Anorectal Transplantation

The First Long-term Success in a Canine Model

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Objective: Anorectal transplantation is a challenging procedure but a promising option for patients with weakened or completely absent anorectal function.

Summary Background Data: We constructed a canine model of anorectal transplantation, evaluated the long-term outcomes, and controlled rejection and infection in allotransplantation.

Methods: In the pudendal nerve function study, 6 dogs were randomly divided into 2 groups, transection and anastomosis, and were compared with a control using anorectal manometry, electromyography, and histological examination. In the anorectal transplantation model, 4 dogs were assigned to 4 groups: auto-transplant, allotransplant with immunosuppression, allotransplant without immunosuppression, and normal control. Long-term function was evaluated by defecography, videography, and histological examination.

Results: In the pudendal nerve function study, anorectal manometry indicated that the anastomosis group recovered partial function 6 months postoperatively. Microscopically, the pudendal nerve and the sphincter muscle regenerated in the anastomosis group. Anorectal transplantation was technically successful with a 3-stage operation: colostomy preparation, anorectal transplantation, and stoma closure. The dog who underwent allotransplantation and immunosuppression had 2 episodes of mild rejection, which were reversed with methylprednisolone and tacrolimus. The dog who underwent allotransplantation without immunosuppression had a severe acute rejection that resulted in graft necrosis. Successful dogs had full defecation control at the end of the study.

Conclusions: We describe the critical role of the pudendal nerve in anorectal function and the first long-term success with anorectal transplantation in a canine model. This report is a proof-of-concept study for anorectal transplantation as a treatment for patients with an ostomy because of anorectal dysfunction.

Keywords: anal reconstruction, anorectal transplantation, colostomy, functional recovery, preclinical animal experiment, vascularized composite tissue allotransplantation

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The incidence of fecal incontinence in the general population may be as high as 17%, and the number of patients undergoing colostomy for this reason is steadily increasing.[1] The major reasons for colostomy are congenital anorectal malfunction (atresia or Hirschsprung disease), intractable anal dysfunction secondary to inflammatory bowel diseases (Crohn disease or ulcerative colitis), severe trauma, and previous abdominoperineal excision (Miles operation) for pelvic malignant disease.[2] The potential number of ostomy patients is estimated at approximately 200,000 in Japan, 650,000 in Europe, and 700,000 in the United States. Most patients also have psychosocial sequelae and/or poor quality of life.[3–5] Various techniques for anal reconstruction have been reported to minimize the deleterious effects of a definitive colostomy such as sphincteroplasty, gracilis or gluteus maximus muscle transfer, and artificial sphincter implantation; however, none is considered the criterion standard therapy.[6–9]

Recently, allotransplantations of nonvital organs, such as the face, larynx, trachea, hand, uterus, and penis, have been performed successfully.[10–15] The development and evaluation of surgical and interventional techniques proceed through stages that are similar to those used for drug development, but with important differences.[16]

The technical feasibility of anorectal transplantation in rat, porcine, and human cadaver models has been reported, but the functional and immunological outcomes are unclear.[17–25] We performed preclinical research to evaluate anorectal transplantation and used dogs as the optimal animal species because of their similar defecation control to humans.[26,27] This report describes 2 phases in

the study. The pudendal nerve function study involved 6 dogs divided into 2 groups; one in which the nerve was resected versus the other in which the nerve was transected and anastomosed versus a control group. We assessed functional outcomes in this group using histology, electromyography, and manometry. In addition, the transplant model involved 4 dogs, with 1 dog each undergoing autotransplant versus allotransplant with immunosuppression versus allograft without immunosuppression versus control. This group was assessed with defecography and videography.

METHODS

Animals

Twelve healthy adult male beagle dogs weighing 8 to 12 kg were included in this study. The number of dogs was minimized to address the ethics requirements. Seven dogs were used in the pudendal nerve function study (3 in the transection group, 3 in the anastomosis group, and 1 in the control group). Another 5 dogs underwent anorectal transplantation (1 undergoing autotransplantation, and 2 donors for 2 recipients undergoing allotransplantation). All animal experiments were approved by the Animal Experimental Committee of Kyoto University and the University of Tokyo Animal Care and use Committee. All efforts were made to minimize animal suffering. The dogs were fasted for 24 hours preoperatively and given water *ad libitum*. The dogs were allowed free cage activity. Buprenorphine sustained release (40 µg/kg) injections were given subcutaneously twice daily and carprofen (4.4 mg/kg) was administered orally once daily for 7 days after surgery. All dogs were euthanized 1 year after surgery, at the study endpoint, except for the dog who underwent allotransplantation without immunosuppression who experienced severe rejection of the graft and was euthanized at graft failure.

Presurgical Preparation

All surgical procedures and physiological measurements were performed under general anesthesia. Dogs were premedicated with 0.25 to 0.05 mg/kg atropine sulfate, intramuscularly, followed by anesthesia induction with 15 mg/kg ketamine hydrochloride and 3 mg/kg xylazine hydrochloride or propofol, and endotracheal intubation. Continuous monitoring was performed by electrocardiography and oxygen saturation by reflectance oximetry using a sensor clipped to the ear. The perianal region was shaved, and the animals were placed in the supine position. The perineal and anal regions were then disinfected with 70% ethanol and iodine tincture, and covered with sterile drapes. Sevoflurane (0.5%–1.0%) or isoflurane (2%) and nitrous oxide were used for anesthetic maintenance, with mechanical ventilation. Fentanyl (10 µg/kg/h) and rocuronium bromide 0.5 mg/kg were administered for analgesia and muscle relaxation, respectively.

