Blackwell Publishing

Asian J Androl 2006; 8 (5): 543–548
DOI: 10.1111/j.1745-7262.2006.00192.x

AJA
www.asiaandro.com

·*Original Article*·

Porcine vesical acellular matrix graft of tunica albuginea for penile reconstruction

Kwan-Joong Joo[1], Byung-Soo Kim[2], Jeong-Ho Han[3], Chang-Ju Kim[4], Chil-Hun Kwon[1], Heung-Jae Park[1]

[1]*Department of Urology, Kangbuk Samsung Hospital, Sungkyunkwan University School of Medicine, 108 Pyung-Dong, Jongro-Ku, Seoul 110-746, Korea*
[2]*Department of Chemical Engineering, Hanyang University College of Engineering, 17 Hangdang-Dong, Seongdong-Ku, Seoul 133-791, Korea*
[3]*Department of Pathology, Samsung Medical Center, Sungkyunkwan University School of Medicine, 50 Ilwon-Dong, Gangnam-Ku, Seoul 135-710, Korea*
[4]*Department of Physiology, Kyung Hee University College of Medicine, 1 Hoegi-Dong, Dongdaemun-Ku, Seoul 130-701, Korea*

Abstract

Aim: To characterize the feasibility of the surgical replacement of the penile tunica albuginea (TA) and to evaluate the value of a porcine bladder acellular matrix (BAM) graft. **Methods:** Acellular matrices were constructed from pigs' bladders by cell lysis, and then examined by scanning electron microscopy (SEM). Expression levels of the mRNA of the vascular endothelial growth factor (VEGF) receptor, fibroblast growth factor (FGF)-1 receptor, neuregulin, and brain-derived neurotrophic factor (BDNF) in the acellular matrix and submucosa of the pigs' bladders were determined through the reverse transcription-polymerase chain reaction (PCR). A 5 mm × 5 mm square was excised from the penile TA of nine rabbits. The defective TA was then covered in porcine BAM. Equal numbers of animals were sacrificed and histochemically examined at 2, 4 and 6 months after implantation. **Results:** SEM of the BAM showed collagen fibers with many pores. VEGF receptor, FGF-1 receptor and neuregulin mRNA were expressed in the porcine BAM; BDNF mRNA was not detected. Two months after implantation, the graft sites exhibited excellent healing without contracture, and the fusion between the graft and the neighboring normal TA appeared to be well established. There were no significant histological differences between the implanted tunica and the normal control tunica at 6 months after implantation. **Conclusion:** The porcine BAM graft resulted in a structure which was sufficiently like that of the normal TA. This implantation might be considered applicable to the reconstruction of the TA in conditions such as trauma or Peyronie's disease. *(Asian J Androl 2006 Sep; 8: 543–548)*

Keywords: tissue engineering; extracellular matrix; penis; reconstructive surgical procedure; graft survival

Correspondence to: Prof. Heung-Jae Park, Department of Urology, Kangbuk Samsung Hospital, Sungkyunkwan University School of Medicine, 108 Pyung-Dong, Jongro-Ku, Seoul 110-746, Korea.
Tel: +82-2-2001-2240, Fax: +82-2-2001-2247
E-mail: hjae.park@samsung.com
Received 2005-11-20 Accepted 2006-03-18

1 Introduction

A variety of conditions, most notably trauma, penile neoplasm, congenital anomalies, or Peyronie's disease might necessitate surgical penile reconstruction. A vari-

ety of operative approaches have been developed in order to facilitate the functional and aesthetic restoration of the male genitalia [1]. However, restorative operations in such cases have generally been challenging as a result of the limited availability of the existing penile tissue [2, 3]. The acellular matrix, using urinary tract tissue or small intestinal submucosa (SIS), might constitute possible options for the treatment of such patients.

