Transplant International

ORIGINAL ARTICLE

A novel rat microsurgical model to study the immunological characteristics of male genital tissue in the context of penile transplantation

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ABSTRACT

Penis transplantation represents an exciting new avenue for restoration of male genitalia and function after devastating tissue loss. This animal model is designed to fill a critical void to study immunologic aspects related to reconstructive transplantation of male genitalia. A rat penile graft dissection was designed based on the internal pudendal arteries and dorsal penile vein and includes the skin of the prepuce. A nonsuture cuff technique was used to anastomose the graft vessels to the recipient superficial epigastric and femoral vessels. Seventy-seven penile transplantations were performed. Graft design yields suitable caliber and length of vessels at the radix of the penis. Anastomosis of the dorsal penile vein and the internal pudendal arteries insures optimal graft perfusion. The nonsuture cuff technique allows for successful microvascular anastomosis by a single surgeon with an average overall operative time of 2.5 h. Long-term graft survival (>30 days) was observed in syngeneic transplants. We have established a robust murine model with ideal vascular perfusion of penile tissue to study the unique immunobiology of male genitourinary allotransplantation. Heterotopic inset further allows for visual monitoring of graft viability, while the native penis serves as an optimal control.

Transplant International 2020; 33: 796–805

Key words
animal model, microsurgery, penile transplantation, rat, transplantation model, vascularized composite allotransplantation

Received: 14 June 2019; Revision requested: 8 July 2019; Accepted: 2 March 2020; Published online: 2 April 2020

Introduction

The field of vascularized composite allotransplantation (VCA) saw its first breakthrough surgery in 1998 [1] when a team led by J.M. Dubernard performed a successful hand transplant. Hand transplants were followed by facial transplants, the first of which was reported in 2005 [2]. Uterine [3] and penile transplants were more recent additions to the expanding field of VCA. These are grafts that enable their recipients to conceive and bear children, breaking new ground for reconstructive and restorative allotransplantation [4,5]. Penile transplantation has become a clinical reality with successful

doi:10.1111/tri.13603

efforts reported in South Africa and the USA in 2014 [6], 2016 [7], 2017, and 2018.

Due to the success of the procedure in the short term, penile transplantation has garnered support as a viable option for reconstruction of the penis, groin, and perineum in patients with extensive injuries that cannot be reconstructed with conventional operative techniques [8]. Early reports demonstrate that penile transplants can result in restoration of normal urinary and a satisfying level of sexual function [6]. Furthermore, they show that immunosuppression protocols used in other types of VCA sufficiently prevent rejection in the first year post-transplant [6]. However, many questions regarding the immunogenicity of penile grafts remain, as data discussing the immunological aspects specific to penile transplants remain limited.

To study the immunological characteristics of the unique components of penile transplants—namely, urethral lining tissue and the corporal bodies—animal models are crucial. Several rodent penile transplantation models have been developed, each with relevant limitations when applied for immunological research [9-12]. Building on the extensive experience of our group in rodent microsurgery and surgical model development [13-19], we present a novel, highly reproducible technique for a heterotopic penile transplantation in rats with a >90% success rate using a nonsuture cuff technique for revascularization (Fig. 1). The method is specifically designed to accommodate immunological research pertaining to transplantation of the male genitalia and has been used as such [20]. A key component of the study design is the placement of the graft on the dorsal aspect of the thigh, facilitating simple visual monitoring of the graft.

Materials and methods

Male, 8- to10-week-old animals with fully grown genitalia were used for this model. For the study of transplant rejection, a fully mismatched strain combination [Brown Norway (BN) to Lewis (LEW)] was utilized.

Animals were housed in pathogen-free facilities and were cared for in accordance to the Johns Hopkins University Animal Care and Use Committee (Protocol no. RA16M178), in compliance with the guidelines published by the National Institutes of Health (NIH Publication no. 86-23, revised 1985). All surgeries were performed using microsurgical instruments (S&T AG, Neuhausen, Switzerland).

Donor penis procurement

The donor is sedated with 4% isoflurane gas anesthesia, and the animal is maintained on 2% inhaled isoflurane throughout the procedure. The surgical area in the anterior groin is shaved, and the operative field is scrubbed once with 70% alcohol and once with 10% povidone-iodine. The rat is positioned in a stable, supine position to expose the operative field using a sterile field drape, sterile instruments, and a high magnification surgical microscope (40×). Toe pinch withdrawal reflex is tested to monitor adequate depth of anesthesia prior to starting the procedure.

A midline incision (~2 cm) is made in the pubic skin cranial and caudal to the penis (Fig. 2a). The hairless prepuce is separated from hair-bearing skin using scissors, cutting the skin proximal to the orifice of the bilateral preputial sebaceous glands (Fig. 2b). Microforceps and micro-cautery are used to proximally dissect the penis down to the pubic bone, and the venous plexus that covers the base of the penis is exposed (Fig. 2c). Cautery is used to divide bilaterally the venous pudendal plexus and the inferior external pudendal vein. A symphysiotomy is then performed using a Mayo scissor (Fig. 2d).

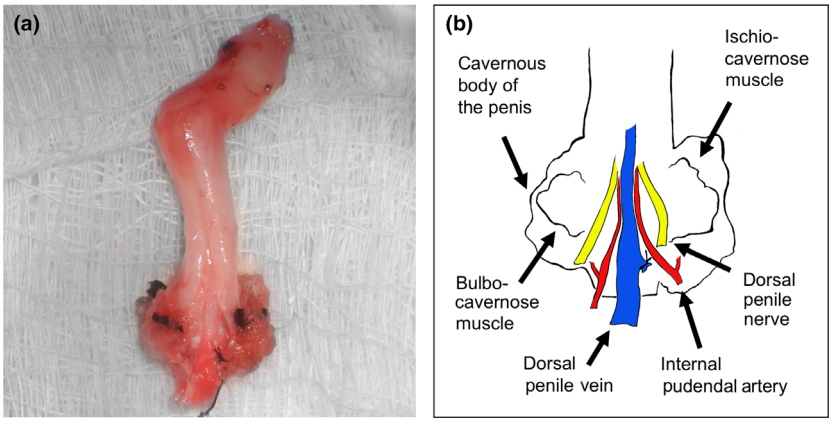

Figure 1 Graft design. The graft is designed to use the dorsal penile vein and the bilateral internal pudendal arteries. Both dorsal penile nerves are included in the graft.

