

BASIC SCIENCE

Dynamic Penile Corpora Cavernosa Reconstruction Using Bilateral Innervated Gracilis Muscles: A Preclinical Investigation



Zhuming Yin, MD,^{1,2} Liqiang Liu, MD,¹ Bingjian Xue, MD,¹ Jincal Fan, MD,¹ Wenlin Chen, MD,³ and Zheng Liu, MD¹

ABSTRACT

Introduction: Prosthesis-assisted penile reconstruction has been performed extensively to restore a cosmetically acceptable phallus. However, a large number of patients will undergo revision surgery for various prosthesis-related complications.

Aim: To develop a 1-stage prosthesis-free dynamic cavernosa reconstruction method using bilateral innervated gracilis muscles and to investigate the feasibility and reliability of the surgical design.

Methods: 10 fresh cadavers were dissected to assess the availability of bilateral gracilis muscles for functional cavernosa rebuilding. 11 mongrel female dogs were involved in the penile reconstruction surgery. The neophallus consisted of bilateral gracilis muscles as the neo-cavernosa, a right gracilis skin flap as the neourethra, and a lower abdominal flap with an anterior rectus sheath as the skin envelope and neo-tunica albuginea. The function and structure of the neo-phalli were assessed 7 months postoperatively.

Main Outcome Measures: The neurovascular pedicle length of the gracilis muscles and the volume of the gracilis venter musculi were measured in the cadaveric investigation. The average dimensions of the canine neo-phalli at rest and during electrostimulated erection were obtained and the muscular fatigue-resistant curve was drawn. Histologic evaluations also were performed.

Results: The neurovascular pedicle length and volume of the gracilis muscles were sufficient to yield a nearly normal appearance of the neo-cavernosa in the cadaveric and animal studies. The muscular fatigue-resistant curve demonstrated adequate length, stiffness, and duration of erection of the neo-phalli to accomplish normal coitus. Histologic evaluations showed an intact neourethra and nearly normal muscle structure in the inner layer of the canine neo-cavernosa, except for significantly increased amount of collagen fibers and type I/III collagen ratio in the outer layer of the neo-cavernosa. The percentage of type II (fatigue-prone) muscle fibers did not change significantly.

Conclusion: Our preclinical investigation proves that corpora cavernosa reconstruction using bilateral innervated gracilis muscles is technically feasible and functionally efficacious. **Yin Z, Liu L, Xue B, et al. Dynamic Penile Corpora Cavernosa Reconstruction Using Bilateral Innervated Gracilis Muscles: A Preclinical Investigation. Sex Med 2018;6:162–170.**

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Key Words: Penile Reconstruction; Corpora Cavernosa Reconstruction; Erectile Function; Gracilis Muscle; Preclinical Study

INTRODUCTION

As the number of patients with penile loss or gender dysphoria continues to increase, the demand for anatomic, functional, and

esthetic penile reconstruction is rising. Fortunately, with a better understanding of the male genital anatomy and evolving surgical procedures, penile reconstruction can be conducted successfully by various flap procedures and erectile prosthesis implantation,

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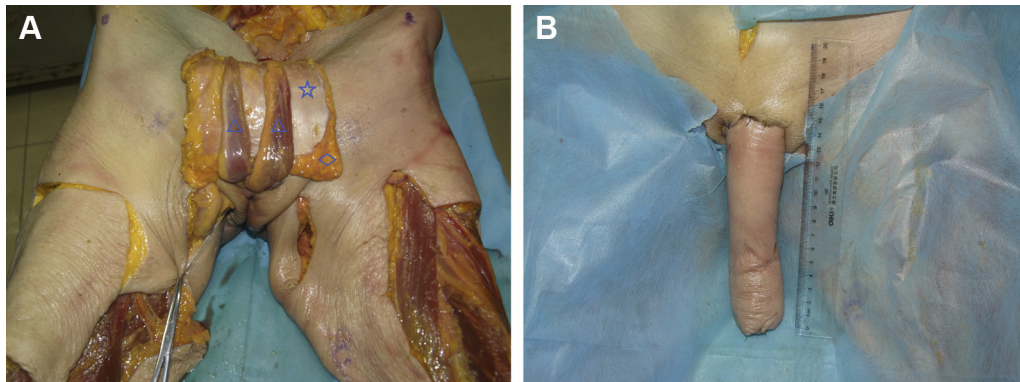


Figure 1. Design of the dynamic corpora cavernosa reconstruction in the cadaveric study. Panel A shows the intraoperative design. The reconstructed penis consisted of bilateral gracilis muscles (triangles) as the neo-cavernosa and a unilateral island pedicled anterolateral thigh flap (rhombus) with part of the fascia lata (star) as the skin envelope and neo-tunica albuginea. Panel B show the postoperative outcome.

resulting in nearly normal urinary and sexual function.^{1–3} However, prosthesis insertion for corpora cavernosa reconstruction must be performed in a 2nd stage after penile shaft reconstruction according to most surgeons.¹ In addition, the prosthesis implantation procedure is prone to multiple complications involving prosthesis extrusion, infection, and dysfunction, which could necessitate at least 1 revision surgery.^{4,5}

Autologous costal cartilage implantation could be advantageous for patients who desire 1-stage prosthesis-free phalloplasty.⁶ However, using the rod-like cartilage implant as static support for the neophallus cannot simulate the dynamic function of the penile cavernosa, which is characterized by a self-controlled directional volume switch for erection. In this respect, penile allotransplantation could offer an ideal solution for functional cavernosa reconstruction,⁷ but the technique is still in its infancy, and many logistical, procedural, and ethical issues remain controversial, which renders the novel technique prohibitive for reconstructive surgeons.