Pudendal Nerve Function Study

Following a circumanal skin incision, the anal canal and the sphincter muscles were circumferentially dissected from the surrounding tissue. The posterior wall of the anal segment was separated until identification of the anterior surface of the coccygeal muscle. The levator ani muscle was identified at the lateral and posterior wall, and the anterior wall was then dissected free, behind the prostate. The neurovascular bundles of the pudendal arteries, veins, and nerves run bilaterally inside the ischial tuberosity and reach the external anal sphincter muscle at the 2 o'clock and 10 o'clock positions (Fig. S1A, http://links.lww.com/SLA/C267). We then dissected the pudendal nerves to avoid damage (Fig. S1B, http://links.lww.com/SLA/C267). In the transection group (N = 3), we transected bilateral pudendal nerves and resected a 1-cm width (Fig. S1C, http://links.lww.com/

SLA/C267). In the anastomosis group (N = 3), we transected bilateral pudendal nerves and then anastomosed the nerves end-to-end with epineural sutures (10-0 Ethilon; Johnson & Johnson, Tokyo, Japan) using a surgical microscope (OME-9000; Olympus Medical Systems Corp., Tokyo, Japan) (Fig. S1D, http://links.lww.com/SLA/C267). The sham operation was performed similarly but without neurotomy. All wounds were closed using skin staples (3 M Precise Vista Skin Staplers; Medema T/A Omega Medical Supplies Ltd., London, UK).

Three-Stage Operation (Colostomy/Anorectal Transplantation/Stoma Closure)

One week before transplantation, we performed colostomy to keep the surgical site clean post-transplant. The surgical procedure for the anorectal transplantation has been described previously.[27] Briefly, through a circumanal skin incision (Fig. S2A, http://links.lww.com/SLA/C268), we identified the bilateral neurovascular bundles of the pudendal arteries, veins, and nerves (Fig. S2B, http://links.lww.com/SLA/C268), which we clamped and carefully transected. Next, we dissected the rectum and harvested the anorectal graft, which we perfused with 20 mL heparinized saline with a temperature at 4°C. We then prepared the graft for the rectal anastomosis (Fig. S2C, http://links.lww.com/SLA/C268) and anastomosed the graft using DST Series and EEA staplers (Covidien, New Haven, CT) autogenically or allogeneically. Bilateral pudendal arteries, veins, and nerves were anastomosed end-to-end using interrupted sutures (10-0 Ethilon; Johnson & Johnson, Tokyo, Japan) using a surgical microscope. Then, we reconstructed the pelvic floor muscles, including the levator ani, using 4-0 Vicryl sutures (Atom Medical Corp., Kyoto, Japan) and closed the wound (Fig. S2D, http://links.lww.com/SLA/C268). We closed the colostomy 2 weeks after transplantation during laparotomy performed through a peristomal incision.

Postoperative Medication

Dogs in the pudendal nerve function study received 10 mg/kg/day enrofloxacin (Baytril; Bayer Yakuhin, Ltd., Osaka, Japan) intramuscularly for 5 days postoperatively. We also administered 100 IU/kg/day heparin (Mochida Pharmaceutical Co., Ltd., Tokyo, Japan) intravenously to the dog undergoing anorectal autotransplantation, for 1 week postoperatively. Additionally, one of the dogs undergoing allotransplantation received immunosuppression with 0.1 mg/kg/day tacrolimus (Prograf; Astellas Pharma Inc., Tokyo, Japan) and 1.0 mg/kg/day methylprednisolone (Solu-Medrol; Pfizer, Tokyo, Japan), intramuscularly to treat rejection.

Photography and Videography

Macroscopic photographs were taken using a digital camera (COOLPIX S8200; Nikon Corporation, Tokyo, Japan) intraoperatively and postoperatively. Videos of the dogs' postoperative defecation appearance were taken using a digital video camera (HDR-PJ800; Sony Inc., Tokyo, Japan).

Anorectal Manometry

We evaluated the anal canal rhythm wave (fluctuations in anal canal continuous pressure) and maximum resting pressure (highest pressure along the functional anal canal) in the lateral decubitus position using a 1-channel catheter (P31-10DP; Konigsberg Instruments Inc., Pasadena, CA) and a measurement and analysis system (Anorect.dll; Star Medical Inc., Tokyo, Japan). The anal canal rhythm wave was measured for 5 minutes by positioning the catheter in the anal canal under anesthesia at rest. The wave was classified as normal, abnormal (sudden pressure change in the anal canal), or flat (no pressure change in the anal canal). Maximum resting pressure

was evaluated with the rapid pull-through technique. The catheter was pulled at a speed of 10 mm/s under anesthesia at rest, and pressure was recorded 5 times then averaged.

Electromyography

Electromyography (Neuropack; Nihon Koden, Inc., Tokyo, Japan) was performed under anesthesia at rest in a supine position before anal canal tissue harvest. During tissue sampling, the perianal region was exfoliated, and Alcock's canal was opened closer to the nerve center than the ischial tuberosity. Next, we placed a stimulating electrode on the pudendal nerve on the side of the rejoined nerve or the resection site (which was marked by a suture placed in the surrounding tissue to avoid damaging the nerve during resection) for electromyography. The electromyogram in each dog was a flat wave (negative response) or a reaction wave (positive response).

Defecography

Defecography was performed under anesthesia at rest in lateral recumbency 1 year after transplantation. Approximately 100 mL of barium paste (barium solution, water, and bran) was injected into the rectum. Several dynamic states (at rest, after anal squeezing, straining, and evacuation) were filmed using fluoroscopy (BV Pulsera; Philips Electronics, Tokyo, Japan).