Using micro-forceps, the dorsal penile vein (DPV), dorsal penile nerves (DPN), and the dorsal penile arteries (DPA) are dissected at the base of the penis (Fig. 3a). Starting 5 mm distal from the pubic bone, the DPAs are dissected distally to the point where DPV and the DPAs disappear beneath the tunica albuginea and enter the corpus cavernosus. A 6-0 silk suture is used to manipulate both vessels and nerves while dissecting (Fig. 3b).

The DPV is released from underlying tissue with proximal dissection using a 6-0 silk suture to manipulate the vein in a no-touch fashion. Beneath the vein's bifurcation into the pudendal plexus, the DPV is closely integrated in the cavernous body. Micro-scissors are used to dissect beneath the vein and one of the branches of the venous pudendal plexus is ligated with four 8-0 silk ligatures. The tissue between the ligatures is coagulated and then transected with scissors (Fig. 3c).

The vein is then dissected proximally into the pelvis to obtain additional length (2 mm), and both DPAs are dissected proximally into the pelvis. Sparing the deep penile artery, all other branches, including the bilateral arteries of the urethral bulb (that tissue is not part of the graft), are coagulated and cut, finishing the dissection at the level of the internal pudendal artery (IPA). The penile nerves that accompany the DPA are also disssected.

Then, the cavernous bodies are dissected, ligated with a single 2-0 silk ligature at the bifurcation, and cut proximally. The ischiocavernosus and bulbocavernosus muscles are ligated bilaterally using 2-0 silk ligatures and transected with the micro-cautery, carefully avoiding the vessels and nerves. The urethra is ligated proximal to the base of the penis with a 2-0 silk ligature and transected proximal to the ligature using the micro-cautery. Both the IPAs as well as the DPV are ligated with 8-0 silk ligatures, and the vessels and nerves are transected with scissors (Fig. 1).

The graft is then flushed through both IPAs with 5 ml of cold (4°C), heparinized (30 IU) saline, and stored at 4°C wrapped in saline-soaked gauze (Fig. 4a,b).

Recipient preparation

The recipient animal is sedated, prepared, and positioned per the donor procedure. The hair in the operative field in the groin region as well as the dorsal aspect of the entire leg is shaved.

Parallel and immediately superficial to the inguinal ligament, a 2 cm incision is made in the groin skin of the animal using scissors and the groin is dissected to expose the inferior external epigastric pedicle. The inferior superficial epigastric artery (SEA) and vein (SEV) are carefully dissected from their origin at the femoral pedicle to the bifurcation in the inguinal fat (Fig. 4c). The superficial femoral artery (SFA) is dissected from the origin of the SEA and SEV to about 2 cm down the leg, and the distal 3 mm of each vessel is skeletonized. The SEA and SEV are then ligated at the level of their respective bifurcations in the distal fat using an 8-0 silk ligature, and the SFA is ligated at the distal end of the dissection (Fig. 4d).

The SEA and SEV are clamped at their proximal origin with a single micro clamp and the SFA proximally with a second clamp. All vessels are cut proximal to

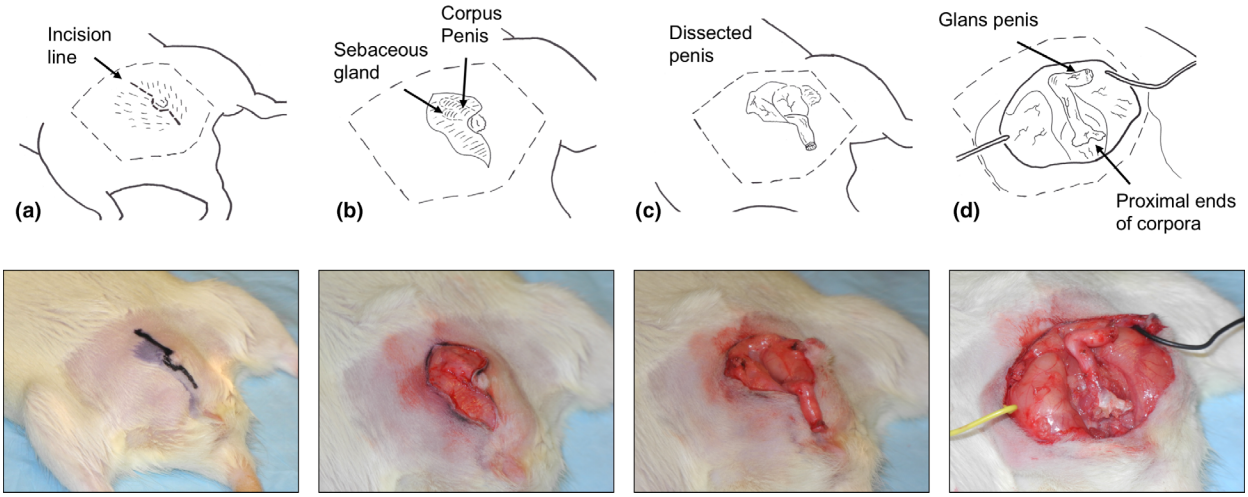

Figure 2 Surgical approach. *Donor procedure:* (a) Ventral view of the incision in the midline across the prepuce. (b) Ventral view after superficial dissection. (c) View after dissection of the entire penis. (d) Graft appearance after ligation of the proximal ends of the corpora.