The gracilis myocutaneous flap was introduced for 1-stage phalloplasty,⁸ but the method does not have extensive application in total penile reconstruction because of the too bulky appearance of the neophallus. However, given its dynamic property and reliable anatomic features, the innervated gracilis muscle could be a technically ideal substitute for the corpora cavernosa. Thus, our preclinical study attempted to develop a 1-stage prosthesis-free dynamic corpora cavernosa reconstruction method using bilateral gracilis muscles and investigate the feasibility and reliability of the surgical design through cadaveric dissection and animal model research.

METHODS

Cadaveric Study and Dissection Techniques

The cadaveric study was approved by the ethics committee of the Plastic Surgery Hospital of the Chinese Academy of Medical Sciences (Beijing, China). 10 fresh cadavers were dissected at the Laboratory of Anatomy at the Peking Union Medical College

(Beijing, China) from February 2014 through June 2015. The average age of the 4 male and 6 female cadavers was 58.0 years (range = 38–73 years).

Dissections were performed to determine the feasibility and reliability of the novel cavernosa reconstruction design. The gracilis muscles and their vessels and nerve pedicles were dissected and measured to assess the availability for functional cavernosa rebuilding. The reconstructed penis consisted of bilateral gracilis muscles as the neo-cavernosa and a unilateral island pedicled anterolateral thigh (ALT) flap with part of the fascia lata as the skin envelope and neo-tunica albuginea (Figure 1, Supplemental Figure 1). Bilateral subcutaneous tunnels at the groin area were dissected, through which the muscles and skin flap were transferred to the pubic symphysis to fashion the neophallus. All findings were documented by digital photography.

Animal Care

All animal care and experimental protocols were approved by the ethics committee of the Plastic Surgery Hospital at the Chinese Academy of Medical Sciences. 11 mongrel female dogs (11–13 months old, weight = 17.4 ± 2.2 kg) were maintained on a commercial paste diet, given deionized water ad libitum, and kept in cages in a $20 \pm 2^\circ\text{C}$ room at 40% to 60% relative humidity with a natural light-dark cycle.

All animals were subjected to penile reconstruction surgery according to the following surgical procedures. Intravenous access was established preoperatively for saline infusion to maintain fluid balance. Penicillin at a dose of 40,000 IU was given intramuscularly before surgery and the antibiotic was administered at the same dose once a day for 5 days postoperatively. Each dog after surgery was equipped with a large-sized Elizabethan collar to prevent the animal from biting and licking the surgical site. The animals were examined daily within 2 weeks after surgery until the wound healed completely. Postoperative complications, such as incision dehiscence and self-bite, were managed immediately once observed.