Numerous materials have been used as grafts for the repair of defects in the tunica albuginea (TA) of the penis. Several synthetic materials, including Gore-Tex, Marlex and Dacron, have been subjected to trials in such operations. These materials are readily available, resilient and inert. However, complications, including inelasticity of the graft and infections, have been associated with the use of these materials [4, 5]. Autologous grafts, including material from the dermis, fascia, veins, tunica vaginalis and dura mater have been proven to be non-immunogenic and are also associated with a low rate of infection [6–9]. However, these grafts might also lack the tensile strength and distensibility of the aforementioned synthetic materials [10]. None of these biological or synthetic materials have been shown to be ideal, nor have any been adopted as established standards.

Acellular matrix, derived from the urinary tract and the SIS, has also been utilized as a scaffold for the stimulation of the growth of the cell components of a variety of urinary tract components [11–13]. We attempted to assess the feasibility of the surgical replacement of the TA of the penis with acellular matrix, and also conducted an evaluation of the value of the porcine bladder acellular matrix (BAM) graft in this regard.

2 Materials and methods

2.1 Preparation of BAM

Bladders were harvested from adult pigs at an abattoir and were then acellularized according to the methods described by Brown *et al*. [14]. In brief, the tissues were placed in a 1% octyl-phenoxy-polyethoxyethanol solution (Triton X-100, Sigma Chemical Co., St. Louis, MO, USA) for 48 h. The specimens were washed with Sorenson's phosphate buffer, and subsequently treated with deoxyribonuclease (DNase, Sigma Chemical Co., St. Louis, MO, USA). The samples were treated with sodium dodecyl sulfate (SDS) solution and then rinsed in phosphate buffered saline for an additional 24 h. The tissues were sterilized with 70% ethanol.

Hematoxylin-eosin (HE) and Masson trichrome staining were used in order to determine the degree of acellularity exhibited by the matrix, as well as the efficacy of the extraction process. Scanning electron microscopy (SEM) was carried out in order to evaluate the ultrastructure, and verify the complete absence of all cellular constituents.

2.2 Evaluation of growth factor expression

The mRNA expression levels of vascular endothelial growth factor (VEGF) receptor, fibroblast growth factor (FGF)-1 receptor, neuregulin and brain-derived neurotrophic factor (BDNF) in the acellular matrices and submucosa of the pigs' bladders were examined by reverse transcription-polymerase chain reaction (RT–PCR).

Total RNA was extracted from the acellular matrix of the pig bladder and submucosa using RNAzol^{TM}B

Table 1. Primer sequences. VEGF, vascular endothelial growth factor; FGF-1, fibroblast growth factor-1; BDNF, brain-derived neurotropic factor; S, sensor; AS, antisensor.

Gene	Sequences	Size (bp)
Cyclophilin	ACCCCACCGTGTTCTTCGAC (S)	300
	CATTTGCCATGGACAAGATG (AS)	
VEGF receptor	ATCTTCCAGGAGTACCCTGA (S)	200
	TTGTTGTGCTGTAGGAAGCT (AS)	
FGF-1 receptor	GACCAGCACATTCAGCTGCA (S)	200
	TTCTCTGCATGCTTCTTGGA (AS)	
Neuregulin	GAAGGGCAAGAAGGACC (S)	200
	TTCATGGGTACATTCTCAG (AS)	
BDNF	CGTGAGTTTGTGTGGACCC (S)	180
	CGCTCTCCAGAGTCCCATGG (AS)	

http://www.asiaandro.com; aja@sibs.ac.cn

(TEL-TEST, Friendswood, TX, USA) according to the manufacturer's instructions. We then carried out reverse transcription of the RNA. The primer sequences for cyclophilin, VEGF receptor, FGF-1 receptor, neuregulin and BDNF are listed in Table 1. PCR was conducted with a GeneAmp 9600 PCR system (Perkin Elmer, Norwalk, CT, USA). The final amount of RT-PCR product for each of the mRNA species was then densitometrically calculated using Molecular Analyst™ software (version 1.4.1; Bio-Rad, Hercules, CA, USA).