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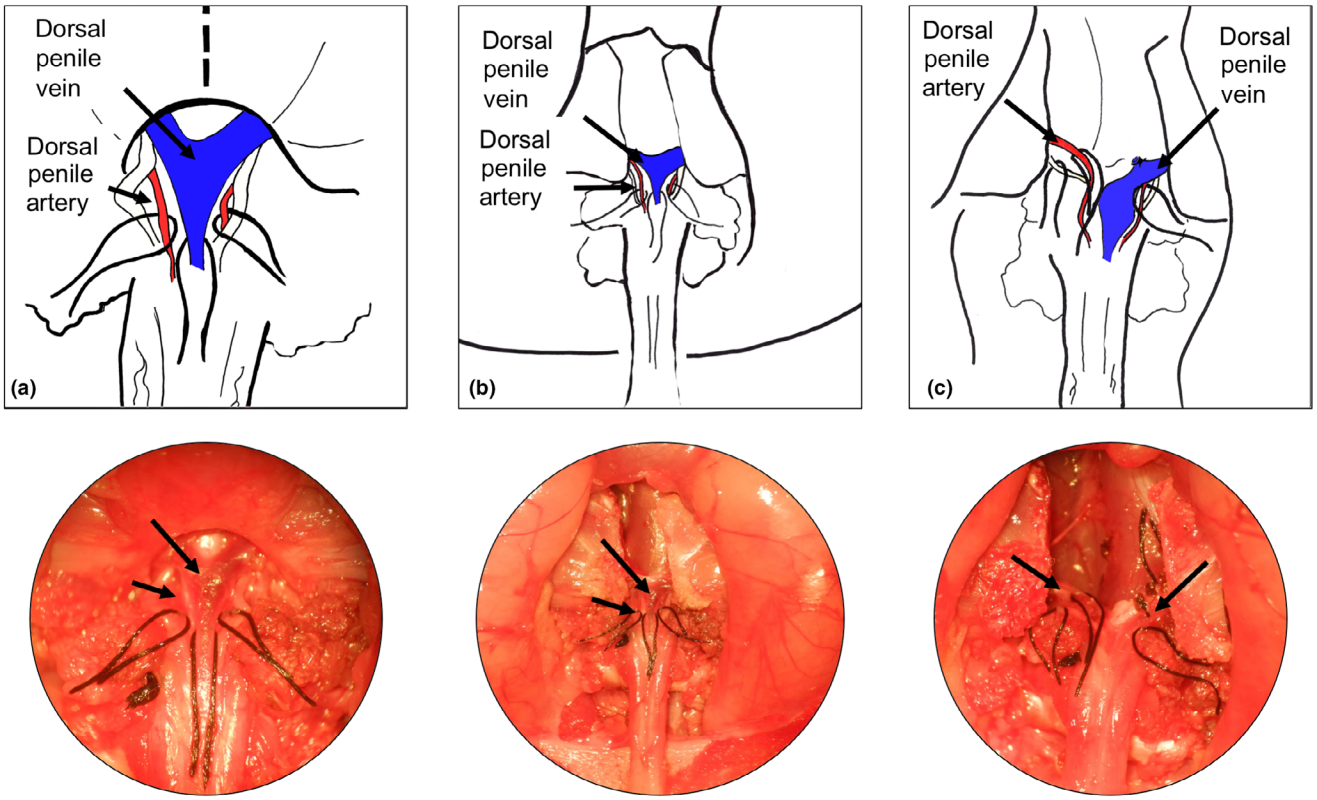

Figure 3 Crucial steps in vascular dissection. (a) Dissected dorsal penile vein, arteries, and nerves. (b) Surgical appearance after cleaving of the symphysis pubis. (c) Surgical appearance after ligation of the right branch of the dorsal penile vein.

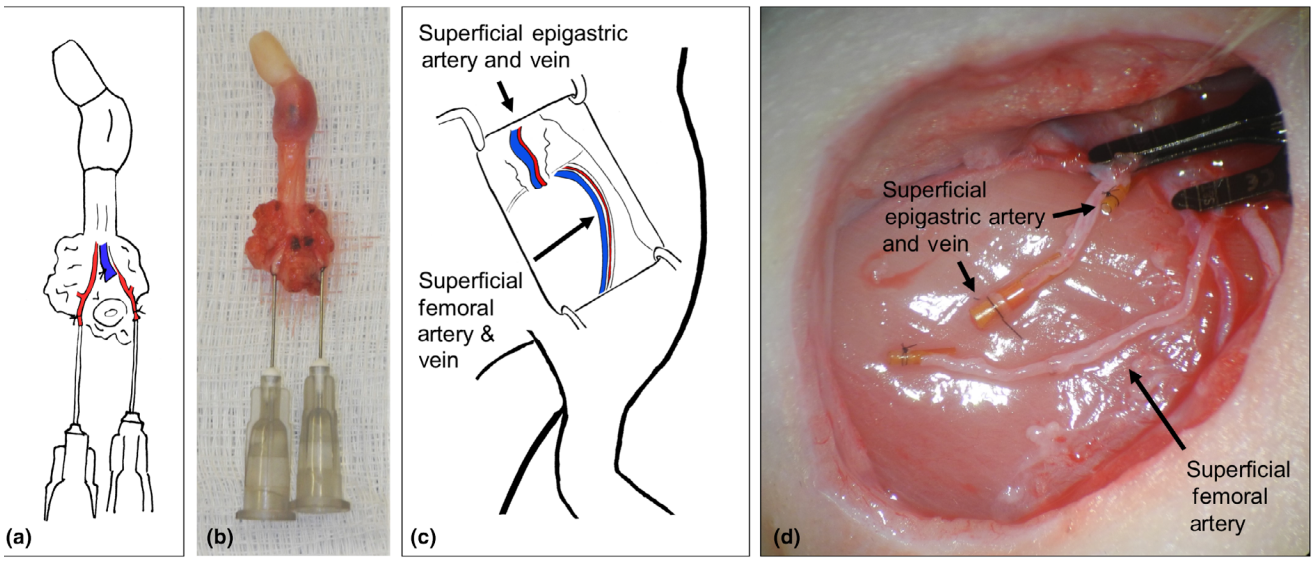

Figure 4 Graft and recipient vessel preparation. (a,b) Explanted graft during perfusion. Blue: dorsal penile vein. Red: bilateral internal pudendal arteries and dorsal penile arteries. (c) Appearance of groin after skin incision. The superficial epigastric artery and vein and the superficial femoral artery and vein are visible. (d) Superficial epigastric artery and vein and superficial femoral artery after dissection and placement of cuffs.

their ligatures. A 27-gauge polymide cuff is placed on both arteries and a 21-gauge cuff on the vein, after which the vessels are covered and protected with a moist gauze (Fig. 4d).

Graft implantation

The recipient groin incision is spread with a retractor, a moist gauze is placed in the space between the recipient's tail and leg, and the graft is placed in the groin with the glans resting on the gauze. The SEA is then clamped with a single micro clamp, which is stabilized in a horizontal position using a mosquito forceps and a stabilizing base. The lateral IPA of the graft is then anastomosed to the recipient SEA using the cuff (Fig. 5a,b).