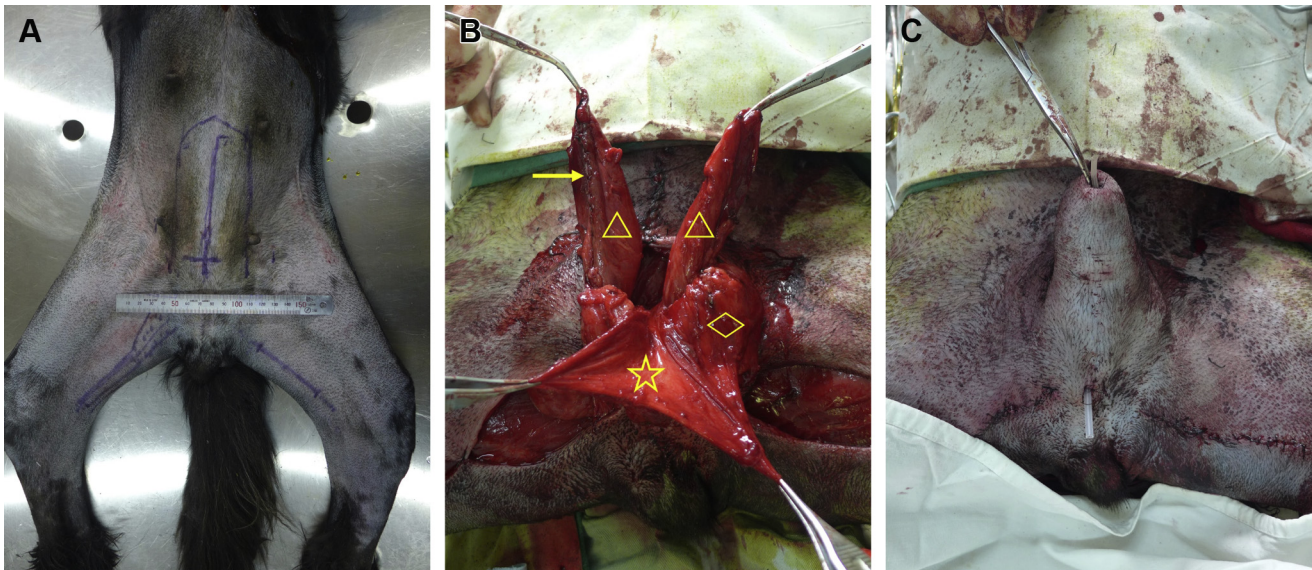


Figure 2. Design of the dynamic corpora cavernosa reconstruction in canine models. Panel A shows the preoperative design. Panel B shows the intraoperative procedures. The neourethra was fashioned by rolling the right gracilis skin flap around a catheter. The neophallus was formed by wrapping the gracilis muscles (triangles) and the neourethra (arrow) with a lower abdominal flap (rhombus) and the anterior rectus sheath (star). Panel C shows postoperative outcome.

Surgical Procedures

The surgical procedures of the animal study were designed based on the result of the cadaveric study. The animals were generally anesthetized with a compound mixture of ketamine, fentanyl, and haloperidol (0.08 mL/kg, intramuscularly). The incisions were made at the proximal and distal sites of the inner thigh, respectively, leaving the skin at the middle site intact. Bilateral gracilis muscles were dissected from distal to proximal insertions, and the neurovascular pedicles were preserved. The distal parts of the muscles were harvested as self-control biopsy specimens. Then, the gracilis muscles were transferred to the pubic area through subcutaneous tunnels, and the proximal ends were anchored to the pubic symphysis. The neourethra was fashioned by rolling the right gracilis skin flap around a catheter. For the skin envelope and neo-tunica albuginea, we initially failed using the ALT flap and part of the fascia lata as described in the cadaveric study. The reason was that neither the vascular pedicle length nor the flap size of the canine ALT flap was sufficient for the neo-phallic skin envelope, which is probably due to the dramatically smaller thigh-to-body length ratio in dogs compared with humans. Therefore, the lower abdominal flap and the anterior rectus sheath were used to wrap the gracilis muscles and the neourethra to form the neophallus (Figure 2, Supplemental Figure 2).

Evaluation of Erectile Function of the Neo-Phalli

The morphology of the neo-phalli was observed 7 months postoperatively. Then, the dogs were reanesthetized using the same method described earlier. A 5-cm incision was made at the inguinal region and the anterior branch of the obturator nerve was dissected and exposed for the electrostimulation erection test

(Supplemental Figure 3). The length, diameter, and stiffness of the reconstructed penises were measured at rest and at tetanically contractile erection by electrostimulation of the motor nerves in the bilateral gracilis muscles. Stiffness was assessed according to a 4-point scale⁹: score 1 = nearly no hardness; score 2 = moderate hardness but cannot complete sexual intercourse; score 3 = sufficient hardness to complete sexual intercourse but less than normal; score 4 = hardness equal to normal. The muscular fatigue-resistant curve was obtained by recording the measurements of the parameters every 5 minutes during 1 hour of continuous electrostimulation (3.0 V, 30 Hz, 0.2 ms).

Histologic Analyses

The animals were euthanized immediately after the neo-cavernosa function assessment. The neo-phalli were harvested en bloc as the neo-cavernosa group, and biopsy specimens of the gracilis muscles in the previous operation were collected as the self-control group. Tissues were fixed with 10% neutral buffered formalin for paraffin embedding. Paraffin-embedded specimens were sectioned at 5 μ m. Hematoxylin and eosin staining was applied to clarify the histologic structure of the neophallus.

Then, the paraffin-embedded sections were stained by a Masson trichrome stain kit (NJJC Bio, Nanjing, China) according to the manufacturer's instructions. The analysis was conducted in the outer layer and inner layer, respectively. The outer layer was defined as the 1-cm-thick superficial layer, and the inner layer was located below the outer layer. 5 slides of each specimen at the intermediate portion were selected and 5 visual fields (magnification = 100 \times) of each layer were scanned under light microscopy. The collagenous material was stained blue and the muscle was stained red. The collagen/muscle ratio was

Table 1. Cadaveric measurement of gracilis muscles (n = 10)

	Left GM		Right GM		P value
	Mean	SD	Mean	SD	
NVL (cm)	11.69	1.47	11.85	1.60	.94
Sy-DFA (cm)	11.44	1.43	11.49	2.01	.28
VML (cm)	28.83	1.46	28.81	1.43	.11
VMW (cm)	3.40	0.63	3.36	0.49	.86
VMT (cm)	1.05	0.24	1.02	0.16	.07

GM = gracilis muscle; NVL = neurovascular pedicle length; Sy-DFA = distance from pubic symphysis to origin of the gracilis branch of the deep femoral artery; VML = length of venter musculi; VMT = thickness of venter musculi; VMW = width of venter musculi.

calculated by Image Pro Plus 6.0 (Media Cybernetics, Rockville, MD, USA), which was defined as (number of blue pixels/number of red pixels) \times 100%. The mean of 50 scans per specimen was calculated for further statistical evaluation.