2.3 Study animals and procedures

Nine young male New Zealand white rabbits, weighing between 1.9 and 2.3 kg each, were used in the present study, and additional three rabbits which had not undergone surgical intervention served as normal controls. Anesthesia was induced and maintained using 5 mg/kg of intramuscular xylazine and 40–50 mg/kg of intramuscular ketamine. After the abdomen and pelvis were prepared by shaving and the application of povidone-iodine, a penile ventral midline incision was made approaching the TA. The tunica was freed from the adjacent nerve fibers and vessels, and a square of the tunica, measuring about 5 mm × 5 mm, was excised. A BAM sheet, which was prepared as described above, was then sutured to the edges of the defect with nylon 5-0 in a running watertight manner. The orientation of the graft surfaces was consistently maintained. The penile wound was then closed with vicryl 4-0 sutures.

The animals were monitored at daily intervals within the first week after surgery and then three times a week until we had verified that wound healing was complete. The operated animals were then divided into three equal-number groups and the grafts were subjected to gross examination 2, 4 and 6 months after implantation. After the animals were sacrificed at 2, 4 and 6 months, their penises were excised and fixed in 10% formalin for later histological examination.

2.4 Histology and staining

The excised penile specimens were then fixed via overnight immersion in 10% buffered formalin, dehydrated in a graded series of ethanol solutions and embedded in paraffin. The 2–3 μm sections were prepared and air-dried onto precoated glass slides. These slides were then stained with HE and Masson trichrome.

3 Results

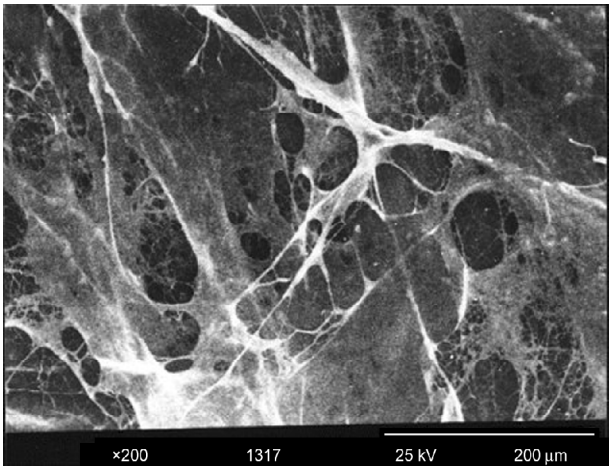

Figure 1. Scanning electron microscopic (SEM) analysis of acellularization. The bladder acellular matrix (BAM) reveals a delicate three-dimensional mesh-like structure composed of collagen fibers. There are many circular acellular pores surrounded by the delicate collagen fibers. Scale bar = 200 μm.

3.1 Preparation and evaluation of acellular matrix

Matrix acellularity was histologically assessed. Matrices were determined to be completely lacking urothelium and smooth muscle after extraction. Cell debris remnants of native bladder tissue were also determined to be completely absent after acellularization, whereas the collagen matrix was verified to have been preserved.

The matrix ultrastructure was visualized by SEM. The urothelium, along with its cellular constituents, could not be detected after extraction. Collagen fibers appeared intact and abundant, with many pores (Figure 1).

The mRNA of the VEGF receptor, FGF-1 receptor and neuregulin were expressed in the porcine BAM; whereas, BDNF was not expressed. However, none of the examined mRNA was expressed in the porcine vesical submucosa (Figure 2).

3.2 Acellular matrix graft of TA

All of the experimental animals survived after their procedures. We witnessed no hematomas, wound infection or dehiscence on the graft sites. The general condition of all animals was favorable.

The graft sites exhibited excellent healing on gross examination, with no contracture. Histologically, the implanted matrix appeared to be thicker than the neighboring TA and we observed subtunical cavernosal fibrosis at the graft site. The fusion between the graft and the