The recipient vein is similarly clamped and stabilized and anastomose the dorsal penile vein to the SEV using the cuff technique [18]. The medial IPA of the graft is then anastomosed to the recipient SFA with the cuff (Fig. 5c).

All vessels are then unclamped at their origin. The entire graft should be perfused within 30 s. Note: Sufficient perfusion is confirmed by (i) oozing from the prepuce, (ii) bright pink coloration of the glans and corpora, (iii) venous return though the recipient vein. Once perfusion is confirmed and hemostasis is obtained, the base of the graft is sutured to the abdominal wall. The stumps of the ischiocavernosus and bulbocavernosus muscles, as well as stumps of the cavernosus bodies, can be used for this purpose.

The animal is then turned over on its side so that the dorsal aspect of the leg on the recipient's side is exposed. A 5 mm-diameter skin defect is created on the caudal dorsal aspect of the thigh followed by a subcutaneous tunnel from the defect to the groin using forceps and curved scissors. The glans penis is gently guided out of the dorsal incision through the subcutaneous tunnel. The edges of the prepuce are sutured into the incision with 6-8 standing 6-0 nylon sutures (Fig. 6). Hemostasis is confirmed, the subcutaneous (fat) layer is closed with a running 4-0 Polysorb suture, and the groin incision is closed with 6-8 standing 4-0 nylon sutures.

Postoperative care

Postoperative analgesia is provided with buprenorphine (0.2 mg/kg subq) every 12 h for the first 7 days. The animals are administered up to 5 ml of normal saline subcutaneous to compensate for perioperative fluid loss and placed in a preheated cage under a heating lamp or on a heating pad to completely recover from anesthesia. For antibiotic coverage, enrofloxacin 10 mg/kg subq is administered daily for 10 days. The surgical site is monitored for infections, and the weight of each recipient animal is obtained every day postsurgery. Weight loss greater than 15% percent must be considered an endpoint.

Results

Using this method, a total of 80 grafts were transplanted with a >90% surgical success rate for the underlying studies. Surgical failures resulted from postoperative bleeding ($n = 4$) and graft thrombosis ($n = 3$). Surgical

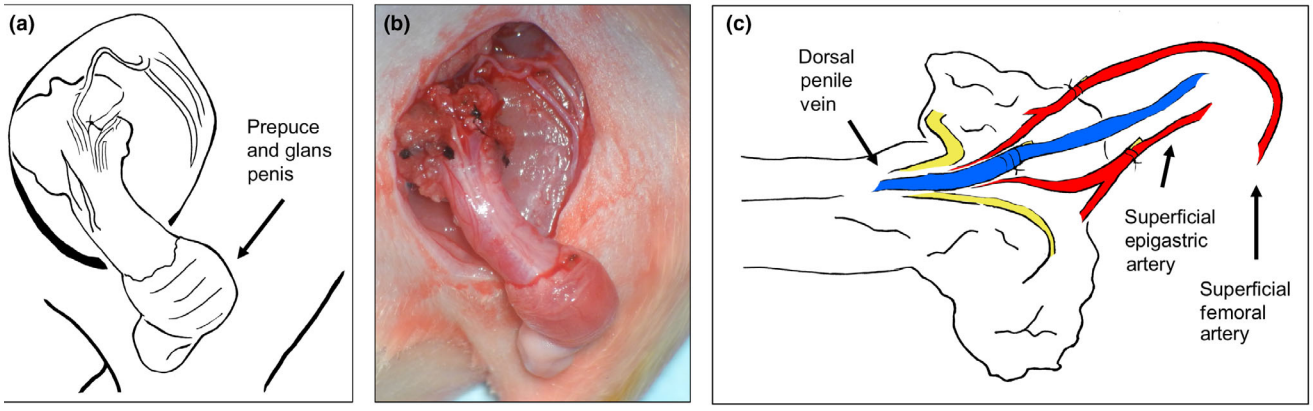

Figure 5 Vascular anastomosis and graft inset. (a,b) Overview of graft placement in the groin. Surgical field and graft after reperfusion. Note the complete return of color to the graft. (c) Graft vessels anastomosed to recipient vessels through nonsuture cuff technique. Blue: dorsal penile vein anastomosed to the superficial epigastric vein. Red: top; superficial femoral artery anastomosed to the medial internal pudendal artery. Bottom; superficial epigastric artery anastomosed to the lateral internal pudendal artery. Yellow: penile nerves.

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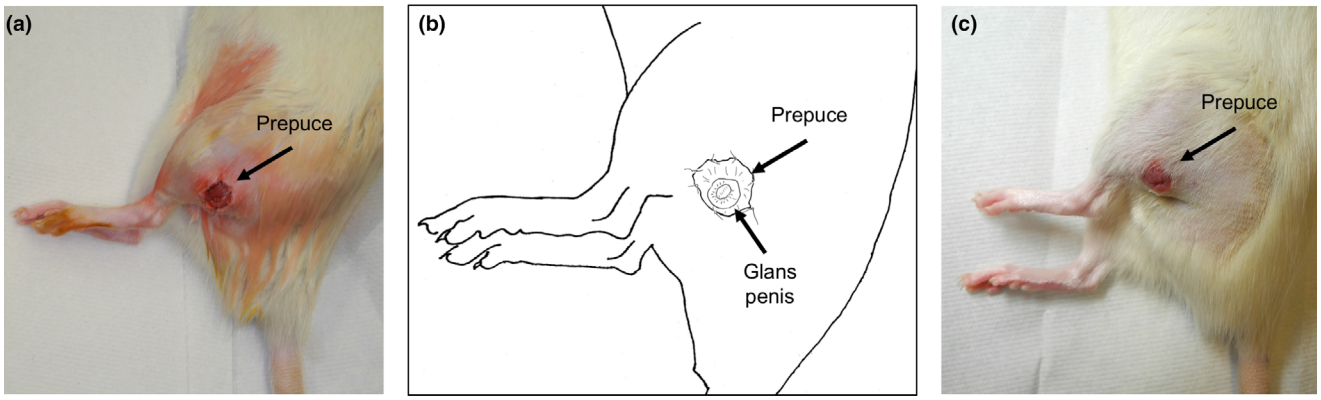

Figure 6 Graft placement on dorsal aspect of the thigh. (a) Syngeneic transplant, graft placement immediately postsurgery. (b) Schematic overview of graft placement. (c) Syngeneic transplant at POD7.

site infections are fully prevented with antibiotics (enrofloxacin). Total graft ischemia time is limited to 45–70 min. Clinical allograft rejection can be monitored easily due to the heterotopic inset location at the dorsal aspect of the thigh in both rejecting and nonrejecting grafts (Figs 6 and 7). After successful surgery, graft viability was monitored by daily visual inspection. All syngeneic grafts remained viable until their respective endpoints of POD 14–POD 90 (Fig. 7). Histological samples of all syngeneic grafts showed viable tissue and no signs of necrosis (Fig. 8).

