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Original Article

Early microrecanalization of vas deferens following biodegradable graft implantation in bilaterally vasectomized rats

Christopher M. Simons[1], Barry R. De Young[2], Thomas S. Griffith[1], Timothy L. Ratliff[1], Erin Jones[3], Surya K. Mallapragada[3], Moshe Wald[1]

[1]*Department of Urology, University of Iowa, Iowa City, IA 52242-1089, USA*
[2]*Department of Pathology, University of Iowa, Iowa City, IA 52242-1089, USA*
[3]*Department of Chemical and Biological Engineering, Iowa State University, Ames, IA, USA*

Abstract

We evaluated a biodegradable graft for reconstruction of rat vasa deferentia with long obstructed or missing segments. A total of 47 Sprague-Dawley rats underwent bilateral vasectomy and were divided into groups according to length of the vas deferens affected (0.5, 1, 1.5 cm). After 8 weeks, poly-(*D,L*-lactide) (PDLA) grafts were used to reconnect the vas deferens. Grafts and adjoining vasa deferentia were excised 8 and 12 weeks later and evaluated microscopically. At 8 weeks, microscopic changes included a robust inflammatory response around the grafts. All grafts were still intact but in the early stages of degradation. No microtubules, indicative of vas deferens recanalization, were identified. One specimen showed evidence of healing and neovascularization at the interface zone between the vas deferens and the graft. At 12 weeks, grafts were further degraded but still present. Microscopic evaluation showed decreased inflammation. Seven specimens showed neovascularization at the interface zone; two of these showed distinct epithelialized vas deferens microcanals at the graft edges. One specimen showed a microcanal spanning the entire 0.5-cm graft. A time period of 8 weeks is not ample enough for vas deferens regeneration in the setting of a biodegradable PDLA graft; however, early evidence of re-growth was seen at 12 weeks. A longer healing time should permit further biodegradation of the graft, as well as re-growth and possible eventual reconnection of the vas deferens, allowing passage of sperm. These findings suggest a potential role for biodegradable grafts in the reconstruction of vas deferens with long obstructed segments.

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1 Introduction

In the United States, approximately 500 000 men have a vasectomy each year to block undesired fertility [1]. Surveys suggest that 2%–6% will later seek to have the operation reversed [2]. Vasectomy reversal most commonly takes the form of a vasovasostomy. Although post-vasectomy patients who desire restoration of fertility constitute the greatest number of patients undergoing this operation, others may seek correction of obstructive lesions secondary to genital tract congenital anomalies, infection, trauma, or incidental injury in previous pelvic, inguinal, or scrotal surgery.

The preferred method of surgical reconstruction

Correspondence to: Dr Moshe Wald, Department of Urology, University of Iowa, 200 Hawkins Dr., 3 RCP, Iowa City, IA 52242-1089, USA.
Fax: +1-319-356-3900 E-mail: moshe-wald@uiowa.edu
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of the male genital tract involves removal of the obstructed segment and restoration of continuity of the tract with a primary anastomosis via vasovasostomy or vasoepididymostomy. Vasovasostomy is preferred because it is technically less demanding to perform and is associated with higher postoperative patency and pregnancy rates. However, long gaps created during previous vasectomy or long segments of obstruction can make successful vasovasostomy difficult. Biodegradable grafts can be used as scaffolds to support the growth of the divided vas deferens, or to bridge or narrow the gap. In the latter case, by decreasing the length of the gap, the technical problem of an anastomosis under tension can be avoided.

An earlier study found small epithelialized tubules in vas deferens specimens taken at the time of vasovasostomy [3]. These small channels were independent of sperm granuloma and their formation was termed microrecanalization. The men were treated at 2–14 years post-vasectomy, and the continuity of the channels varied from 200 to 1 140 μm, showing the capacity of the vas deferens to reorganize and regenerate.

Another study evaluated vas replacement with either transplanted native vasa (contralateral side) or transplanted blood vessels (aorta from female rat donor) [4]. Three graft lengths were examined: 0.5, 1, and 1.5 cm. Evidence of functional patency (smear examination to detect sperm distal to graft) ranged from 10% in the 1.5-cm group to 55% in the 0.5-cm group. Anatomic patency ranged from 25% and 30% in the 1- and 1.5-cm groups, respectively, to 75% in the 0.5-cm group.