Histological Examination of Anorectal Tissues

In the pudendal nerve function study, anorectal tissues were transected in the sagittal plane and fixed in 20% buffered formaldehyde for 2 days. After dehydration with ethanol, the tissues were embedded in paraffin, and 6-μm thick sections were made in the coronal and horizontal planes and stained with Masson's trichrome. Sections were cut at several levels and meticulously examined to identify any pathological findings. The rectum and anal canal, including mucous membranes and sphincter muscles, were examined at ×200 magnification using light microscopy. The degree of histological change was graded from 1 to 4 as follows: 1, no morphological change; 2, mild interstitial fibrosis and sphincter muscle atrophy (< approximately 25% of the area); 3, moderate interstitial fibrosis and sphincter muscle atrophy (approximately 26%–50% of the area); and 4, severe interstitial fibrosis and sphincter muscle atrophy (approximately >50% of the area). Twenty to twenty-five sections for each sample were simultaneously evaluated to determine the histological stage and were graded by 3 senior histologists, who were blinded to the origin of the sections. Results are described as means ± standard deviations.

Transmission Electron Microscopic Examination of the Pudendal Nerve

In the pudendal nerve function study, the pudendal nerves were harvested 1 cm peripheral to the site where the nerves were transected and anastomosed 1 year earlier. Samples were removed, cut into 3-mm sections, fixed in 2.5% glutaraldehyde in 0.1 mol/L phosphate-buffered saline (pH: 7.4) for an additional 48 hours, and then fixed in 1% osmium tetroxide at 4°C for 1 hours. Sections were dehydrated with a graded ethanol series, substituted with QY-2 (Nissin EM, Tokyo, Japan), and then embedded in epoxy resin (Quetol 651; Nissin EM, Tokyo, Japan). Semithin sections were cut, stained with 1% toluidine blue, and then evaluated under light microscopy. Ultrathin sections were cut, stained with uranyl acetate and lead citrate, and examined using a JEM-1400 electron microscope (JEOL Ltd., Tokyo, Japan). For the quantitative analysis, photographs of each nerve section were taken from 12 random fields in each section at ×800 magnification and evaluated in each region using ImageJ software, version 1.62 (National Institutes of Health, Bethesda, MD). Morphometric indices were assessed regarding the

thickness of the myelin sheath, ratio of unmyelinated and myelinated nerve fibers, and the numbers of nerve fibers in each photograph. Data were expressed as means ± standard deviation. All measurements were performed by an investigator who was blinded to the grouping information for each section.

Statistical Analysis

Results are described as means ± standard deviations. Comparisons between 2 groups were performed using the unpaired Student *t* test. Comparisons of >2 groups were made by analysis of variance with Bonferroni correction. Statistical significance was defined as $P < 0.05$.

Role of the Funding Source

The funder of the study had no role in the study design, data collection, data analysis, data interpretation, or writing the report. The corresponding author had full access to all data in the study and had final responsibility for the decision to submit for publication.

RESULTS

Pudendal Nerve Function Study

Anorectal manometry confirmed normal anal canal rhythm waves in all dogs preoperatively (Fig. 1A). There was no normal rhythm wave in the transection group (N = 3) 12 months postoperatively, indicating no recovery of the anal canal rhythm wave (Fig. 1B). We began to see a normal rhythm wave in the anastomosis group (N = 3) 6 months postoperatively (Fig. 1C). The resting pressure in the transection group and the anastomosis group was 26 ± 2.2 mm Hg and 32 ± 5.5 mm Hg preoperatively; 20 ± 1.5 mm Hg and 20 ± 3.7 mm Hg 1 week postoperatively; 18 ± 2.0 mm Hg and 19 ± 2.3 mm Hg 1 month postoperatively; 20 ± 3.7 mm Hg and 26 ± 9.6 mm Hg 6 months postoperatively; and 12.7 ± 3.2 mm Hg and 22 ± 6.9 mm Hg 1 year postoperatively, respectively. The transection group had a significantly lower resting pressure postoperatively vs preoperatively. The anastomosis group showed no significant differences 6 months postoperatively versus preoperatively (Fig. 1D).

We used electromyography to confirm neuromuscular transmission from the pudendal nerve to the anal sphincter 1 year postoperatively (Fig. S3A, http://links.lww.com/SLA/C269). The transection group had negative responses (Fig. S3B, http://links.lww.com/SLA/C269). Even though 1 dog had a unilateral response and no response in the right pudendal nerve, overall, the anastomosis group had positive responses (Fig. S3C, http://links.lww.com/SLA/C269).

Pathologically, compared with the control (Fig. 2A), the transection group had severe atrophy of the internal anal sphincter and interstitial expansion with blue-stained collagen fibers in the submucosa (Fig. 2B). In contrast, the anastomosis group had mild atrophy (Fig. 2C). Regarding the external anal sphincter, compared with the control (Fig. 2D), the transection group had severe atrophy (Fig. 2E), and the anastomosis group had mild atrophy (Fig. 2F). The degree of internal and external anal sphincter muscle atrophy is summarized in Figure 2G and H, respectively.