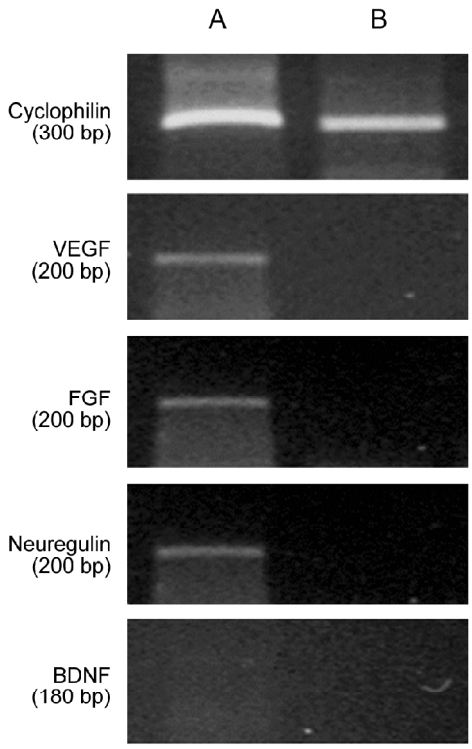

Figure 2. Results of RT-PCR analysis of the mRNA levels of vascular endothelial growth factor (VEGF) receptor, fibroblast growth factor (FGF)-1 receptor, neuregulin, and brain-derived neurotrophic factor (BDNF). Cyclophilin mRNA was also reverse-transcribed and amplified as the internal control. Lane A: Acellular matrix of pig bladder; Lane B: Submucosa of pig bladder.

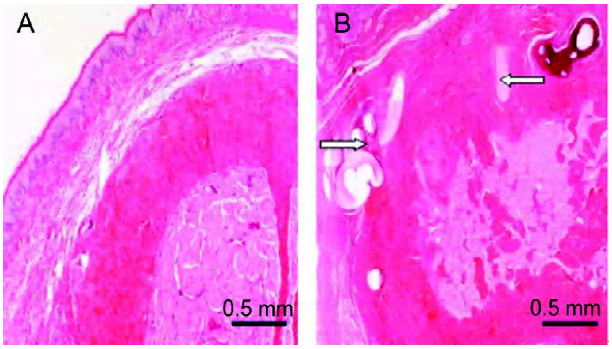

Figure 3. Magnified tunica albuginea (TA) and corpus cavernosum of rabbit. (A): Normal rabbit penis; (B): Rabbit penis 6 months after implantation. The white arrows indicate the implanted acellular matrix at the TA (Hematoxylin-eosin stain, ×20).

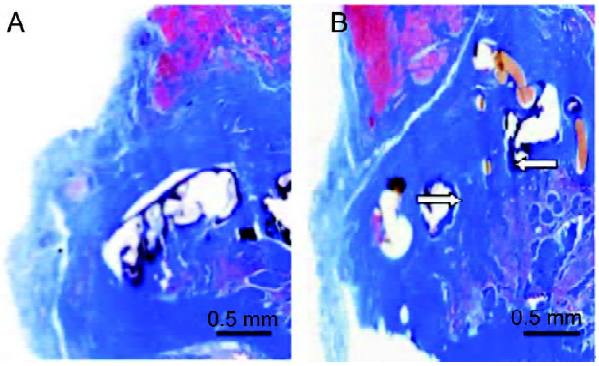

Figure 4. Cross sections of rabbit penis. (A): Normal rabbit penis; (B): Rabbit penis 6 months after implantation. The white arrows indicate the implanted acellular matrix at the TA (Masson Trichrome stain, × 20).

neighboring normal TA appeared to be well established. The inflammatory status of the graft site in the implanted group was determined to be more severe when compared with the control group 2 months after the implantation. Thereafter, the inflammation of the graft site was observed to gradually decrease. Orientation of the fibrocytes, capillaries and collagen fibers began to be observable at 2 months, and progressed gradually, achieving completion between 4 and 6 months. The amount of collagen-like normal TA had increased by 6 months after implantation (Figure 3). We noted no progression of fibrosis, nor did we observe any inflammatory changes in the corpus cavernosum. Histologically, the implanted acellular matrices were similar to the normal control tunica 6 months after implantation (Figure 4).

4 Discussion

Penile reconstruction using conventional methods, including autologous free flaps or prosthetic devices, have proven largely unsatisfactory, as the result of the limited availability of native penile tissue [1, 2]. The replacement of penile tissue with alternative materials remains a challenging prospect, due principally to the unique anatomical architecture of the corporal bodies. Penile tissue, especially the TA, is required in order to cover the defect in many instances in which penile reconstruction is necessitated.