Earlier studies have evaluated possible biomedical applications of biodegradable polymer grafts, specifically their use in guiding and accelerating peripheral nerve growth [5, 6]. These biodegradable polymer substrates have been shown to significantly enhance neurite alignment and outgrowth.

Poly(lactic acid) is currently one of the most widely investigated and commonly used synthetic bioerodible polymers used for applications such as sutures, drug delivery systems, fracture fixation devices, ligament and tendon replacement, and tissue engineering. Lactic acid exists in two stereoisomeric forms, and polymers derived from the D and L monomers are semicrystalline materials. Hydrolysis of poly-(*D,L*-lactide) (PDLA) yields lactic acid, which is biocompatible.

This study explored the use of a biodegradable PDLA graft for reconstruction of the vas deferens in rats after vasectomy involving vasal segments of varying lengths. The primary endpoint was histological evidence of vas deferens re-growth. A further point of interest was whether rats with graft-reconstructed vasa deferentia were able to impregnate female rats.

2 Materials and methods

The study protocol was approved by the Institutional Animal Care and Use Committee of the University of Iowa (Iowa, IA, USA) (Figure 1).

2.1 Rats

A total of 47 adult male Sprague-Dawley rats (purchased from Harlan Sprague Dawley, Inc., Indianapolis, IN, USA) weighing 225–250 g were randomly divided into two groups of 18 and a third group of 11. For all operations, anesthesia was induced using intraperitoneal pentobarbital (40 mg kg^{-1}). Twelve female Sprague-Dawley rats were housed with male rats following insertion of the biodegradable grafts to detect pregnancy as a functional outcome of the reconstructive surgery. The housing protocol is described below.

2.2 First surgical procedure (bilateral vasectomy)

Using a low midline abdominal incision, rats in all three groups were subjected to bilateral vasectomy with different lengths of vas obstruction. The first group had

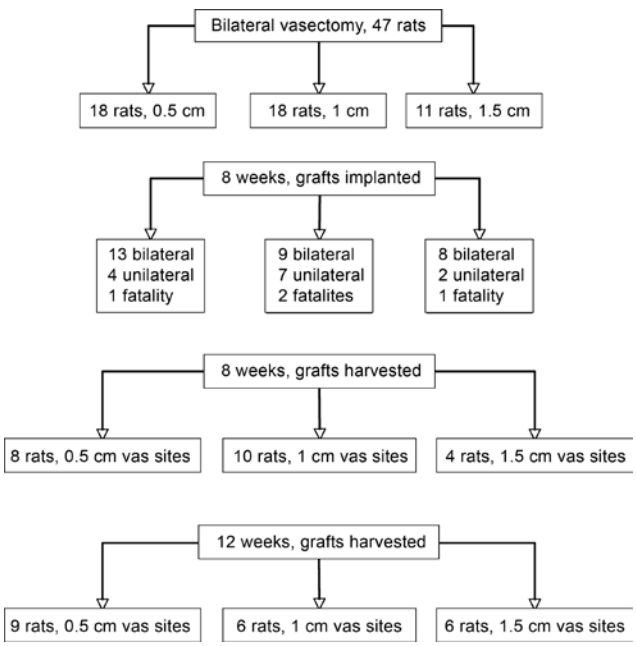

Figure 1. Study timeline.

a vasectomy length of 0.5 cm, the second group 1.0 cm, and the third group 1.5 cm. These lengths represent 10%, 20%, and 30% of the total vas length in the rat [4]. Following an established technique [7], a segment of vas deferens was isolated from surrounding tissues and a 4-0 silk ligature was placed on the abdominal side for future identification of the vasectomy site. This was followed by complete circumferential fulguration with a hand-held electrocautery device to generate the desired length of obstruction without excision of vasal tissue. Severed vasa deferentia were returned to their anatomical position, and the skin was closed using 4-0 monocryl.

2.3 Biodegradable grafts

The biodegradable grafts used in this study were designed and constructed at Iowa State University (Ames, IA, USA). Porous conduits for grafting were made of PDLA (Birmingham Polymers Lactel PDLA), inherent viscosity 63 mL g^{-1} in $CHCl_3$ at 30ºC). PDLA was dissolved in chloroform at 30% (w/v). Sodium chloride was ground in a mortar and pestle and sieved through a #140 (106 μm) mesh. Sieved NaCl was added to the polymer/chloroform solution to obtain finished conduits with 75% porosity. Polyvinyl alcohol-coated glass capillary tubes were dipped into the PDLA/salt suspension (Figure 2). Tubes were soaked in water to remove NaCl and polyvinyl alcohol and conduits were dried in a desiccator. Desiccated conduits were stored in sampling tubes at 20ºC.