This advanced rat penile transplant model was designed for the assessment of immunobiologic features of tissues specific to the male genitalia, such as urethra and corpora, in the setting of vascularized composite allotransplantation. The design enables transplantation of the complete penis on a pedicle that ensures optimal perfusion of both superficial and deep graft tissues through both penile arteries (Fig. 1). The used technique results in successful donor and recipient procedures, including microvascular anastomosis by a single surgeon with an average operative time of 2.5 h.

Discussion

For the reconstruction of devastating injuries with extensive tissue loss, hand and face transplants have evolved as valid treatment options for cases not amenable to conventional reconstructive methods. More recently, penile transplantation has proven to be clinically viable in the short term with the use of conventional immunosuppressive protocols [6,7].

The goal of penile tissue transplantation is trifold: to restore body image, regain voiding function, and enable sexual intercourse. All of these functions can only be regained when the patient's immunological response to the graft is successfully controlled. The primary, overarching goal in penile transplantation and (reconstructive) transplantation as a whole is thus a state of immune quiescence that allows for acceptance of the transplant with reasonable amounts of maintenance immunosuppression. Despite highly encouraging early results in four human recipients, little is known about the long-term outcomes of penile transplantation and the accompanying immunosuppressive

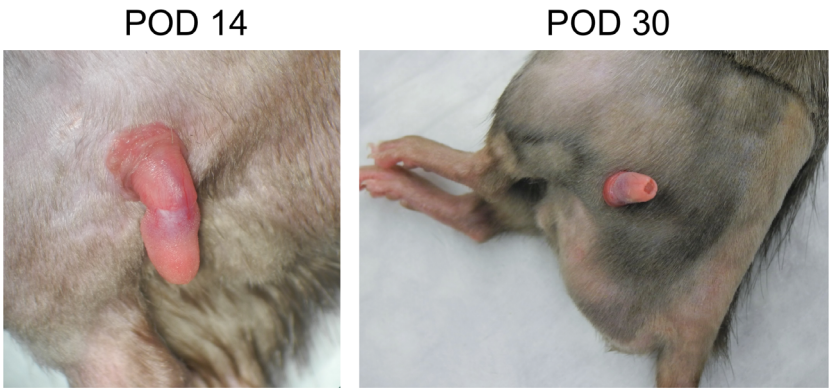

Figure 7 Clinical images of syngeneic grafts 14 and 30 days post-transplant. Graft color is indicative of ample perfusion at all timepoints. No sign of rejection is visible at any timepoint.

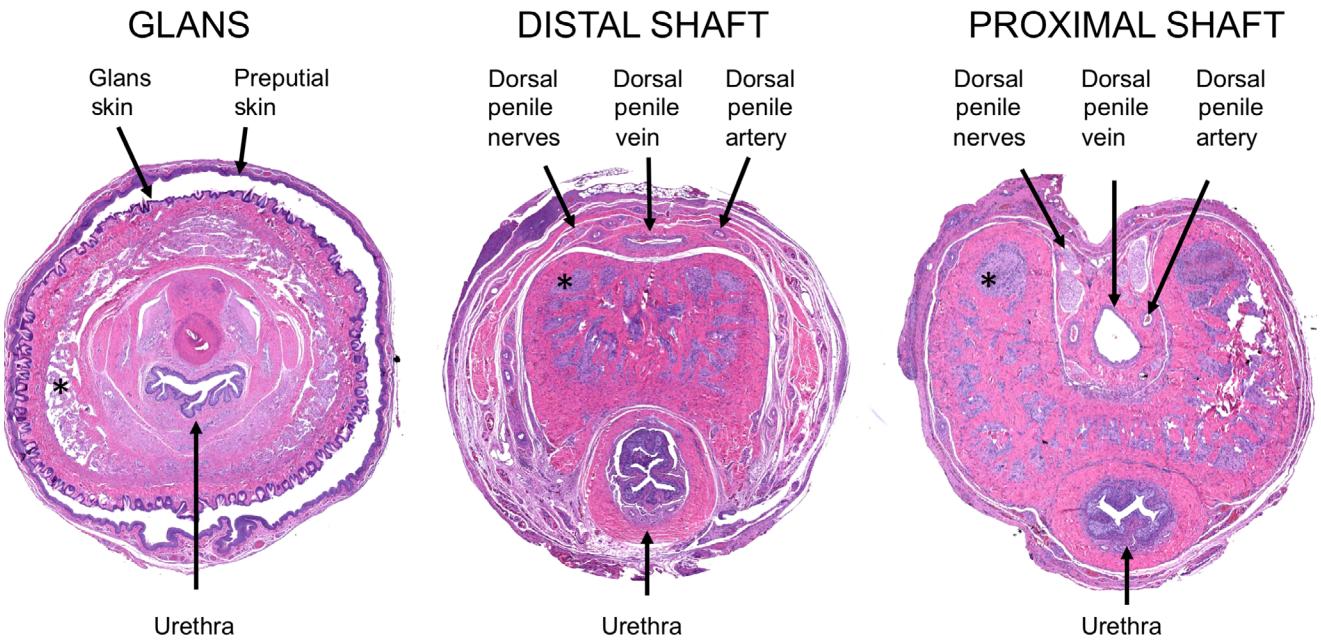

Figure 8 Histology images of a syngeneic penile graft at postoperative day 14. *Left.* Cross section of the penis at the level of the glans features double layer of squamous epithelium including glans (inner) and preputial skin (outer). The vascular channels of the glans are open (*). *Center.* Cross section of the penis at the level of the distal shaft allows visualization of the dorsal neurovascular bundle. Vascular channels of the corpora cavernosa show fibrous obliteration (*). *Right.* Cross section of the penis at the levels of the proximal shaft. Dorsal vessels and nerves are of larger diameter are visible and patent. As in the distal shaft, vascular spaces of the corpora cavernosa show fibrous obliteration of the lumen, associated with impaired corporal outflow that is associated with the model design (*). All tissues appear fully viable on histology.