Pathologically, regarding the pudendal nerve, compared with the control (Fig. 3A), the transection group had significantly decreased thickness of the myelin sheath and lower numbers of fibers (Fig. 3B). In contrast, the anastomosis group had mild decreased thickness of the myelin sheath and maintained the number of fibers (Fig. 3C). The thickness of the myelin sheath (μm) was 1.1 ± 0.5 in the control, 0.3 ± 0.18 in the transection group, and 0.6 ± 0.4 in the anastomosis group. The anastomosis group had significantly greater nerve thickness versus the transection group, but lower
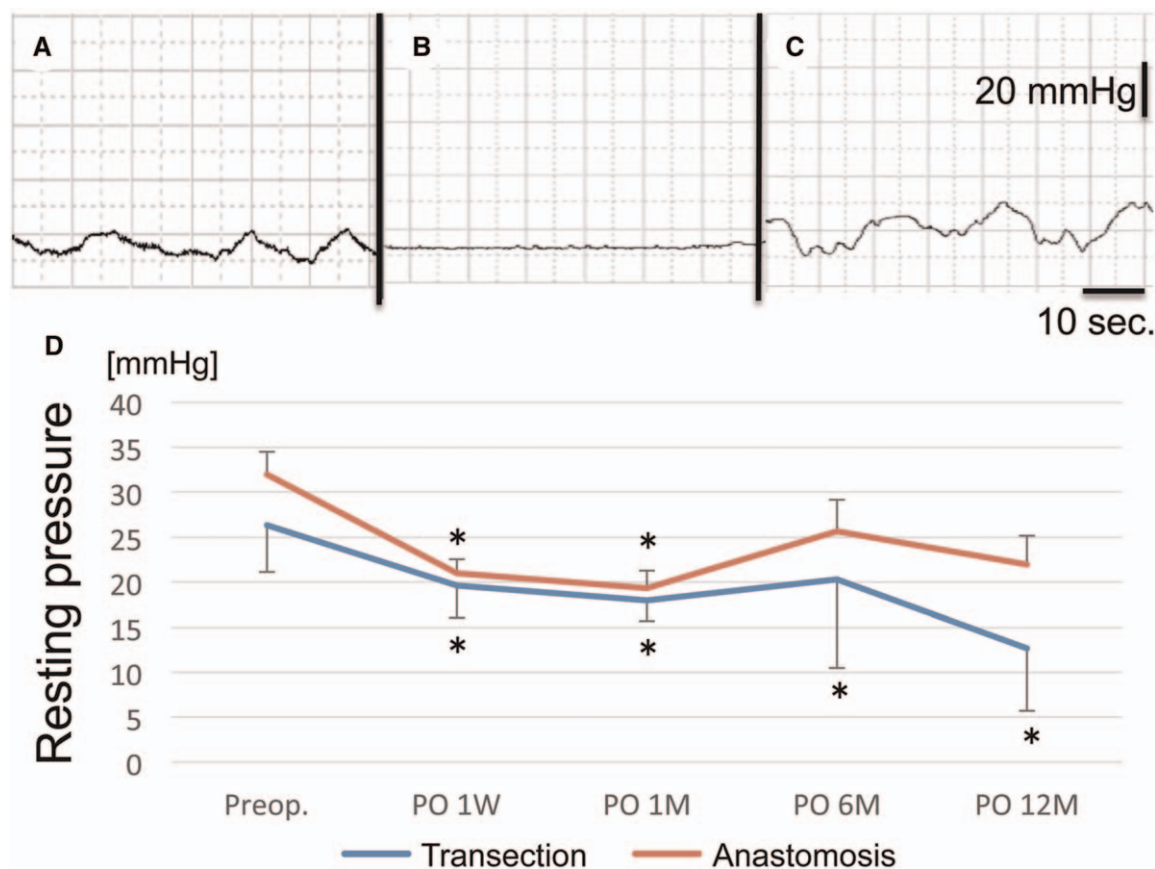
FIGURE 1. Anorectal manometry in the pudendal nerve function study. (A) Normal anal canal rhythm waves. (B) No recovery of the anal canal rhythm wave. (C) Recovery of normal anal canal rhythm waves. Horizontal bar = 10 seconds and vertical bar = 20 mm Hg. The resting pressure in the transection group and the anastomosis group is summarized in Graph D.

than in the normal control (Fig. 3D). The number of unmyelinated and myelinated (and total) fibers, respectively, was 19 ± 3.5 and 15 ± 3.4 (34 ± 6.3) in the control, 0 ± 0 and 7.5 ± 5.0 (7.5 ± 5.0) in the transection group, and 12 ± 6.0 and 22 ± 8.1 (33 ± 11) in the anastomosis group. The transection group had significantly fewer nerve fibers versus the anastomosis group and the control, with no difference between the anastomosis group and the control (Fig. 3E).

Electron microscopy revealed that, compared with the control (Fig. 3F), nerve fibers were clearly destroyed in the transection group (Fig. 3G). In contrast, the anastomosis group maintained nerve fibers, and Schwann cells were observed, suggesting nerve regeneration (Fig. 3H and I).

Anorectal Transplantation

Anorectal transplantation was technically successful with the 3-stage operation. In a preliminary experiment without this method, the surgical site was infected by stool, the wound reopened, and the graft failed (data not shown). Dogs undergoing autotransplantation were generally healthy and had good wound healing (Fig. 4A–C). One of the dogs undergoing allotransplantation received immunosuppression with 0.1 mg/kg/day tacrolimus just after transplantation. He was generally healthy but acute and chronic mild rejection occurred 4 days and 4 months after transplantation, respectively (Fig. 4D and E). Rejection was diagnosed according to the signs of redness, swelling, and easy bleeding of the graft. Both episodes were successfully treated

with 1.0 mg/kg/day methylprednisolone and 0.2 mg/kg/day tacrolimus intramuscularly for 10 days. Good wound healing followed this therapy (Fig. 4F). The other dog undergoing allotransplantation did not receive immunosuppression, as a positive control. He developed general fatigue and developed severe rejection 1 day after transplantation (Fig. 4G). Because the graft almost failed, we euthanized this dog, as the endpoint. The dog undergoing autotransplantation and the dog undergoing allotransplantation with immunosuppression began to defecate through the anorectal graft after stoma closure and achieved perfect control at the study endpoint (Video 1 (Video 1(.mp4 file). Controlled Defecation 1 Year after Anorectal Autotransplantation. This video has no audio and no interviewers/interviewees, and was filmed at the Laboratory of Veterinary Surgery, Graduate School of Agricultural and Life Science, The University of Tokyo, Tokyo, Japan on 5 March 2015.), http://links.lww.com/SLA/C271 and 2 (Video 2(.mp4 file). Controlled Defecation 1 year after Anorectal Allotransplantation with Immunosuppression. This video has no audio and no interviewers/ interviewees, and was filmed at the Laboratory of Veterinary Surgery, Graduate School of Agricultural and Life Science, The University of Tokyo, Tokyo, Japan on 26 May 2015., http://links.lww.com/SLA/C272)).