2.4 Second surgical procedure (biodegradable graft placement)

At 8 weeks after the initial bilateral vasectomy, all animals underwent bilateral placement of PDLA graft at the previous vasectomy site. The incision was made through the previous scar and the previously placed silk tie was located. The cauterized segment of the vas deferens was then carefully removed. Finally, a specially prepared biodegradable graft of corresponding length (0.5, 1, 1.5 cm) was microsurgically sewn into the gaps in the vasa of all three groups using 7-0 prolene sutures. This type of suture was experimentally found to be the most appropriate for handling the grafts, as 9-0 and 10-0 sutures did not penetrate the graft material very well. Each graft was approximately 4 mm in diameter, slightly larger than the diameter of the vas deferens. This was consistent with previous biodegradable graft studies for nerves, in which grafts were slightly larger in diameter than the nerve to be reconstructed. A Wild M651 operating microscope (Wild Leitz, Willodale, Ontario, Canada) was used to make an end-to-end anastomosis between the vas and graft using two to three sutures at both the abdominal and testicular ends. This sequence was then repeated on the contralateral side. After successful microsurgical implantation, animals were returned to the animal storage facility and two animals housed to a cage as they had been housed preoperatively. After 1 week to allow for healing, rats were housed with one female Sprague-Dawley rat per cage for 10 days to account for the rat average estrous cycle of 4–5 days [8]. If pregnancy was not achieved after 10 days, the female rat was rotated to another cage for another 10-day period as recommended by a veterinarian at our institution. Pregnancy was determined by palpation of a gravid uterus and swollen mammary glands.

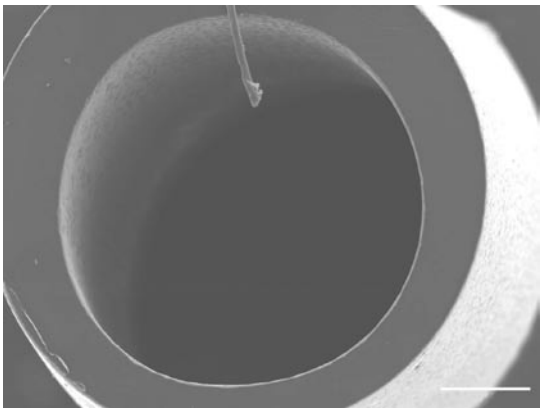

Figure 2. Scanning electron microscope image of a polymer conduit. Bar = 200 μm.

2.5 Third surgical procedure (harvesting of reconstructed segments)

At 8 weeks after the grafts were sutured in place, 22 of the 47 rats, representing approximately half of the animals in each group, were randomly selected to be sacrificed. Reconstructed vas segments were harvested from each rat. The harvested vasa were immediately placed in formalin for storage. Rats were then killed by lethal intraperitoneal injection of pentobarbital, followed by confirmation of death by a physical method.

At 4 weeks after the initial harvest–12 weeks since reconstruction of the divided vas deferens–vasa were harvested from the remaining 21 rats as described above. Four rats died from anesthesia-related complications.

Thirteen rats had unilateral graft placement because of technical difficulties. The grafts and adjoining vasa deferentia harvested were evaluated microscopically with hematoxylin and eosin staining by a single pathologist.

3 Results

3.1 Grafts harvested 8 weeks after placement

In all, 22 specimens were reviewed at 8 weeks, including 0.5-cm grafts (n = 8), 1-cm grafts (n = 10), and 1.5-cm grafts (n = 4). All grafts were intact and identifiable with no gross signs of degradation (Figure 3). On visual inspection, two specimens were found to contain a green, purulent-appearing material. The graft and vas were not easily visible in another 12 specimens because they were surrounded by a tough brown sac-like tissue encasement. Incision of the sac-like tissue revealed the vas and graft surrounded by caseous material. Transverse sections were obtained from throughout the specimen, including representative samples from the vas, the vas–graft interface at each anastomosis, and the graft itself. Microscopic evaluation of the sections by a single pathologist revealed an intense inflammatory response. The grafts, although present, were all in various early stages of degradation. No microtubules, indicative of vas deferens recanalization, were identified. One 1.5-cm graft showed evidence of healing and neovascularization at the interface zone between vas deferens and graft (Figure 4). No female rats were impregnated by any animal in this study group.