Picrosirius red staining also was conducted using a specialized kit (NJJC Bio), which was used according to the manufacturer's instructions. 50 visual fields (magnification = 400 \times) of each specimen were selected as described earlier under polarized light microscopy. Type I collagen was stained yellow and red and type III was stained green. The collagen type I/III ratio was calculated by the same software, and the ratio was defined as (number of yellow and red pixels/number of green pixels) \times 100%. The mean of 25 scans was calculated for further statistical evaluation.

Tissue sections were immunostained by anti-MY-32 antibody to detect the amount of type II (fatigue-prone) muscle fibers using streptavidin-peroxidase immunohistochemical staining kits (NJJC Bio). Briefly, after a dewaxing and antigen retrieval procedure, endogenous peroxidase activity was blocked with 3% H₂O₂ at room temperature for 10 minutes. The slides were incubated with 10% normal goat serum for 10 minutes and then with anti-MY-32 mouse monoclonal antibody (diluted 1:200; Sigma, St Louis, MO, USA) at 4°C overnight. Then, the sections were incubated with a secondary antibody for 10 minutes, and the reaction was visualized with the 3,3'-diaminobenzidine complex followed by incubation with horseradish peroxidase and streptavidin. A single pathologist who was unaware of the corresponding data assessed the results. The percentage of positive muscle fibers per visual field (200 \times) was counted, and the average value of the 25 percentage counts of each specimen was calculated for further statistical evaluation.

Statistical Analysis

Normality of the data in the cadaver dissection and the animal experiment was detected by the Shapiro-Wilk test. Unpaired t-test was used for the comparison of neophallus measurements and immunostaining results. 1-way analysis of variance and Bonferroni post hoc analysis were applied to assess the significance of the distinction of the Masson trichrome and picrosirius red staining results. Significance was assumed with a *P* value less than .05.

Table 2. Measurement of the neophalli at rest and electrostimulated erection (n = 10)

	Rest		Erection		P value
	Mean	SD	Mean	SD	
Length (cm)	8.70	0.62	10.42	1.03	<.001
Diameter (cm)	4.02	0.53	4.20	0.45	.173
Stiffness	1.23	0.47	3.20	0.45	<.001

RESULTS

Bilateral Innervated Gracilis Muscles Are Anatomically Feasible for Cavernosa Reconstruction

The cadaveric investigation was performed to clarify the anatomic features of the innervated gracilis muscles for dynamic cavernosa reconstruction. The major neurovascular bundle of the gracilis muscle was defined as the neurovascular pedicle. As presented in [Table 1](#), the mean neurovascular pedicle length of the left gracilis muscles was 11.69 \pm 1.47 cm, and the distance from the pubic symphysis to the origin of the gracilis branch of the deep femoral artery was 11.44 \pm 1.43 cm. On the right side, the neurovascular pedicle length was longer than the distance from the pubic symphysis to the origin of the gracilis branch of the deep femoral artery, indicating that the neurovascular pedicle length was sufficient to be transferred to the pubic area through subcutaneous tunnels. In addition, the gracilis venter musculi yielded a satisfactory volume of the neo-cavernosa by dimensional measurements ([Table 1](#)). The surgical outcome demonstrated a nearly normal appearance of the reconstructed penis with appropriate length and diameter ([Figure 1](#)).

Bilateral Innervated Gracilis Muscles Are Functionally Feasible for Cavernosa Reconstruction

The animal study was conducted to simulate the novel surgical design of dynamic cavernosa reconstruction. 1 of the 11 dogs died within 24 hours postoperatively of respiratory failure. 3 dogs had incision dehiscence, and 2 of the 3 had partial flap loss because of unstable fixation and self-bite, but all wounds healed after secondary surgeries.

7 months postoperatively, all neo-phalli displayed a nearly normal appearance while the animals were standing and moving ([Video 1](#)). The average length of the reconstructed penises at rest was 8.70 \pm 0.62 cm but lengthened significantly to 10.42 \pm 1.03 cm when the motor nerves were continuously stimulated (*P* < .05; [Table 2](#)). As expected, the diameter showed an insignificant response to electrostimulation (*P* > .05). The mean hardness at erection was graded as 3.20 \pm 0.45, indicating adequate stiffness to accomplish normal coitus.

