Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.JournalofSurgicalResearch.com

Decellularized carotid artery functions as an arteriovenous graft

Hualong Bai, MD, PhD,[a,b] *Alan Dardik, MD, PhD,*[c,d,e,**] *and Ying Xing, MD, PhD*[a,*]

[a] *Department of Physiology, Basic Medical College of Zhengzhou University, Henan, China*
[b] *Department of Vascular Surgery, First Affiliated Hospital of Zhengzhou University, Henan, China*
[c] *The Vascular Biology and Therapeutics Program, Yale University School of Medicine, New Haven, Connecticut*
[d] *Department of Surgery, Yale University School of Medicine, New Haven, Connecticut*
[e] *Department of Surgery, VA Connecticut Healthcare System, West Haven, Connecticut*

ARTICLE INFO

Article history:
Received 24 May 2018
Received in revised form
16 July 2018
Accepted 2 August 2018
Available online xxx

Keywords:
Arteriovenous graft
Decellularized
Patency
Rat

ABSTRACT

Background: Prosthetic arteriovenous grafts (AVG) continue to have a high rate of failure in clinical use, yet there is continued clinical demand for them. However, there is no small animal model of AVG to test novel tissue-engineered vascular grafts. We established a new rat arteriovenous graft model to compare the healing of decellularized carotid artery (CA) to autologous CA.

Materials and methods: The infrarenal vena cava and aorta of Wistar rats were exposed and dissected free below renal artery. A longitudinal 1 mm venotomy and arteriotomy were made on the anterior walls. The conduit was either autologous CA or heterologous decellularized CA; a conduit was sewn to the inferior vena cava and aorta in end-to-side fashion. Rats were sacrificed on postoperative day 21 for examination.

Results: All rats survived without heart failure. Conduits had 100% patency rate (day 21) in both the control and decellularized CA groups ($n = 6$). Both control and decellularized CA showed similar rates of reendothelialization, inflammatory cell infiltration, and cell turnover. The outflow vein beyond the autologous or decellularized conduits showed similar neointimal thickness and cell turnover.

Conclusions: Decellularized CA may be a viable tissue engineering graft for use as an arteriovenous graft for dialysis access. The rat aorta-vena cava graft is a useful model to test new materials including tissue-engineered grafts for use as AVG.

Introduction

Although the native arteriovenous fistula is the best conduit for dialysis access, the fistula is still associated with a very high rate of failure; to examine fistula failure, several animal models have been developed, including the needle puncture fistula in both mouse[1] and rat,[2] the carotid artery (CA)-jugular vein fistula,[3] the femoral artery-vein fistula,[4] and the rat tail artery-vein fistula.[5] All of these models create fistulae by suturing the artery and vein directly together without any intervening conduit. However, arteriovenous fistulae require an available superficial vein for clinical use, which is not

* *Corresponding author*. Department of Physiology, Basic Medical College of Zhengzhou University, Henan, China 450001. Tel.: +86 0371 67781958; fax: +86 0371 67781959.
** *Corresponding author*. Yale University School of Medicine, 10 Amistad Street, Room 437, PO Box 208089, New Haven, CT 06520. Tel.: +1 203 737 2082; fax: +1 203 737 2290.
E-mail addresses: alan.dardik@yale.edu (A. Dardik), xingy@zzu.edu.cn (Y. Xing).
0022-4804/$ – see front matter © 2018 Published by Elsevier Inc.
https://doi.org/10.1016/j.jss.2018.08.008

always available. Accordingly, arteriovenous grafts (AVG) are also frequently used for dialysis access; however, there is also a high rate of AVG failure.[6,7] To study mechanisms of AVG failure, several large animal models have been used, including a porcine model[8] and canine models.[9-11] However, there is currently no rodent AVG model reported.

Arteriovenous fistulae are thought to be better than prosthetic grafts for dialysis access because fistulae are associated with higher primary patency and reduced rates of infection.[12] However, many patients lack suitable veins to construct fistulae as the veins are frequently scarred, have small diameter, or were used for previous procedures. Typical secondary patency rates for AVG have been reported to be 41% to 85% at 1 y and 26% to 83% at 2 y; infection rates range from 8% to 41%.[13] These high rates of infection are a typical limiting factor for use of prosthetic grafts for dialysis access.[14] Because both Dacron and expanded polytetrafluoroethylene continue to be associated with high rates of thrombosis and infection, there has been recent interest in tissue engineering of small diameter vascular grafts.[15-18] Decellularized vessels are composed of decellularized grafts that retain the extracellular matrix, maintaining the structural architecture and mechanical properties of native tissues with reduced immunogenicity.[19] Decellularized vessels showed promising results in animal studies.[20,21] However, there is still no small animal model examining decellularized vessels for use as an AVG.