treatments. Currently, we are unaware of any animal studies that address penile transplant outcomes or the effectiveness of immunosuppressive treatments in the setting of penile transplantation. To enable researchers that aim to expand the limited knowledge, our group sought to design a male genital transplant model. Considering that rodent studies are currently the main *in vivo* model for transplant immunology research and that rat models supply fully mismatched rat strains and combine relative affordability with sufficient penile vessel size, our group used the rat for this penile transplant model. An earlier, single-artery model developed by our group has been used in a previously published study on rat penile rejection in fully mismatched strain combinations [20]. In this study, 25 allogeneic and six syngeneic transplants were clinically and histologically monitored at postoperative days (POD) 3-30. Allogeneic grafts were found to reject in a 4-stage clinical progression. Epidermolysis clinically started at POD 7, and full rejection and necrosis were found to occur between POD 14 and 16. Histological analysis showed that skin and urethral lining tissue were first rejection targets followed by tunica albuginea and corpora cavernosa in a distal to proximal pattern.

Subsequent experiments that involveld greatly extended ischemic times demonstrated a high rate of vascular complications using the single-artery inflow model. These complications necessitated improvements to the graft's blood supply and were resolved by the modification of the model to include a second arterial anastomosis, the technique that is demonstrated in this manuscript. To confirm that the addition of a second arterial anastomosis did not significantly alter the immunological properties of the model, three additional allogeneic transplants were performed and histologically analyzed at POD 3, 5, and 7. These transplants had a clinical (Fig. 9) and histological (Fig. 10) rejection pattern that was the same as the pattern found in the previously published study using the single-artery model [20]. As the rejection pattern was unaltered by the addition of vascular inflow, we conclude that this model provides improvements in risk of tissue ischemia but is similar to the previous model when applied in immunological research. As it involves a second anastomosis, the double-artery surgery is more elaborate than the single-artery approach, but only minimally adds to the surgical time (approximately 5–10 min for harvest and implant combined).

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Although various small [16-19] and large [21] animal models have been described for penile replantation and transplantation, they are limited in their application for immunological research of the vascularized penile graft. The first reported penile transplantation model, described by Koga *et al.* [10] was a nonvascularized model, with the graft placed in a pouch created within the recipient's omentum. Though the graft was reported to revascularize in the omentum, graft monitoring could only be achieved via laparotomy. The model proposed by our group connects the superficial epigastric artery and vein and the superficial femoral artery to the dorsal penile vein and pudendal arteries, using all the existing physiological graft vasculature, which closely resembles clinical graft design and perfusion. The model leads to adequate perfusion and perfect graft survival in a successful syngeneic transplant.

Karamürsel *et al.* [9] designed an autologous transplantation model: They anastomosed the graft's right IPA and IPV to either the femoral or the saphenous artery and vein. The graft was implanted on the ventral aspect of the thigh or rerouted to the pubic region. In this model, there is a considerable size mismatch between recipient and donor vessels, which complicates surgical anastomosis. More importantly, in our experience, graft placement on accessible areas such as the ventral aspect of the thigh allows animals to auto-mutilate the transplanted tissue. Our model is a heterotopic model in the rat groin, which tunnels the glans penis to the dorsal aspect of the thigh, making the graft visible at the dorsal aspect of the hind limb (Figs 6 and 7). This vascularized design facilitates daily graft inspection in the conscious animal and obviates the need for repeated anesthesia while keeping the animal from damaging the graft.

Sonmez *et al.* [12] described a heterotopic allogeneic penile transplantation model; the authors anastomosed the graft's corpus spongiosum and dorsal penile vein to the saphenous artery and vein. The graft was placed in the pubic region, after rerouting of the recipient's native penis into the scrotum. While the size match between corpus spongiosum and saphenous artery may be more