The muscular fatigue-resistant curve was drawn to determine the duration of the erection-like tetanic contraction of bilateral

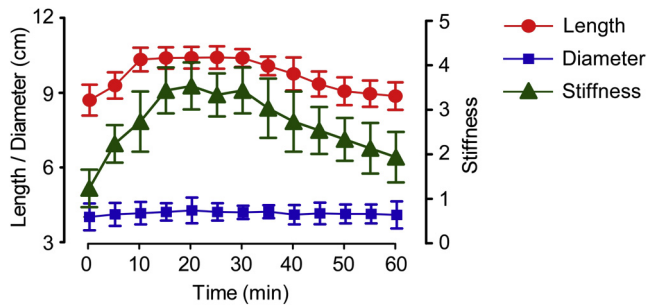


Figure 3. Muscular fatigue-resistant curve of the neo-phalli. Measurements of length, diameter, and stiffness of the reconstructed penises were recorded every 5 minutes during 1 hour of continuous electrostimulation ($n = 10$). Data are presented as mean \pm SD for the 3 parameters. Length and diameter are presented on the left y scale and stiffness is presented on the right y scale.

gracilis muscles (Figure 3). The neo-phalli achieved maximum length within 10 minutes, whereas it took approximately 15 minutes to achieve peak stiffness under 60-minute continuous electrostimulation. The duration of the platform stage of maximum length and stiffness was 20 minutes, which is sufficient for satisfactory coitus.

Histologic Structure of the Gracilis Muscle Fulfills the Requirement of Dynamic Cavernosa Reconstruction

To further investigate the feasibility and reliability of the novel dynamic cavernosa reconstruction, the histologic architecture of the neo-phalli was analyzed by hematoxylin and eosin, Masson trichrome, and picrosirius red staining. Hematoxylin and eosin staining of the canine neo-phalli showed nearly normal muscle structure in the inner layer of the neo-cavernosa and an intact urethra without stricture (Figure 4A, C, D). However, in the 1-cm-thick outer layer of the gracilis muscles, the width of pink-stained perimysium and endomysium expanded significantly (Figure 4B), suggesting enhanced fibrosis of the outer part of the neo-cavernosa.

This finding was confirmed by Masson trichrome staining (Figure 5). The amount of collagen fibers was shown to increase significantly in the outer layer of the neo-cavernosa (from 0.92 ± 0.05 to 0.69 ± 0.18 ; $P < .01$, outer layer vs control), whereas fibrosis of the inner layer did not differ significantly from the control group (from 0.54 ± 0.16 to 0.69 ± 0.18 ; $P > .05$, inner layer vs control). The neo-tunica formed by the rectus sheath also was observed in the form of a 100- to 400- μ m-thick collagen cap outside the neo-cavernosa (Figure 5A), providing the neophallus with sufficient hardness for satisfactory sexual intercourse.

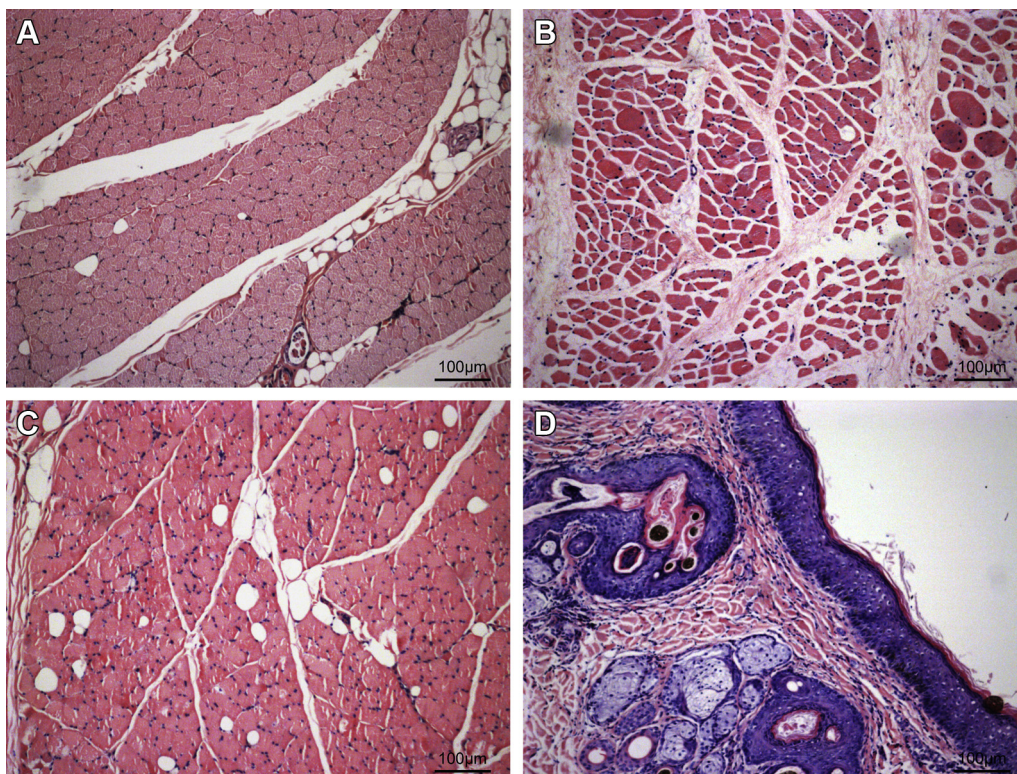


Figure 4. Histologic structure of the neo-phalli. Hematoxylin and eosin stain (magnification = 100 \times) shows nearly normal muscle structure and an intact urethra. Panel A shows the self-control group. Panel B shows the outer muscle layer of the neo-cavernosa. Panel C shows the inner muscle layer of the neo-cavernosa. Panel D shows the neourethra.

