early plate removal and subsequent free ambulation⁴⁸. Since, soft tissue damage in knee transplantation is extensive, open osteotomies have to be performed and we did not remove the osteosynthesis plates the reduction of material properties in both tibia and femur after 16 weeks osseous integration time was expected.. Nevertheless, osseous union was successful, and the resulting reconstruction was sufficiently strong to resist fracture at the bone junction site. Indeed, only 20% of specimens subjected to cantilever bending failed through the osteotomy site. The ultimate stress values were still satisfactory

and clinically bone strength was sufficient to withstand the strong muscle forces of the rabbit's hind limb.

Histologic examination revealed viable bone in all specimens after isolating the knee on a single pedicle. This has also been demonstrated previously^{23–25, 29, 30, 44}. To the existing literature we provide new information on bone turnover and mineralization. Both are increased after orthotopic transplantation of vascularized knee autotransplantation. Giessler et al. also found significantly increased mineral apposition rates after segmental vascularized femur autotransplantation⁴⁷. In the present study, separate quantitative analysis of periosteal and endosteal bone surfaces revealed that higher mineralization and bone formation rates occurred only on endosteal surfaces. This was likewise observed in murine segmental bone transplantation⁴⁹ and may explain the findings after vascularized segmental bone autotransplantation observed by Giessler et al.³⁴. The predominance of endosteal new bone formation in our model is particularly interesting, since this is the surface most benefiting from neoangiogenesis from implanted vascularized tissue.

Knee joint transplantation requires extensive dissection of soft tissues. This seems to lead to considerable scarring and to severe knee contractures in the rabbit model. No other rabbit study reports this important measure of functional outcome. In the canine, normal weightbearing and motion has been reported by some^{22, 24, 25} and severe dysfunction described by others²³. We also found joint cartilage to be viable, but with evidence of arthritic change and reduced thickness as in another rabbit study³⁰. Less cartilage disorganization has been seen in dogs^{22–25}. We believe this may well be related to the rabbits' normal resting posture, with the knee maximally flexed.

Conclusion

In conclusion, we have developed a rabbit knee transplantation model, and report the resulting vascularity, bone histomorphometry, bone material properties and cartilage properties in orthotopic vascularized knee composite tissue autotransplants. This model can be used to evaluate experimental knee allotransplantation. We plan to use this model to test the potential role of surgical angiogenesis and short term immunosuppression to maintain joint viability and function. In this preliminary study we have described use of rigid bone fixation allowing osseous healing as well as the required methods to evaluate healing, bone material properties, cartilage integrity, joint contracture and quantitative measures of bone viability and remodeling. Radiographic, histologic, histomorphometric, microangiographic and biomechanical data are all promising and merit the future progression of the principle of surgical angiogenesis with short-term immunosuppression from our earlier work on bone allotransplants to composite tissue whole joint allotransplantation.

Acknowledgments

The authors wish to thank Synthes USA for the donation of locking plates and screws.

Grant sponsor: National Institutes of Health; Grant number: AR49718

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e3093241-001-0

Figure 1.

Operative technique: Through a medial skin incision all muscles were detached from the knee and the joint graft was isolated on its pedicle (popliteal vessels with multiple epiphyseal and metaphyseal branches). The knee was replanted and osteosynthesis of femur and tibia was accomplished using locking plates. The saphenous AV-bundle was raised from the lower leg and a fascial flap based on the superficial inferior epigastric vessels was harvested. The muscles were reattached to the graft and the saphenous AV bundle and SIEF-flap were pulled through tibia and femur, respectively.

Bone Healing Score Results

Figure 2.
Median bone healing scores of 10 rabbit knee autografts during follow-up (16 weeks).
Complete healing (24 points) was observed in all cases.

Figure 3.
Median arthrosis scores (0; no arthrosis, 5; complete destruction) of 10 rabbit knee autografts during follow-up (16 weeks).

Figure 4.

A; Visualization of a patent intramedullary SIEF-Flap with subsequent capillary sprouting (black arrows) after femur clarification with the Spaltzeholz-technique. B; Histologic view (10 × magnification) of a patent intramedullary arteriovenous saphenous bundle (H&E-stain, black artefacts due to persistent Microfil® in the vessels).

Figure 5. Ultimate stress (MPa) at the root and at the fracture site with cantilever bending testing (*, significant difference).

Table 1

Histomorphometric results: All measurements were performed separately for femur and tibia as well as endosteal (endo) and periosteal (peri) layers. (sL.Pm; single label perimeter, dL.Pm; double label perimeter, BFR; bone formation rate, MAR; mineral apposition rate, s.; significant differences, n.s.; no significant difference).

Bone	Parameter		Surgical Leg	Contralateral Leg	Significance
Femur	sL:Pm	endo	13.97 ± 4.14	5.31 ± 1.63	s.
		peri	6.09 ± 1.16	11.58 ± 3.09	n.s.
	dL:Pm	endo	26.08 ± 470.	6.42 ± 1.93	s.
		peri	15.24 ± 2.75	18.29 ± 9.02	n.s.
	BFR	endo	74.25 ± 12.63	20.83 ± 11.38	s..
		peri	36.24 ± 6.43	80.76 ± 66.65	n.s.
	MAR	endo	2.99 ± 0.32	2.69 ± 0.55	n.s.
		peri	2.44 ± 0.16	2.37 ± 0.78	n.s.
Tibia	sL:Pm	endo	11.22 ± 2.13	5.55 ± 1.34	s.
		peri	8.77 ± 2.23	9.74 ± 2.94	n.s.
	dL:Pm	endo	21.39 ± 3.72	5.37 ± 1.44	s.
		peri	16.34 ± 3.73	19.59 ± 8.09	n.s.,
	BFR	endo	116.69 ± 57.81	14.59 ± 5.04	n.s.
		peri	83.29 ± 47.10	78.52 ± 55.14	n.s.
	MAR	endo	4.31 ± 1.14	2.39 ± 0.34	n.s.
		peri	3.57 ± 0.82	2.25 ± 0.55	n.s.