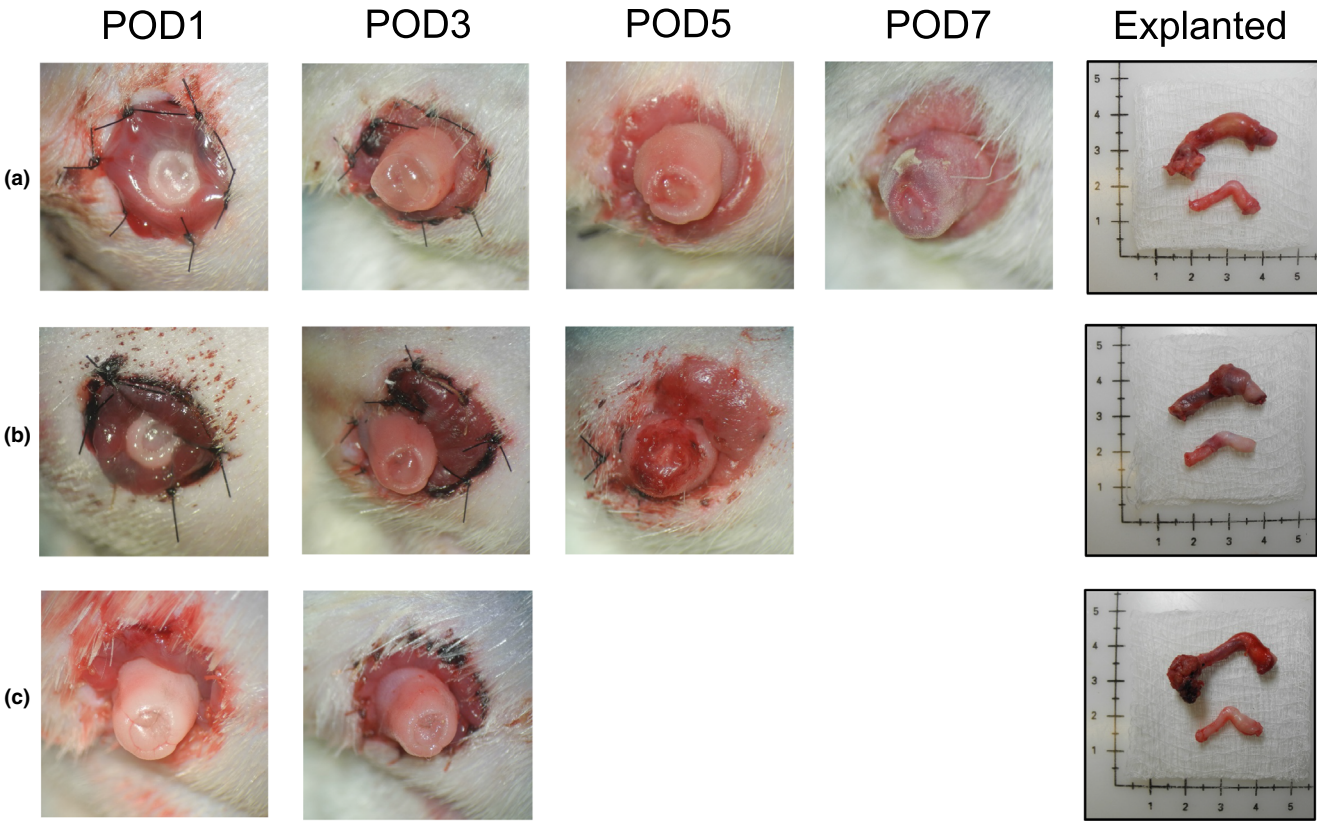

Figure 9 Allogeneic (BN into LEW) penis transplants POD1–7 using a double-artery model. Rows a–c. POD1: Normal graft appearance. POD3: Erythema and edema of glans and preputial skin. POD5: Increased edema and erythema. POD7: Erythema, edema, generalized epidermal sloughing. Explanted column: grafts are compared with native penises. Marked edema is visible after explantation at all timepoints (a: POD7, b: POD5, c: POD3).

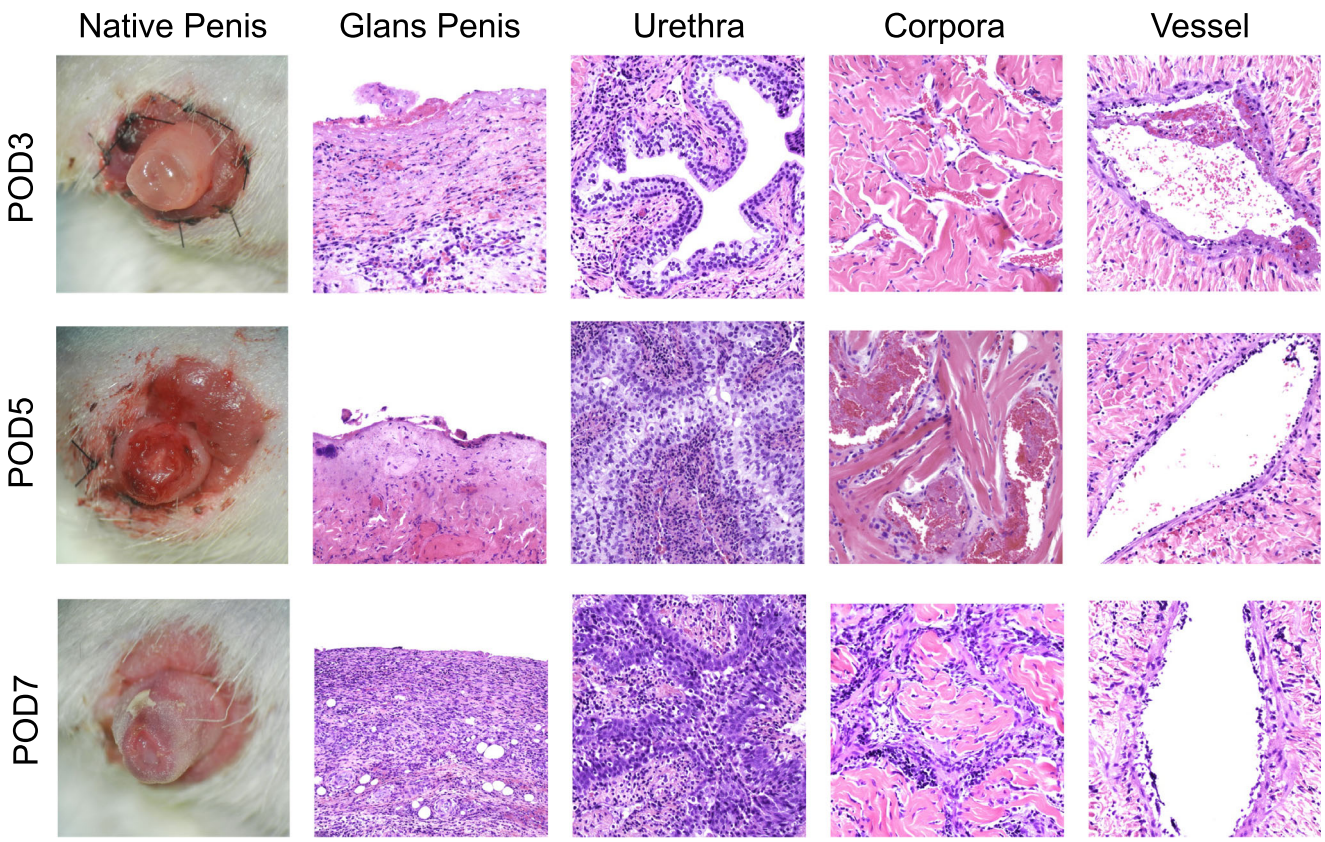

Figure 10 Clinical and histological images of allogeneic rat penile transplants. POD3: Grade II rejection; histological sloughing of epidermis, mild-moderate inflammation of the skin. POD5: Grade III rejection; dense inflammation of the skin, moderate urethritis, minimal inflammation of corpora. POD7: Grade III rejection; dense severe inflammation of the skin, severe urethritis, increased inflammation of corpora.

adequate, nonphysiological arterial perfusion via the corpus spongiosum may lead to non-descript histological changes and may further alter the rejection process, thus limiting the value of results obtained. The model as described by our group uses physiological bilateral vascular perfusion, which is enabled by the use of the earlier described cuff technique (Figs 1 and 5). With the cuff technique, the 0.1–0.2 mm vessels of the penile pedicle can be anastomosed reliably with minimal to no blood loss.