3.2 Grafts harvested 12 weeks after placement

A total of 21 specimens were reviewed at 12 weeks, including 0.5-cm grafts (n = 9), 1-cm grafts (n = 6), and 1.5-cm grafts (n = 6). The grafts were still present but had degraded further than grafts in the 8-week group. Granulomas were evident in 17 specimens. Sac-like tissue encasements were not seen in specimens obtained at 12 weeks. One specimen showed gross signs of infection, including the presence of purulent material that resembled an abscess. Microscopic evaluation showed the early stages of resolution and less inflammation than in the 8-week group. Seven specimens (33% of the study group) showed neovascularization at the interface zone, including two (10% of the study group) that showed distinct epithelialized vas deferens microcanals at the graft edges (Figure 5). Further exploration of one of these specimens showed that the microcanal had grown continuously for the entire length of the 0.5-cm graft. An example of a native rodent vas deferens is depicted in Figure 6. No female rats cohabitated with this group were impregnated.

4 Discussion

To our knowledge, this study is the first to examine the use of a biodegradable graft for reconstruction of long obstructions of the vas deferens where primary repair was not an option. The time periods of 8 weeks between vasectomy and reversal followed by 8 and 12 weeks until harvesting of graft and vas were chosen to approximate the human time course as patients usually present for reversal several years after vasectomy. The

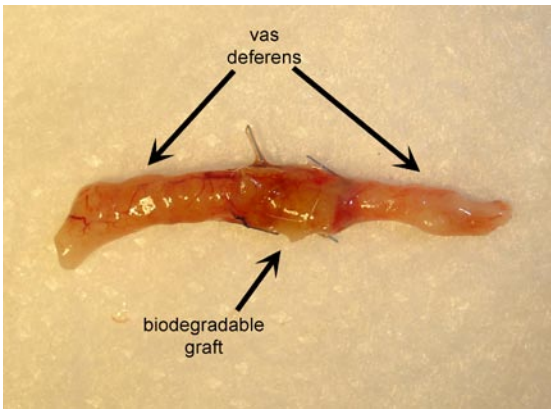

Figure 3. Gross appearance of 0.5-cm poly-(*D,L*-lactide) (PDLA) graft after harvest at 8 weeks. Note the vas deferens attached to both ends of the graft in an end-to-end anastamosis.

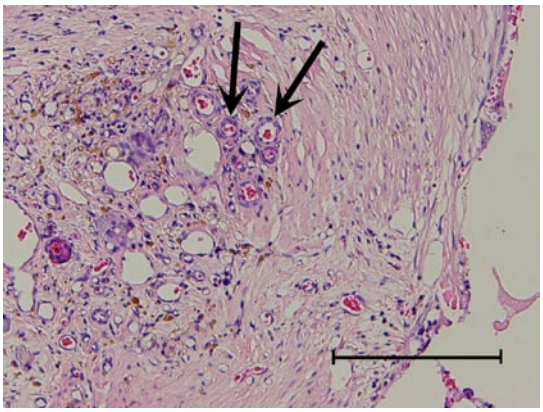

Figure 4. A 1.5-cm poly-(*D,L*-lactide) (PDLA) graft at 8 weeks, with evidence of neovascularization (arrows) (magnification × 200). Bar = 0.5 mm.