In the specific case of Peyronie's disease, the results of medical treatment tend to be rather poor. Tunical plication is associated with a reasonable rate of success, but penile shortening is a primary disadvantage of this operation. Other alternative surgical procedures include the incision or the complete removal of plaque, coupled

with the grafting of the resultant defect. In cases of congenital genital anomalies, including epispadias, micropenis or aphallia, penile tissue is necessitated for the reconstruction of the penis or for the correction of deformities. Moreover, penile tissue can be used in order to obscure the defect in the penile structure in cases of trauma. Corporal defects have also previously been repaired with autologous grafts in patients who required repairs involving penile prostheses with cylinder extrusions [6, 7].

The ideal graft materials for such procedures should be non-cytotoxic, elicit a minimal degree of inflammatory reaction with good tissue acceptance, be easy to procure, be inexpensive, strong and easy to handle during procedure. Attempts at reconstructive procedures have utilized a variety of autologous tissues and synthetic materials. Autologous grafts, including material derived from the dermis, fascia, veins, tunica vaginalis and dura mater are non-immunogenic and tend to be less likely to elicit infections [6–9]. However, the harvesting of autologous grafts might serve to prolong the operative time, and these grafts might also lack the tensile strength and distensibility associated with the synthetic materials [10]. Several synthetic materials, including Gore-Tex, Marlex and Dacron, have been used in these procedures as well. These materials are readily available, resilient and inert. However, complications have been observed in association with their use, including graft inelasticity and the formation of a reactive capsule around the patch [4]. It was reported in a previous study that the infection rate in 57 patients with cavernous fibrosis who received a prosthesis composed of synthetic graft material was 30% [5].

Research is currently underway to identify the optimal graft for penile reconstruction procedures. These potential alternatives have become more feasible as the result of recent advances in experimental and clinical applications in the fields of both tissue engineering and biochemicals [15]. Tissue engineers have recently developed collagen-based acellular matrices and non-immunogenic membranes, which are derived from homologous or heterologous tissues, including the aorta, bladder, urethra and small bowel [11–13, 16]. These acellular matrices permit the regeneration of the native epithelial tissue, which then functions as a scaffold, promoting angiogenesis and the growth of smooth muscle bundles [17]. It is believed that a variety of growth factors facilitate these reactions. In the present study, the mRNA of the VEGF receptor, FGF-1 receptor and neuregulin were expressed in the porcine BAM, but were determined not to be expressed in the porcine vesical submucosa. For this reason, we utilized the BAM as a graft material for implantation. In another study, the mRNA of the insulin-like growth factor and the heparin-binding epidermal growth factor were expressed after the implantation of the acellular matrix [18].

The acellular matrix has recently been recognized as a promising candidate for use in the reconstruction of the urinary tract [13]. Acellular matrices derived from the bladder, urethra, corpus cavernosum and SIS were shown to be able to achieve adequate structural and functional parameters, with no complications, in a variety of genitourinary tract reconstruction trials [11–13, 18, 19]. Although SIS grafts have reportedly been used successfully in patients with penile curvature [20], acellular matrix grafting of the TA has not yet been clinically applied.

In another experimental study, histological analysis revealed the acute infiltration of inflammatory cells on the acellular matrix ten days after implantation, and angiogenesis was verified by observation of the development of several new vessels. Then, at 3 weeks, the matrix was replaced gradually with the host tissue [18]. In the present study, inflammation, as well as the formation of fibrocytes, capillaries, and collagen fibers was observed two months after implantation and the inflammation of the graft site gradually decreased. The graft was gradually replaced with normal tissue, under the influence of the surrounding tissue.

BAM grafts were shown to form an adequate structure, similar to that of the normal TA and we witnessed no complications in conjunction with this technique. Discreet clinical investigations will be required in order to verify the results of this research. The implantation of the BAM might prove applicable to the reconstruction of the TA in situations including trauma, congenital anomalies or Peyronie's disease.

Acknowledgment

The present study was supported by the Samsung Biomedical Research Institute grant (No. SBRI c-AO-038-1).

