Porcine vesical acellular matrix graft of tunica albuginea for penile reconstruction

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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
ity 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-polyethoxycetanol 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™B
(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

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
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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

4 Discussion

Penile reconstruction using conventional methods,
with the grafting of the resultant defect. In cases of congenital genital anomalies, including epispadias, micropenis or aphantia, 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.

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References

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