We have previously shown that acellular pericardial patches and Dacron patches both showed a neointimal formation and reendothelialization with adaptation to the microenvironment in which they were implanted.[22,23] These results suggest that decellularized tissues can be successfully used as off-the-shelf tissue-engineered vascular graft (TEVG) for use in vascular surgery. We established a rat AVG model and hypothesized that decellularized CA can be successfully used as an AVG with similar adaptation to the autologous CA.

Methods

Animal model

Male Wistar rats (6-8 wk) were used for implantation of the AVG. Microsurgical procedures were performed aseptically in a dedicated facility using a dissecting microscope. Anesthesia was given via 3% sodium pentobarbital (0.1-0.15 mL/100 g IP). A midline incision was made in the neck, and the right CA was exposed and harvested; after flushing out the blood, the CA was stored in saline on ice and the neck incision was closed. A midline abdominal incision was made and the infrarenal inferior vena cava (IVC) and the aorta were exposed. The site of AVG implantation was approximately 3 mm below the level of the origin of the renal veins; the IVC and aorta were dissected free at this site, and all lumbar veins and arteries at this level were ligated and divided using 6-0 nylon sutures. The infrarenal IVC was clamped and a longitudinal 1 mm venotomy was made on the anterior wall of the IVC. The control CA or decellularized CA was sewn to the IVC in end-to-side fashion with running 10-0 nylon sutures. The aorta was then clamped at the same level and a longitudinal 1 mm arteriotomy was made on the anterior wall and the control or decellularized CA was similarly sewn to the aorta in end-to-side fashion with running 10-0 nylon sutures. After completing the anastomoses, the clamps on the IVC were removed first and then the clamps of the aorta were removed. After assurance of hemostasis, the abdomen was then closed and the rat was allowed to recover from anesthesia.

Rats were sacrificed on postoperative days 21 and the aorta, IVC and graft were explanted for analysis as described in the following. No immunosuppressive agents, antiplatelet agents, antibiotics, or heparin were given at any time.

Decellularization of rat CA

The right CA (1 cm length) from male Wistar rats (6-8 wk) was dissected and carefully removed using sterile technique; the CA were then stored at 4°C in phosphate-buffered saline (PBS) (pH 7.4; Invitrogen, Carlsbad, CA) containing penicillin 100 U/mL and streptomycin 100 μg/mL (Invitrogen). Decellularization of CA was accomplished as described previously.[19] Briefly, CA were incubated in 250 mL CHAPS buffer (8 mM CHAPS, 1 M NaCl, and 25 mM EDTA in PBS) for 12 h, followed by a 60 min wash, and then incubated in 10 mL sodium dodecyl sulfate buffer (1.8 mM sodium dodecyl sulfate, 1 M NaCl, and 25 mM EDTA in PBS) for 24 h, followed by a 24-h wash with PBS to completely remove the detergent. Decellularized CA were stored at 4°C until use.

Confirmation of decellularization

Control and decellularized CA were fixed in 10% neutral-buffered formalin for 24 h, embedded in paraffin, cut into 5-mm sections, and stained with H and E and elastin van Gieson's. Sections were also stained for CD31 for endothelial cells, α-actin for smooth muscle cells, and collagen-1 for collagen using immunohistochemistry; and double stained with Von Willebrand factor (vWF) and α-actin using immunofluorescence.

Histology

Rats were anesthetized with isoflurane inhalation, and tissues were fixed by transcardial perfusion of PBS followed by 10% formalin. Tissue was removed and fixed overnight in 10% formalin followed by a 24-h immersion in 70% percent alcohol. Tissue was then embedded in paraffin and sectioned (5 μm thickness). Tissue sections were deparaffinized and stained with hematoxylin and eosin.

Immunohistochemistry

Tissue sections were deparaffinized and then incubated using primary antibodies overnight at 4°C. After overnight incubation, the sections were incubated with EnVision reagents for 1 h at room temperature and treated with Dako Liquid DAB Substrate Chromogen System (Dako). Finally, the sections were counterstained with Dako Mayer's Hematoxylin.

Immunofluorescence

Tissue sections were deparaffinized and then incubated with primary antibodies overnight at 4°C. Sections were