References

1 Perovic S. Phalloplasty in children and adolescents using the extended pedicle island groin flap. J Urol 1995; 154: 848–53.

2 Young VL, Khouri RK, Lee GW, Nemecek JA. Advances in total phalloplasty and urethroplasty with microvascular free flaps. Clin Plast Surg 1992; 19: 927–38.
3 Nukui F, Okamoto S, Nagata M, Kurokawa J, Fukui J. Complications and reimplantation of penile implants. Int J Urol 1997; 4: 52–4.
4 Landman J, Bar-Chama N. Initial experience with processed human cadaveric allograft skin for reconstruction of the corpus cavernosum in repair of distal extrusion of a penile prosthesis. Urology 1999; 53: 1222–4.
5 Knoll LD, Furlow WL, Benson RC Jr., Bilhartz DL. Management of nondilatable cavernous fibrosis with the use of a downsized inflatable penile prosthesis. J Urol 1995; 153: 366–7.
6 Melman A, Holland TF. Evaluation of the dermal graft inlay technique for the surgical treatment of Peyronie's disease. J Urol 1978; 120: 421–2.
7 Gelbard MK, Hayden B. Expanding contractures of the tunica albuginea due to Peyronie's disease with temporalis fascia free grafts. J Urol 1991; 145: 772–6.
8 Leungwattanakij S, Tiewthanom V, Hellstrom WJ. Evaluation of corporal fibrosis in cadaveric pericardium and vein grafts for tunica albuginea substitution in rats. Asian J Androl 2003; 5: 295–9.
9 Kelami A. Surgical treatment of Peyronie's disease using human dura. Eur Urol 1977; 3: 191–2.
10 Seftel AD, Oates RD, Goldstein I. Use of a polytetrafluo roethylene tube graft as a circumferential neotunica during placement of a penile prosthesis. J Urol 1992; 148: 1531–3.
11 Probst M, Piechota HJ, Dahiya R, Tanagho EA. Homologous bladder augmentation in dog with the bladder acellular matrix graft. BJU Int 2000; 85: 362–71.
12 Smith TG 3rd, Gettman M, Lindberg G, Napper C, Pearle MS, Cadeddu JA. Ureteral replacement using porcine small intestine submucosa in a porcine model. Urology 2002; 60: 931–4.
13 El-Kassaby AW, Retik AB, Yoo JJ, Atala A. Urethral stricture repair with an off-the-shelf collagen matrix. J Urol 2003; 169: 170–3.
14 Brown AL, Farhat W, Merguerian PA, WilsonGJ, Khoury AE, Woodhouse KA. 22 week assessment of bladder acellular matrix as a bladder augmentation material in a porcine model. Biomaterials 2002; 23: 2179–90.
15 Cross WR, Thomas DF, Southgate J. Tissue engineering and stem cell research in urology. BJU Int 2003; 92: 165–71.
16 Campodonico F, Benelli R, Michelazzi A, Ognio E, Toncini C, Maffezzini M. Bladder cell culture on small intestinal submucosa as bioscaffold: experimental study in engineered urothelial grafts. Eur Urol 2004; 46: 531–7.
17 Sutherland RS, Baskin LS, Hayward SW, Cunha GR. Regeneration of bladder urothelium, smooth muscle, blood vessels and nerves into an acellular tissue matrix. J Urol 1996; 156 (2 Pt 2): 571–7.
18 Sievert KD, Bakircioglu ME, Nunes L, Tu R, Dahiya R, Tanagho EA. Homologous acellular matrix graft for urethral reconstruction in the rabbit: histological and functional evaluation. J Urol 2000; 163: 1958–65.
19 Kwon TG, Yoo JJ, Atala A. Autologous penile corpora cavernosa replacement using tissue engineering techniques. J Urol 2002; 168: 1754–8.
20 Kropp B, Cheng EY, Pope JC 4th, Brock JW 3rd, Koyle MA, Furness PD 3rd, *et al*. Use of small intestinal submucosa for corporal body grafting in cases of severe penile curvature. J Urol 2002; 168 (4 Pt 2): 1742–5.

Edited by Prof. Guang-Huan Sun