Defecography showed a dilated rectum and closed anal canal before defecation (Fig. S4A, http://links.lww.com/SLA/C270) and contracted rectum and open anal canal after defecation (Fig. S4B, http://links.lww.com/SLA/C270) 1 year after autotransplantation.
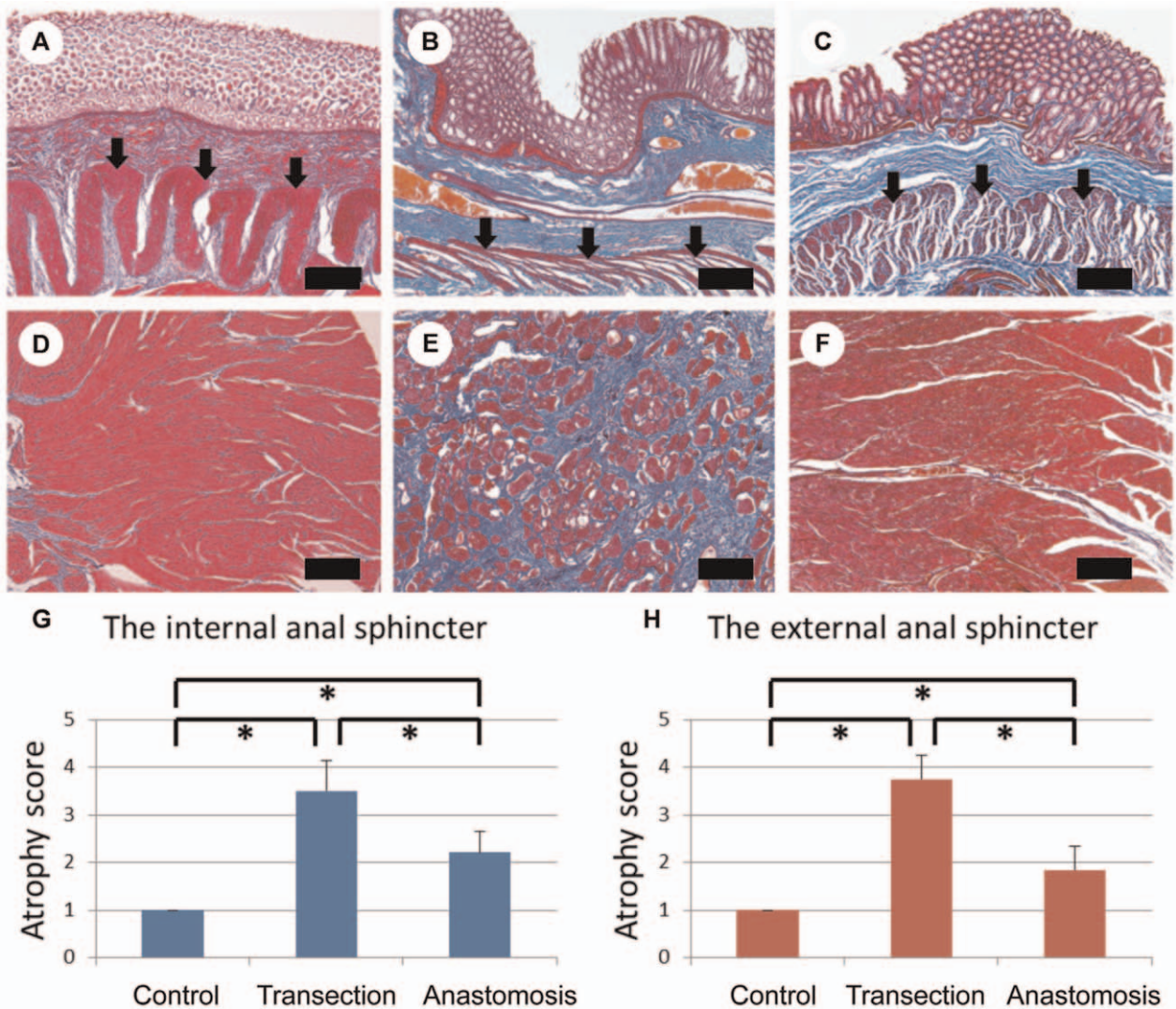
FIGURE 2. Pathological evaluation of the internal and external sphincter muscles in the pudendal nerve function study. The internal sphincter muscle in the control (A), transection group (B), and anastomosis group (C), and the external sphincter muscle in the control (D), transection group (E), and anastomosis group (F) were examined histologically using Masson trichrome staining. Collagen fibers were stained blue, nuclei were stained black, and the background was stained red. Bar = 1 mm. Arrows indicate the internal sphincter muscle layer (A–C). The atrophy scores for the internal and external sphincter muscles are summarized in Graph G and H, respectively.

These findings were also confirmed 1 year after allotransplantation with immunosuppression (Fig. S4C and D, http://links.lww.com/SLA/C270). These findings, the "beak sign," indicate that the anorectal graft functioned to retain stool.

Pathologically, compared with normal mucosal epithelium (Fig. 5A), submucosal tissue (Fig. 5B), and external anal sphincter (Fig. 5C), the control dog and the autotransplant dog showed no shedding of mucosal epithelium (Fig. 5D), slight edema and cell infiltration in the submucosa (Fig. 5E), and mild atrophy in the external anal sphincter (Fig. 5F). Findings were similar in the mucosa (Fig. 5G), submucosa (Fig. 5H), and external anal sphincter (Fig. 5I) in the dog undergoing allotransplantation with immunosuppression. In contrast, the dog undergoing allotransplant without immunosuppression showed severe shedding of the mucosal epithelium (Fig. 5J),

edema and cell infiltration in the submucosa (Fig. 5K), and external anal sphincter atrophy (Fig. 5L).