Zhao *et al.* [21] described an orthotopic penile transplantation model in the Beagle dog; anastomoses of the deep dorsal vein, dorsal arteries and nerves, as well as the corpora cavernosa and urethra were performed. The recipients were catheterized. Eight grafts were lost early after surgery; in the twelve remaining recipients, urinary catheters were removed at POD 7 and the authors reported physiological urination with a linear stream. This model shows promise for translation, but is very resource-intensive and could be limited by the large bone marrow-containing baculum in the dog penis. As a first platform for immunology research in penis transplantation, we believe the rat is the best model system.

Given our model's intended use for immunological research, our group deemed orthotopic placement too great a burden on the recipient animal. Orthotopic placement requires recipient penile amputation and carries a significantly increased risk for surgical failure. In addition to vascular anastomosis, orthotopic placement requires coaptation of erectile tissue and the urethra. These added surgical procedures create considerable risks of urinary retention and hematoma. This is illustrated by the experience of Seyam *et al.* [11], who transected and replanted rat penises immediately distal to the bulb with anastomoses of the dorsal vein, dorsal nerves, and tunica albuginea. They report that initial attempts to anastomose the urethra resulted in animal death from urinary retention and describe that anastomoses of the dorsal penile arteries could not be performed, resulting in compromised graft viability.

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Limitations

Like every heterotopic transplant model, the one described in this article has certain limitations regarding its functionality: There is no voiding through the urethra, nor is there erectile function. Fibrosis of the erectile bodies and minor signs of inflammation of urethral tissue is observed in syngeneic controls, which can possibly be attributed to the heterotopic design. Finally, it is important to note that the bone marrow-containing baculum in the rat penis can possibly be a confounding factor in the rejection process. These findings need to be taken into account when interpreting the outcomes in studies using our model.

Conclusions

In summary, we developed a method for penile transplantation in rats using a nonsuture cuff technique, which has proven to be a feasible model with a high success rate. Given its heterotopic placement, the model is best suited for immunological or tissue preservation research. This manuscript is intended to enable future research into the specific immunological aspects of penile transplantation.

Authorship

SAJF: created the surgical model, performed all of the operations, collected samples, and wrote the manuscript. GJF: assisted with model design, assisted with operations, and edited the manuscript. AM: did histopathological analysis and assisted with manuscript figures. MC: assisted with sample collection and edited the manuscript. JWE: assisted with administration and critical manuscript revision. BCO: assisted with model design and assisted with operations. DV: assisted with study design and critical revision. WPAL: assisted with study design. RJR: assisted with study design. DSC: assisted with study design, experimental oversight, and critical revision. GB: designed the research study and performed experimental oversight and critical revision of the manuscript.

Funding

The authors have declared no funding.

Conflicts of interest

The authors have declared no conflicts of interest.

REFERENCES

1. Dubernard J-M, Owen E, Herzberg G, *et al.* Human hand allograft: report on first 6 months. *Lancet* 1999; **353**: 1315.
2. Dubernard J-M, Lengelé B, Morelon E, *et al.* Outcomes 18 months after the first human partial face transplantation. *N Engl J Med* 2007; **357**: 2451.
3. Brännström M, Johannesson L, Dahm-Kähler P, *et al.* First clinical uterus transplantation trial: a six-month report. *Fertil Steril* 2014; **101**: 1228.
4. Johannesson L, Kvarnström N, Mölne J, *et al.* Uterus transplantation trial: 1-year outcome. *Fertil Steril* 2015; **103**: 199.
5. Brännström M, Johannesson L, Bokström H, *et al.* Livebirth after uterus transplantation. *Lancet* 2015; **385**: 607.
6. van der Merwe A, Graewe F, Zühlke A, *et al.* Penile allotransplantation for penis amputation following ritual circumcision: a case report with 24 months of follow-up. *Lancet* 2017; **390**: 1038.
7. Cetrulo CL, Li K, Salinas HM, *et al.* Penis transplantation: first US experience. *Ann Surg* 2018; **267**: 983.
8. Morrison SD, Shakir A, Vyas KS, Kirby J, Crane CN, Lee GK. Phalloplasty: a review of techniques and outcomes. *Plast Reconstr Surg* 2016; **138**: 594.
9. Karamürsel S, Karamürsel T, Çelebioğlu S. Rat penis as a replantation model. *Ann Plast Surg* 2005; **55**: 503.
10. Koga H, Yamataka A, Wang K, *et al.* Experimental allogenic penile transplantation. *J Pediatr Surg* 2003; **38**: 1802.
11. Seyam RM, Kattan SA, Assad LW, El-Sayed RM, Almohanna FH. Penile autotransplantation in rats: an animal model. *Urol Ann* 2013; **5**: 255.
12. Sonmez E, Nasir S, Siemionow M. Penis allotransplantation model in the rat. *Ann Plast Surg* 2009; **62**: 304.
13. Ibrahim Z, Cooney DS, Shores JT, *et al.* A modified heterotopic swine hind limb transplant model for translational vascularized composite allotransplantation (VCA) research. *J Vis Exp* 2013.
14. Oh B, Furtmüller GJ, Sosin M, *et al.* A novel microsurgical model for heterotopic, en bloc chest wall, thymus, and heart transplantation in mice. *J Vis Exp* 2016; e53442.
15. Cardini B, Oberhuber R, Hein SR, *et al.* Mouse model for pancreas transplantation using a modified cuff technique. *J Vis Exp* 2017.
16. Oberhuber R, Cardini B, Kofler M, *et al.* Murine cervical heart transplantation model using a modified cuff technique. *J Vis Exp* 2014; e50753.
17. Cheng C, Lee C, Fryer M, *et al.* Murine full-thickness skin transplantation. *J Vis Exp* 2017.
18. Furtmüller GJ, Oh B, Grahammer J, *et al.* Orthotopic hind limb transplantation in the mouse. *J Vis Exp* 2016.
19. Sucher R, Oberhuber R, Margreiter C, *et al.* Orthotopic hind-limb transplantation in rats. *J Vis Exp* 2010.
20. Fidder SAJ, Furtmüller GJ, Simons B, *et al.* Characterization of clinical and histological rejection of male genital tissues using a novel microsurgical rat penile transplantation model. *Transplantation* 2019; **103**: 2245.
21. Zhao Y, Hu W, Zhang L, *et al.* Penis allotransplantation in beagle dog. *Biomed Res Int* 2016; **2016**: 1.