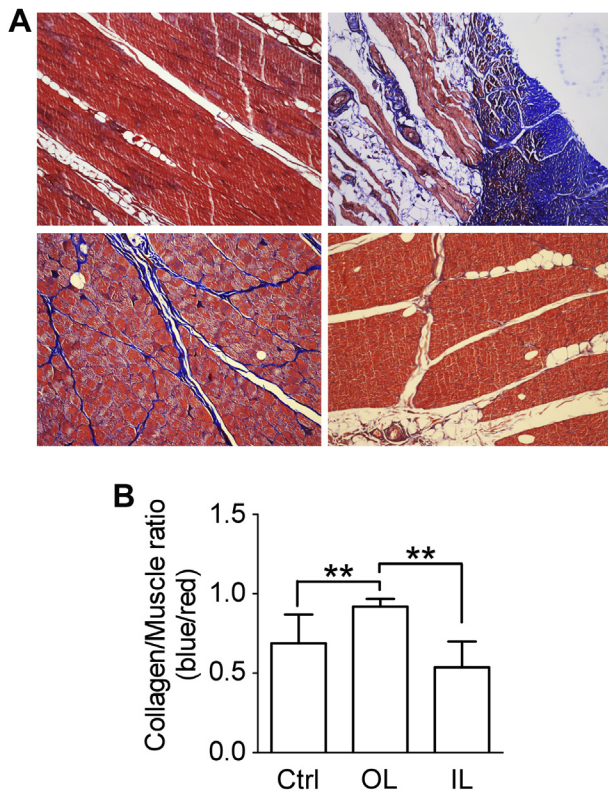


Figure 5. Evaluation of fibrosis of the neo-cavernosa. The amount of collagen fibers was tested by Masson trichrome staining ($n = 10$). Panel A shows representative images (magnification = $100\times$) of Masson trichrome staining of the self-control group (top left), the neo-tunica albuginea (top right), the outer layer (bottom left), and the inner layer (bottom right) of the neo-cavernosa group. Panel B shows quantification of the collagen/muscle ratio in the self-control group and the outer and inner layers of the neo-cavernosa. $**P < .01$. Ctrl = control group; IL = inner layer of neo-cavernosa group; OL = outer layer of neo-cavernosa group.

The collagen type discrepancy between the neo-cavernosa and the control group was further detected by picrosirius red staining (Figure 6). The higher type I/III collagen ratio in the outer layer showed statistical significance compared with the ratio in the control group (from 4.37 ± 1.51 to 2.10 ± 0.47 ; $P < .01$, outer layer vs control). The collagen composition of the inner layer was similar to the control group (from 1.84 ± 0.27 to 2.10 ± 0.47 ; $P > .05$, inner layer vs control), which preserved nearly normal elasticity for the neophallus.

To determine the composition difference of muscle fiber types between the neo-cavernosa and the self-control group, anti-MY-32 immunostaining was conducted. As shown in Figure 7, the percentage of type II (fatigue-prone) muscle fibers decreased slightly 7 months postoperatively, but the difference did not approach significance (from $74.94 \pm 1.86\%$ to $77.69 \pm 2.95\%$; $P > .05$, neo-cavernosa vs control). The result indicated that transformation of the gracilis muscle fibers from type II to I (fatigue resistant) did not occur merely by surgical intervention.

DISCUSSION

The ideal goal of penile reconstructive surgery is not only to achieve an esthetically acceptable result but also to allow patients to regain sexual and urinary functions with confidence.^{1,10–12} However, despite all modern advances in urinary and plastic surgery, reconstruction of the corpora cavernosa and restoration of patients' sexual function remain challenging because of the complex structure of the erectile tissue. Currently, implantation of an inflatable or malleable penile prosthesis is the most frequently used strategy and is universally considered the standard technique for cavernosa reconstruction.^{1,2,13} However, the multistage requirement and dramatically high risk of post-operative complications, such as prosthesis malposition, infection, protrusion, and leakage, make cavernosa reconstruction a painstaking surgery. Hence, it is necessary to explore autologous tissues, such as the gracilis muscles, which possess cylindrical morphology and erectile function similar to the corpora cavernosa.

It has been reported that the gracilis myocutaneous flap can be used in total penile reconstruction.⁸ Unfortunately, the unsatisfactory appearance of the neophallus restricts its further application.¹⁴ However, as the muscular part of the flap, the innervated gracilis muscle could be an ideal alternative for dynamic cavernosa reconstruction.