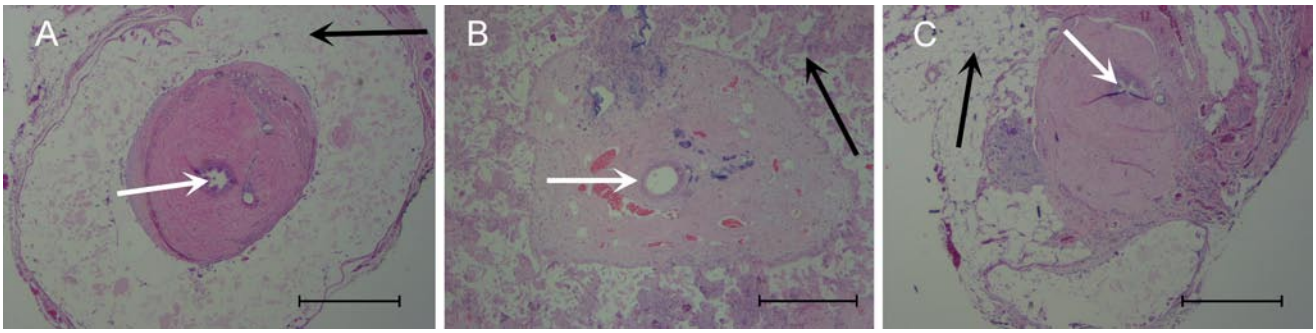

Figure 5. (A): Evidence of microrecanalization at the midpoint of a 0.5-cm poly-(*D,L*-lactide) (PDLA) graft (magnification × 40). Bar = 1 mm. (B): Microcanal at the midpoint of a 0.5-cm PDLA graft (magnification × 200). Bar = 0.5 mm. (C): Microcanal at the interface zone of a 0.5-cm PDLA graft (magnification × 40). Bar = 1 mm. All panels: white arrows, microcanal; black arrows, graft.

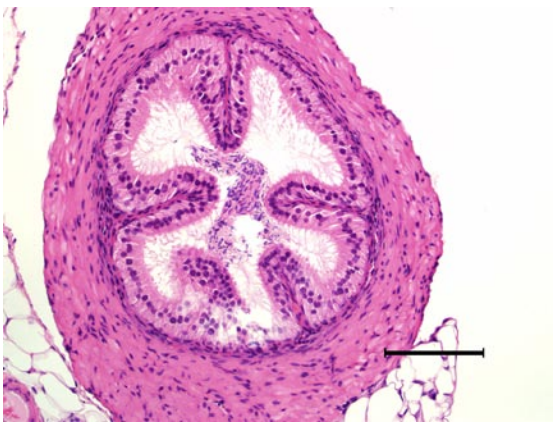

Figure 6. Murine vas deferens (magnification × 20). Bar = 1 mm.

presence of the PDLA graft at 8 and 12 weeks after reconstruction shows that this graft was sufficiently able to avoid dissolution, thus allowing it to serve as a scaffold over which the vas deferens could re-grow. One specimen from the 1.5-cm graft group showed neovascularization after 8 weeks with reduced inflammatory response. As neovascularization is generally regarded as a necessary step for tissue re-growth, its presence suggests the potential for future vas recanalization. Two specimens from the 12-week group, both 0.5-cm grafts, showed a further dampening of the acute inflammatory response and the presence of microrecanalization. One 0.5-cm specimen that was further explored with serial sections showed that the microtubule of the vas was present over the entire length of the graft. This is considered true regeneration rather than preservation of native vas because the graft placement technique was an end-to-end anastomosis that did not allow for any of the vas ends to enter the lumen of the graft at the time of reconstruction. Some sections revealed narrowing at the vas-graft interface but no microscopic evidence of stricture. Overall, most sections contained a patent vas-graft interface.

The ultimate indication of successful vas deferens reconstruction is pregnancy, but none of the female rats that were housed with the study rats became pregnant. Although stricture at the vas-graft interface may have played a role, the physiological stress of multiple operations on the male rats is also a possibility. The graft itself may have presented a functional impediment at this early stage as it is a rigid tube and is not capable of peristalsis as the native vas is. Finally, the time allowed after graft implantation may have simply been too brief.

This study has certain limitations. Smears for sperm in vasal fluid obtained distal to the graft were not performed. This may have detected the presence of sperm and thus could have been a more sensitive indicator of anatomical patency. There were minor surgical limitations, as we were not able to successfully perform bilateral reconstruction on every rat, although the majority of rats underwent bilateral procedures. Still, at minimum every rat received a unilateral biodegradable graft. The main impediment to bilateral graft placement was the presence of extreme inflammation and scarring from previous operations, thus preventing localization and fastidious dissection of the affected vas.

A time period of 8 weeks is not ample enough to allow for regeneration of the vas deferens using biodegradable PDLA grafts in this rat vasectomy model. However, early evidence of vas deferens re-growth was seen at 12 weeks. A microcanal was seen to

connect both ends of the native vas by crossing the entire 0.5-cm graft. This time frame also allowed for further resolution of the intense host inflammatory response seen after graft placement. Although some vas deferens microrecanalization was observed at 12 weeks, keeping the graft in place for longer than 12 weeks should allow re-growth of more microcanals with the possibility of eventual restoration of fertility. Additional studies that include a longer time period between vasectomy and graft placement as well as assessment of the presence of sperm on the abdominal side of the grafts are needed to further evaluate the potential future role of biodegradable grafts in reconstruction of long segments of obstructed vas deferens.

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