DISCUSSION

Anorectal transplantation is expected to become feasible for total anorectal reconstruction, but is behind in its development despite tremendous innovation in vascularized composite allotransplantation in the last decade. Despite the performance of >70 hand (arm) transplants, 36 face transplants, and 11 uterus transplants worldwide,[28] few published studies have evaluated anorectal transplantation[17,18] The reasons are that the surgery is technically difficult and involves multiple disciplines. Corroborating evidence for long-term functional and immunological outcomes is also needed before human anorectal transplantation can be implemented. Our team
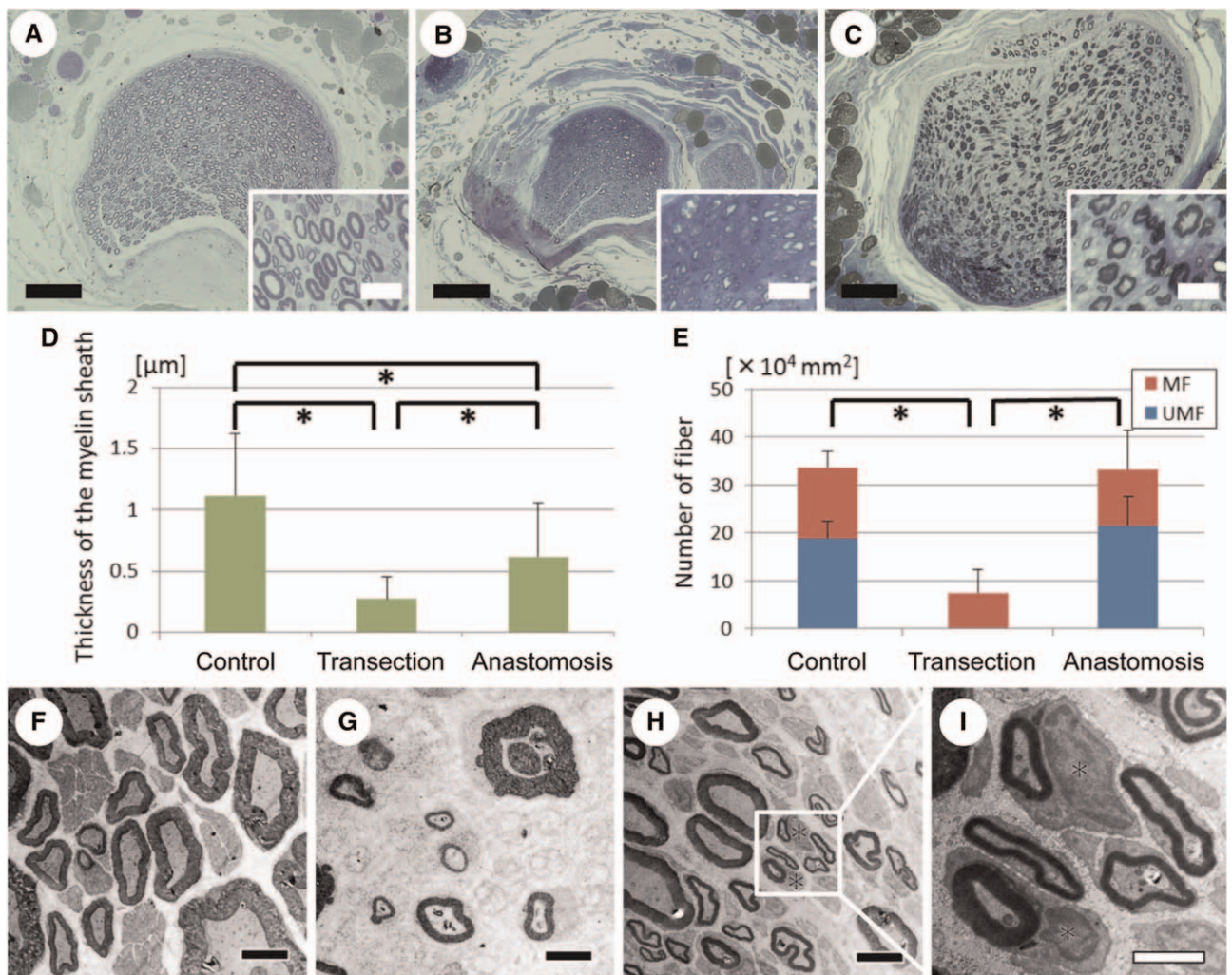
FIGURE 3. Pathological evaluation of the pudendal nerve in the nerve function study. Compared with the control (A), the transection group showed significantly decreased thickness of the myelin sheath and lower numbers of fibers (B). In contrast, the anastomosis group showed mild decreased thickness of the myelin sheath and maintained the number of fibers (C). The thickness of the myelin sheath is summarized in Graph D. The number of myelinated (MF) and unmyelinated (UMF) fibers is summarized in Graph E. Using electron microscopy, compared with the control (F), nerve fibers were clearly destroyed in the transection group (G). In contrast, the anastomosis group maintained nerve fibers, and Schwann cells were observed, suggesting nerve regeneration (H and I).

consists of colorectal surgeons, plastic surgeons, transplant surgeons, and pelvic anatomists, and we have performed preclinical research in anorectal transplantation with a step-by-step logical developmental approach for >8 years.[19–27]

In our pudendal nerve function study, we confirmed that the pudendal nerve was crucial for anorectal function, although this was common knowledge.[29,30] Manometry, electromyography, and histological examination of the dogs' anal sphincters and pudendal nerves suggested functional recovery following anastomoses of bilateral pudendal nerves. The role of the pudendal nerve in anorectal function is well-defined in humans, but in animals, there is a single report of a rat model used to evaluate functional recovery after anorectal transplantation without pudendal nerve anastomosis.[24] Therefore, we reconfirmed and described the critical role of the pudendal nerve in anorectal function in a canine model.