The gracilis muscle has a highly constant and reliable neural regulation and blood supply,^{15,16} which is the anatomic basis of the reconstructive design. Because of its surgical convenience for pedicled transfer, the gracilis myocutaneous flap is widely used in urethral, pelvic, and perineal reconstruction.^{17–19} Our cadaveric investigation showed that the neurovascular pedicle is longer than the subcutaneous tunnel in the pubic area, suggesting the availability of the gracilis muscles to be transferred as the neo-cavernosa. The surgical design also proved to be anatomically feasible in the subsequent study of canine models.

The gracilis muscle can simulate the cavernosum statically and dynamically, which is the functional basis of the reconstructive method. Given its dynamic property, the innervated gracilis muscle is used extensively to restore various muscle deficiencies and chronic muscle denervation.^{20,21} Thus, unlike the autologous cartilage graft, the gracilis muscle can change its volume voluntarily and directionally. When the penile erection is initiated, the influx of blood into the corporal spaces is triggered and the length and stiffness of the penile shaft increase significantly. Similarly, the length and stiffness of the neo-phalli in our study increased dramatically when the bilateral anterior branches of the obturator nerves were electrostimulated. In contrast, the diameter displayed little difference during electrostimulation, which is consistent with our expectation for a directional volume change of the neo-cavernosa. The duration of continuous tetanic contraction, which lasted longer than 20 minutes in our study, also resembles the normal erection time for satisfactory sexual intercourse.²² However, the gracilis muscle contains no

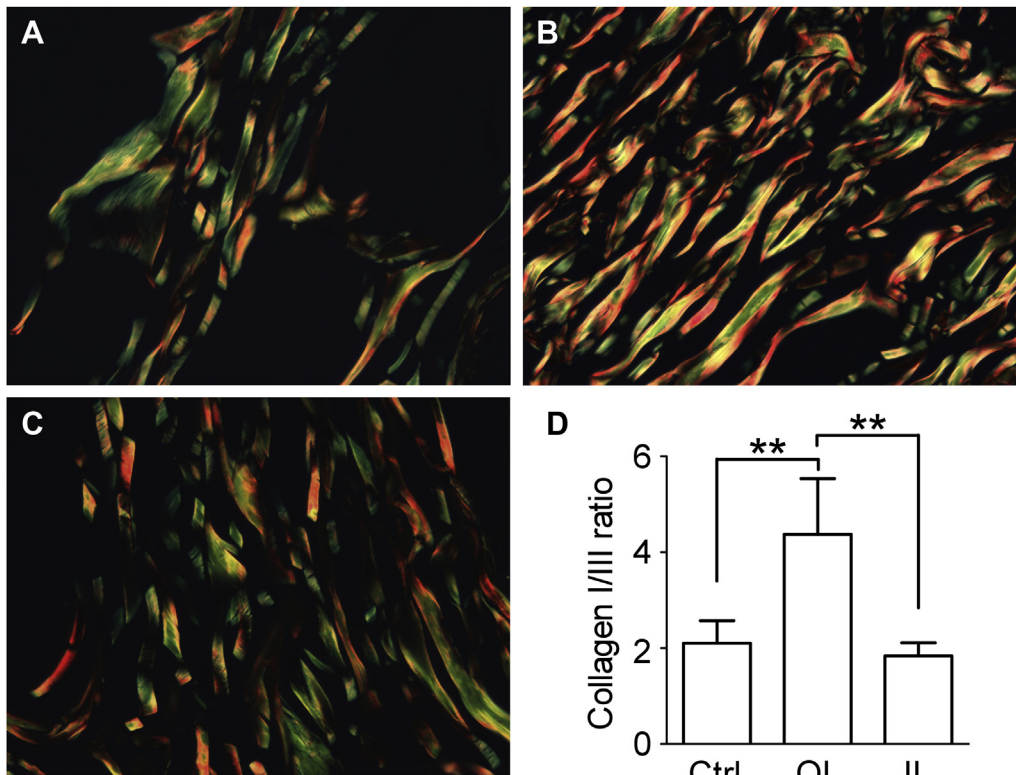


Figure 6. Collagen type investigation of the neo-cavernosa. Collagen type was detected by picosirius red staining ($n = 10$). Panel A shows a representative image (magnification = $400\times$) of the self-control group. Panel B shows a representative image (magnification = $400\times$) of the outer layer of the neo-cavernosa. Panel C shows a representative image (magnification = $400\times$) of the inner layer of the neo-cavernosa. Panel D shows quantification of collagen I/III ratio in the self-control group and the outer and inner layers of the neo-cavernosa. $**P < .01$. Ctrl = control group; IL = inner layer of neo-cavernosa group; OL = outer layer of neo-cavernosa group.

autonomous innervation to trigger sexually induced penile erection. Thus, future studies might concentrate on motor nerve training to achieve tetanic contraction of the neo-cavernosa at patients' will if the surgical design is transferred to clinical application.

