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ORIGINAL ARTICLE

Transplantation of the decellularized tracheal allograft in animal model (rabbit)

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Summary *Background:* It has been difficult to perform tracheal allotransplantation without immunosuppression. To determine whether decellularized trachea can be used in tracheal replacement, we evaluated the viability of decellularized tracheal allografts in a rabbit model of immunosuppressant-free transplantation.
Method: Half allograft (Group 1, $n = 7$) was harvested from adult New Zealand white rabbits, subjected to a detergent–enzymatic method (containing sodium deoxycholate/DNase lavations) of decellularization for as many cycles as needed, and the other half was stored in phosphate-buffered saline at 4°C as a control (Group 2, $n = 7$). Bioengineered and control tracheas were then implanted in 14 age-matched rabbits.
Results: In Group 1 (decellularized), all rabbits survived, whereas in Group 2(control), all rabbits died of airway obstruction between 20 days and 45 days after operation. Histologically, the decellularized allografts displayed complete regeneration of epithelium and cartilage, but the fresh allografts showed inflammatory changes, no epithelium, and no cartilage.
Conclusions: Complete regeneration of epithelium and cartilage tracheal rings occurred after the implantation of decellularized tracheal allografts without immunosuppression. We demonstrate that the decellularized process reduces the allogeneic response to the trachea. Therefore, we believe that the decellularized tracheal allograft is an excellent choice for tracheal replacement. To our knowledge, this is the first study to observe the long-term (1 year) prognosis of this transplanted trachea.

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1. Introduction

Tracheal resection with primary reconstruction is the only curative treatment in patients with a variety of benign or malignant tracheal lesions.[1] Unfortunately, the resectable length of the diseased trachea is usually restricted to approximately 30% of the total length in children and around 6 cm in adults, and any further increase in this resection rate depends on the development of a safe tracheal replacement.[2] This is not yet clinically available because almost every attempt to provide an autologous or synthetic safe and reproducible tracheal graft has been disappointing thus far,[1] casting doubt on the future of long-segment tracheal replacement.[3] Tissue bioengineering has emerged as the most promising technique to create a near-normal trachea,[1,4] and encouraging early experimental results[5] and clinical applications[6] have been reported. To determine whether decellularized trachea can be a realistic substitute in replacement procedures, we evaluated the viability of and the histologic changes in decellularized tracheal allografts in rabbit model of transplantation without immunosuppression.

2. Material and methods

Special humane animal care measures were taken in compliance with the "Principles of laboratory animal care" formulated by the National Society for Medical Research and the "Guide for the care and use of laboratory animals" prepared by the Institute of Laboratory Animal Resources, National Research Council, and published by the National Academy Press, revised 1996.

Each allograft was harvested from adult male New Zealand white rabbits weighing 3170—4575 g. The animals were anesthetized with intravenous administration of pentobarbital (30 mg/kg). They were placed in a supine position under spontaneous ventilation without an endotracheal tube, and either harvesting or transplantation was performed. The cervical trachea was exposed through a midline cervical incision and then harvested from the cricoid cartilage to the carina. To obtain maximum graft viability, grafts were harvested from beating heart donors without warm ischemia.

The harvested segment was then divided into two groups. One half (control) was placed in a stock solution made of phosphate-buffered saline (PBS), containing 1% antibiotic and antimycotic solution. The other half was similarly stored for 24 hours but then submitted to the bioengineering protocol, as described below.

2.1. Matrix bioengineering

The overlying tissue of the harvested tracheas was stripped off, deprived of trachealis muscle, and rinsed four times (for 4 hours each) in PBS containing 1% antibiotic and antimycotic solution. Thereafter, the protocol continued as previously reported.[7] Briefly, the tissue was processed with multiple treatment cycles, including the following steps: the tissue was stored in Aqua milliQ (Millipore) for 48 hours at 4°C and then incubated in 4% sodium deoxycholate for 4 hours and 2000 kU of DNase-I in 1 mol/L NaCl (Sigma Chemical Company) for 4 hours. The presence of cellular elements and major histocompatibility complex (MHC) cells were verified histologically after each cycle. The bioengineering process lasted for 35 days, corresponding to 17 detergent—enzymatic method (DEM) cycles, after which the bioengineered tracheal matrices showed few residual nuclei of chondrocytes but a complete removal of MHC class I and II antigens (Figure 1A).[8] In contrast, MHC class I and II expression was ubiquitous in control tracheas recipient.[8]

The transplantation was performed with recipient rabbits of matched age. Under anesthesia, the recipient was placed in a supine position and a six-ring tracheal segment was resected through a midline cervical incision. Allograft transplantation (control, $n = 7$; decellularize, $n = 7$) was performed in an end-to-end continuous fashion using absorbable monofilament (5-0 PDS, Ethicon, USA) (Figure 1B), and the wound was closed in the usual manner. Postoperatively,all the 14 recepient rabbits were observed for 3 hours before being returned to their individual cages. They received standard feed and water. Analgesics (buprenorphine, 0.05 mg/kg) were administered twice a day on postoperative Day 1. Antibioprophylaxis (enrofloxacin, 10 mg/kg) was administered postoperatively over 5 days. No immunosuppressive agents or steroids were given. Examination with a flexible bronchoscope was performed on the 5th and 15th postoperative days and every 3 months thereafter, and each tracheal graft was examined grossly and microscopically (pathologic examination with hematoxylin and eosin staining)when the animal died or was sacrificed from Day 20 to Day 365.

3. Results

Table 1 summarizes the results for the 14 transplant recipients. In the decellularized group (Group 1), the rabbits were alive for the duration of the experiment and were subsequently killed for the histologic examinations. In control group (Group 2), however, all the seven rabbits died

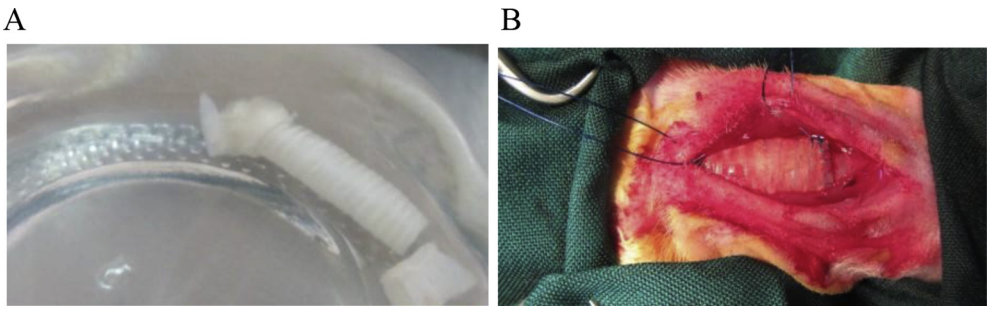

Figure 1 (A) A decellularized trachea. (B) Transplantation of decellularized tracheal allograft.

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