In our anorectal transplantation model, colostomy was necessary to maintain surgical site cleanliness peritransplant, similar to recipient patients who have undergone previous colostomy. Dogs undergoing autotransplantation and allotransplantation with immunosuppression regained good function 6 months postoperatively. Allotransplanted dogs had slightly slower recovery versus the autotransplanted dog likely because of the mild rejection. Eventually, dogs could sense the need to defecate, then pose and defecate, as shown in the videos. Interestingly, histological examination of the graft revealed both external and internal sphincter muscle regeneration. To our knowledge, this is the first report showing that both sympathetic and parasympathetic functional recovery after nerve anastomosis.

The indications for anorectal transplantation must include the advantages and disadvantages because the procedure is performed not to save a patient's life but to improve quality of life. Other candidates are patients with congenital anal dysfunction caused by anal atresia or Hirschsprung disease, and intractable anal fistulae secondary to inflammatory bowel disease, trauma, and rectal or
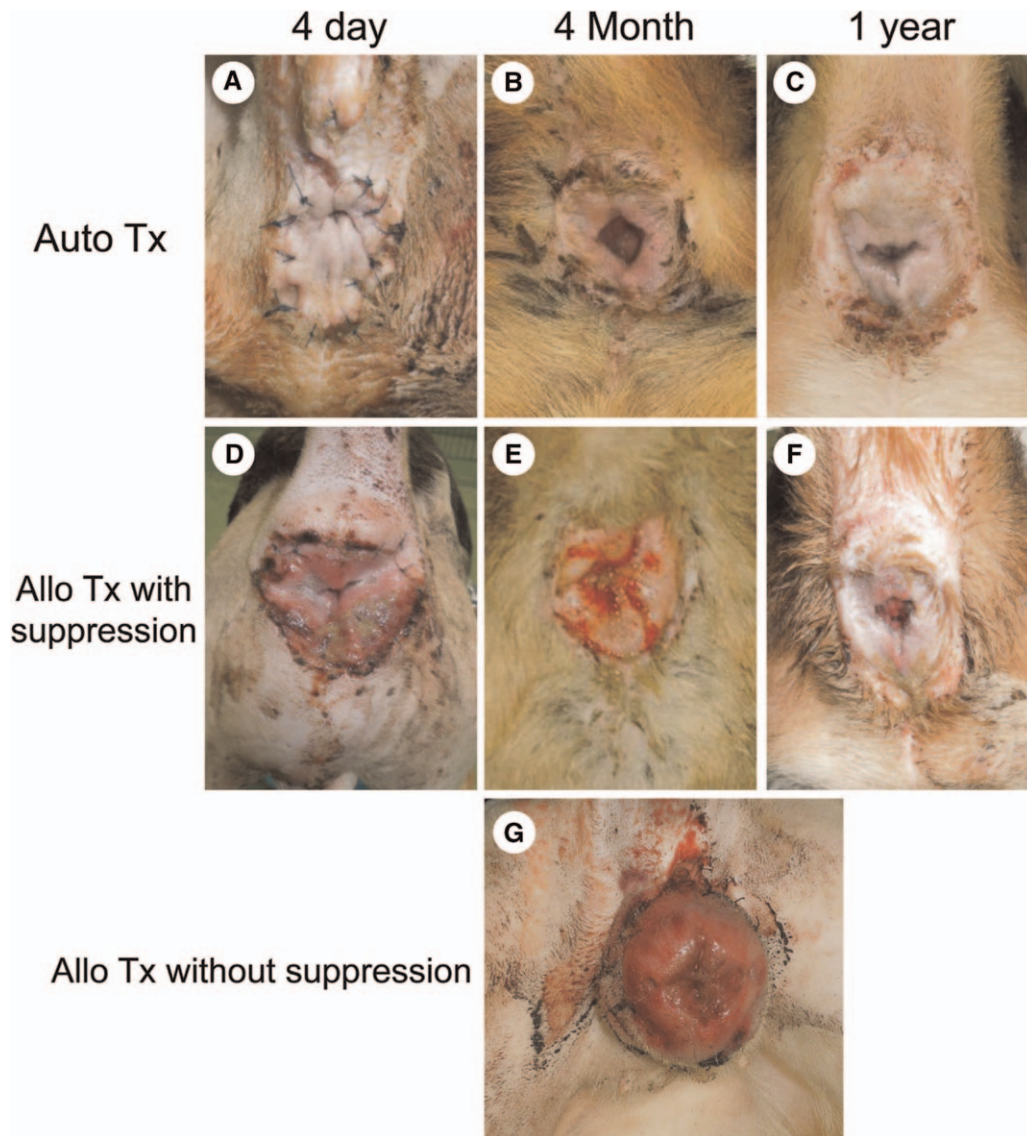
FIGURE 4. Postoperative course after anorectal transplantation. Perianal wounds healed well after autotransplantation. Photographs were taken 4 days (A), 4 months (B), and 1 year after surgery (C). There were 2 episodes of mild rejection in the dog who underwent allotransplantation with immunosuppression; acute and chronic rejection occurred 4 days (D) and 4 months (E), respectively, postoperatively. Both episodes were successfully treated with low-dose immunosuppression (F). Severe rejection occurred in the dog who underwent allotransplantation without immunosuppression, 1 day after surgery (G).

perianal cancer. Postoperative immunosuppression has side effects such as hypertension, renal function impairment, neutropenia, secondary cancers, and diabetes. However, these risks regarding anorectal transplantation are considered much milder than those associated with intestinal transplantation because approximately 80% of all immune cells reside in the intestine and are repopulated after transplantation with recipient cells.[31] In fact, low doses of methylprednisolone and tacrolimus suppressed episodes of rejection in our dog after allogeneic anorectal transplantation. Moreover, recent innovations in immune tolerance or interspecies chimerism are expected to resolve these immunological problems.[32,33]