The architecture of the gracilis muscle is similar to the corporal body, which is the histologic basis of the application of the gracilis muscles in cavernosa reconstruction. The corpus cavernosum is composed of a cylindrical sponge-like tissue containing many sinusoid spaces separated by connective tissue septa. The contour of the gracilis muscle resembles the cavernosum in morphology. In addition, the unipennate spiral type of the muscle fiber bundles,¹⁶ like a loosely coiled spring, endows the neo-cavernosa with the ability to change its volume within a large range. Moreover, the dimensional measurements of the gracilis muscles in our cadaveric study demonstrated sufficient length and volume for cavernosa reconstruction, which was substantiated by the animal study. The amount of total collagen fibers and type I/III collagen ratio were found to increase significantly in the 1-cm-thick outer layer of the gracilis muscles compared with the inner layer, suggesting enhanced type I collagen accumulation in the outer layer. The growing concentration of collagen fibers from the inner layer to the outer

layer, especially type I collagen fibers, provides the neo-cavernosa with adequate stiffness and appropriate elasticity allowing for satisfactory sexual intercourse. To maintain the length and stiffness of the neo-cavernosa during coitus, large quantities of fatigue-resistant (type I) muscle fibers are needed, which means a smaller proportion of fatigue-prone (type II) muscle fibers. However, the immunostaining test showed an unchanged ratio of type II muscle fibers in the neo-cavernosa, which could be of little benefit to patients. However, the drawback can be solved by low-frequency electrical training-assisted fiber type transformation in future practices.^{23,24} Notably, the intact neourethra fashioned by the right gracilis skin flap also reflects the feasibility of the surgical design, although anastomosis to the native urethra was not performed because urinary assessment was beyond the scope of the cavernosa reconstruction investigation.

The ALT flap has been used widely as a reliable option for patients seeking total phallic reconstruction.^{25–27} Initially, the island ALT flap with its pedicle was transferred as the skin envelope in our cadaveric investigation. However, we had to use the inferiorly based pedicled abdominal flap instead of the ALT flap in the animal study, because the pedicle length and flap size of the canine ALT flap were not sufficient for wrapping bilateral gracilis muscles. Despite the satisfactory surgical outcome in

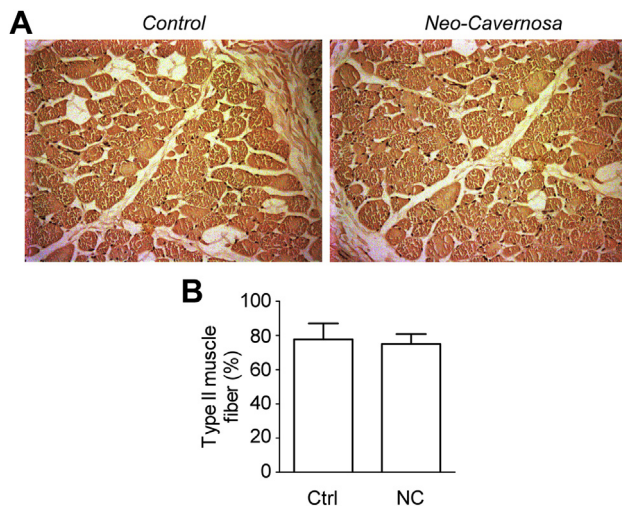


Figure 7. Muscle fiber type investigation of the neo-cavernosa. Muscle fiber type was detected by anti-MY-32 immunostaining ($n = 10$). Panel A shows representative images (magnification = $200\times$) of the self-control group (left) and the neo-cavernosa group (right). Panel B shows quantification of the type II muscle fiber ratio in the self-control and neo-cavernosa groups. Ctrl = control group; NC = neo-cavernosa group.

canine models, further modifications on the skin envelope still need to be made. Hence, the radial free forearm flap, regarded as the workhorse flap in total phallic reconstruction,^{1,3} might be combined with bilateral innervated gracilis muscles to improve the long-term appearance and function of the neophallus.

The previous literature showed a lower rate of prosthesis infection and extrusion in the patients with erectile dysfunction than in patients undergoing total penile reconstruction.^{4,28,29} The most possible reason considered was the absence of the tunica albuginea in reconstruction cases.¹³ Therefore, the tunica albuginea is an indispensable layer of the neophallus, restricting the position and limiting the diameter expansion of the neo-cavernosa. Our animal study used the rectus sheath as the neo-tunica, forming a rigid support surrounding the gracilis muscles. Histologic analysis demonstrated an intact layer mainly composed of collagen fibers. The functional investigation showed significant increases in the length and stiffness of the neo-phalli with little change in diameter at erection, confirming the efficacy of the deep fascia as the neo-tunica. Future clinical practice might involve foreign materials, such as the acellular dermal matrix, as a substitute for the autologous fascia to decrease donor site morbidity.

CONCLUSION

Our preclinical investigation proves that dynamic corpora cavernosa reconstruction using bilateral innervated gracilis muscles is feasible and efficacious and paves the way to the clinical application for the novel surgical design as an excellent alternative for 1-stage prosthesis-free penile reconstruction.

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SUPPLEMENTARY DATA

Supplementary data related to this article can be found at <https://doi.org/10.1016/j.esxm.2018.01.002>.