Anorectal transplantation was inspired by the 1901 achievements of John D Rushmore,[34] who introduced anorectal autotransplantation to treat frequent fecal discharge secondary to complex

perineal trauma. Experimental anorectal allotransplantation was first attempted in 2000.[17] However, human anorectal allotransplantation has not yet been performed clinically. Because rats and pigs do not have the capacity to inhibit defecation, as do people, these animals are not particularly useful as models for human applications. Dogs living with people and having defecation control are the most suitable laboratory animals for anal function evaluation.[26] We previously developed a canine anorectal transplantation model with short-term observation; however, the longterm outcomes and immune response after allotransplantation remained unclear.[27] In this report, we demonstrated the first long-term outcome after anorectal transplantation in a canine model. It is important to use dogs as an experimental model because they are comparable to humans regarding observing defecation control and have vessel sizes and hemodynamic
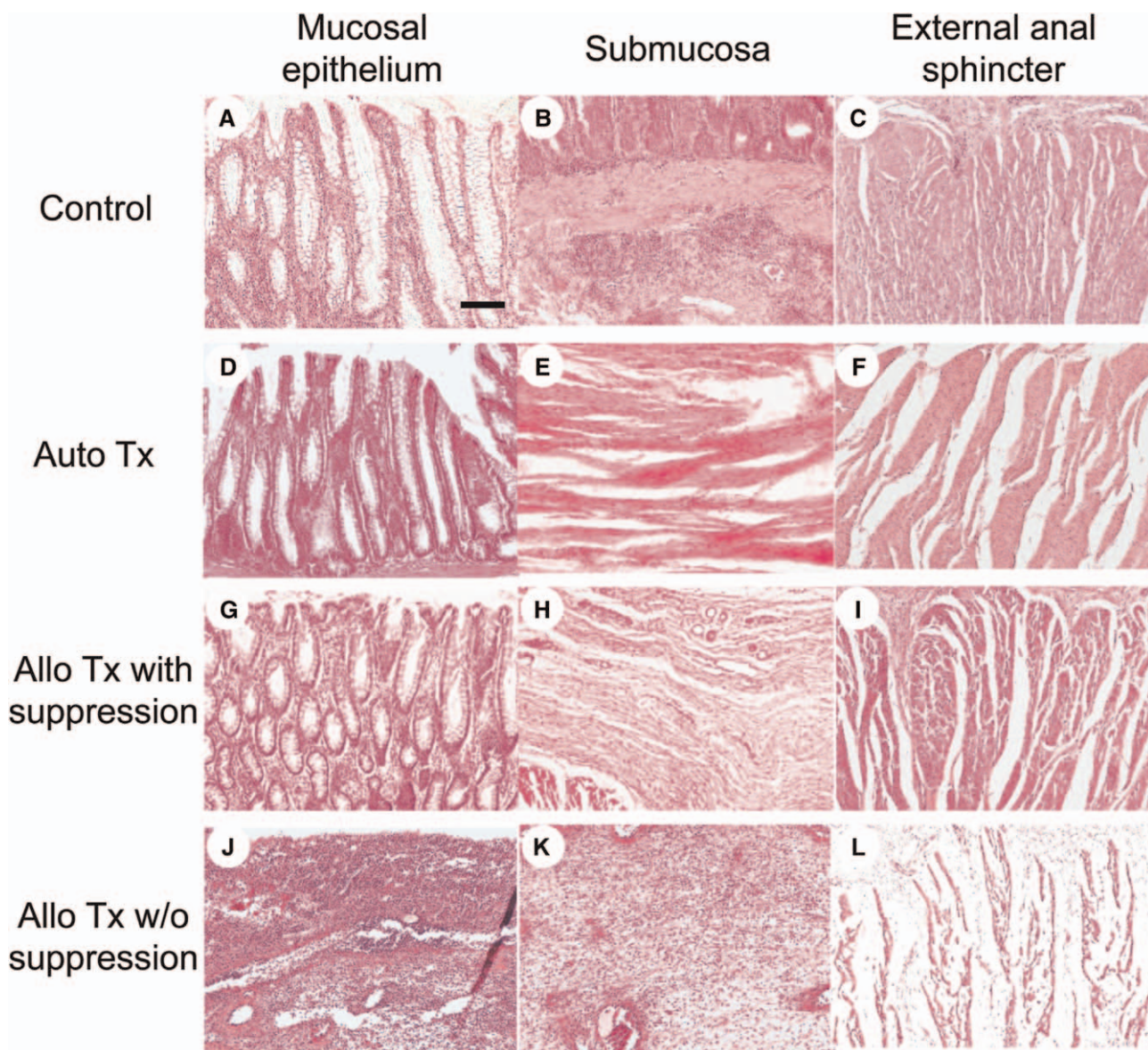
FIGURE 5. Microscopic findings after anorectal transplantation. Compared with normal mucosal epithelium (A), submucosal tissue (B), and external anal sphincter (C) in the control, the dog who underwent autotransplantation showed no shedding of the mucosal epithelium (D), slight edema and cell infiltration in the submucosa (E), and mild atrophy in the external anal sphincter (F). Findings were similar in the mucosa (G), submucosa (H), and external anal sphincter (I) of the dog who underwent allotransplantation with immunosuppression. In contrast, the dog who underwent allotransplantation without immunosuppression showed severe shedding of the mucosal epithelium (J), edema and cell infiltration in the submucosa (K), and atrophy of the external anal sphincter (L).

parameters that approximate those of human vessels.[35] A limitation of this study was the poor understanding of immune response in the canine anorectal transplantation model because knowledge of dog leukocyte antigens is much less clear than for human leukocyte antigens.[36] Therefore, successful human anorectal transplantation with long-term outcomes is expected from both clinical and academic perspectives. This report is a proof-of-concept study for anorectal transplantation as a treatment for patients receiving a colostomy secondary to anorectal dysfunction. Our results confirmed the feasibility of the first human trial taking place in the near future.

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