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Long-term functional reconstruction of segmental tracheal defect by pedicled tissue-engineered trachea in rabbits

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ABSTRACT

Due to lack of satisfactory tracheal substitutes, reconstruction of long segmental tracheal defects (>6 cm) is always a major challenge in trachea surgery. Tissue-engineered trachea (TET) provides a promising approach to address this challenge, but no breakthrough has been achieved yet in repairing segmental tracheal defect. The longest survival time only reached 60 days. The leading reasons for the failure of segmental tracheal defect reconstruction were mainly related to airway stenosis (caused by the overgrowth of granulation tissue), airway collapse (caused by cartilage softening) and mucous impaction (mainly caused by lack of epithelium). To address these problems, the current study proposed an improved strategy, which involved *in vitro* pre-culture, *in vivo* maturation, and pre-vascularization of TET grafts as well as the use of silicone stent. The results demonstrated that the two-step strategy of *in vitro* pre-culture plus *in vivo* implantation could successfully regenerate tubular cartilage with a mechanical strength similar to native trachea in immunocompetent animals. The use of silicone stents effectively depressed granulation overgrowth, prevented airway stenosis, and thus dramatically enhanced the survival rate at the early stage post-operation. Most importantly, through intramuscular implantation and transplantation with pedicled muscular flap, the TET grafts established stable blood supply, which guaranteed maintenance of tubular cartilage structure and function, accelerated epithelialization of TET grafts, and thus realized long-term functional reconstruction of segmental tracheal defects. The integration of all these improved strategies finally realized long-term survival of animals: 60% of rabbits survived over 6 months. The current improved strategy provided a promising approach for long-term functional reconstruction of long segmental tracheal defect.

1. Introduction

The treatment of long segmental tracheal defect (>6 cm) in clinic requires the transplantation of effective tracheal substitute [1,2]. However, up to date, no ideal tracheal substitutes are available due to the lack of suitable autologous tissue donor [3,4], limited source and immuno-rejection of allogenous trachea [5,6], as well as inferior biological function of synthetic tracheal grafts [7,8]. Therefore, the treatment of long segmental tracheal defect still remains a great challenge in tracheal surgery. In recent years, tissue-engineered trachea (TET) generated using autologous cells has been proposed as an ideal tracheal substitute because of proper biological structure and function similar to native trachea and autologous availability [9]. Some recent studies even had reported the application of TET in individual clinical case [10,11], providing a promising strategy for reconstruction of long segmental tracheal defect despite that the long-term clinical efficacy is still unknown.

Since the first attempt to repair tracheal defects in rats using tissue engineering approach by Vacanti CA et al in 1994 [12], several research groups, including ours, have extensively explored the methods and strategies for tracheal reconstruction in different animal models. Nevertheless, only limited success was achieved in repairing partial tracheal defects with the use of tissue-engineered cartilage patch [13–16]. No breakthrough has been achieved so far for the treatment of the segmental tracheal defect and the longest survival time only reached 60 days post-operation [17–20].

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The leading reasons for the failure of segmental tracheal defect reconstruction were mainly related to airway stenosis (caused by the overgrowth of granulation tissue at anastomosis site), airway collapse (caused by cartilage softening) and mucous impaction (mainly caused by the lack of epithelium) [19,21,22]. According to our previous study and other reports, the overgrowth of granulation tissue at anastomosis site was the result of acute and subacute inflammatory reaction, which mainly caused by the surgical trauma and the degradation byproduct from implanted biodegradable scaffolds in TET [23,24]. Of course, the severe inflammatory reaction was also the important reason that interferes in cartilage formation and maturation [25,26]. On the other hand, it has been speculated that the lack of vascularization in transplanted TET was the crucial reason leading to the cartilage softening and epithelialization delay [27].

To address the above-mentioned problems and the possible reasons, this study proposed an improved tracheal tissue engineering strategy from the following aspects. Firstly, the TET construct was pre-cultured *in vitro* for 2 weeks before *in vivo* implantation to alleviate the inflammatory reaction caused by scaffold fibers [26]. Secondly, the construct was implanted into a muscular flap close to trachea for another 4 weeks to promote *in vivo* cartilage maturation and pre-vascularization of TET graft before repairing tracheal defect. Finally, a silicone stent was placed inside TET graft at the early stage of postoperation to further reduce overgrowth of granulation, prevent stenosis, and maintain the patency of airway. In this study, a rabbit model with 1.5 cm length segmental tracheal defect was established to evaluate the feasibility and long-term efficacy of this improved TE strategy.

2. Materials and methods

2.1. General experimental design

Total 20 New Zealand white rabbits in 1-month old were used in this research. The mean weight was 1.50 ± 0.12 kg. Animals were divided randomly into experimental group ($n = 10$, repair with vascularized TET graft) and control group ($n = 10$, repair with free TET graft). All animals received humane care in compliance with the "Guide for Care of Laboratory Animals" formulated by the National Ministry of Science (2006).

The total experimental procedures included three steps: (1) *In vitro* preparation and culture of tubular cell-scaffold constructs; (2) *In vivo* maturation of engineered tubular cartilage in subcutaneous or intramuscular environment; (3) The reconstruction of segmental tracheal defects (with vascularized or free TET grafts) and the postoperative evaluation. The overview of the total experimental design was shown in Fig. 1.

2.2. Isolation and culture of chondrocytes

Cartilage slice with a size of about 2.0 × 2.0 cm was obtained from autologous auricle of rabbit and minced into pieces sized about 1.0 mm^3. The cartilage pieces were pre-treated with 0.25% trypsin (Hyclone), washed with phosphate buffered solution (PBS), and digested with 0.25% collagenase type II (Worthington Biochemical Corp., Freehold, NJ) to isolate chondrocytes as previously established methods [26]. Then, the cells were harvested, cultured, and expanded according to reported methods [26]. The chondrocytes in passage one were harvested for the construction of tubular cartilage.

2.3. Preparation of scaffolds and cell-scaffold constructs

Sixty milligrams of unwoven polyglycolic acid (PGA) fibers (synthesized by Dong Hua University and National Tissue Engineering Center of China, Shanghai, China) were wrapped around a silicone tube (6 mm in diameter, 20 mm in length) to form a cylindrical scaffold (Fig. 2). The scaffold was disinfected with 75% ethanol solution for 60 min, and was washed twice with PBS. The harvested chondrocytes were resuspended in Dulbecco's modified Eagle's medium (DMEM, Gibco BRL, Grand Island, NY) containing 10% fetal bovine serum (FBS, Hyclone) to a final concentration of 6.0×10^7 cells/ml, and 1.0 ml cell suspension was seeded evenly onto the scaffold to form cell-scaffold construct (Fig. 2). All the constructs were incubated for 4 h, and then cultured in DMEM containing 10% FBS as previous report [26,28] in the condition with 5% CO_2 at 37 °C for 2 weeks.

2.4. Implantation surgery

The rabbit was anesthetized with intramuscular injection of ketamine (5 mg/kg) and xylazine (0.05–1 mg/kg). A midline incision was made in the anterior neck. In experimental group, sternohyoid muscle of one side was separated and wrapped the chondrocyte-scaffold construct by suturing the opposite sides of the muscle with 3-0 silk suture (Fig. 3A1). In control group, subcutaneous tissue was separated to form a pocket and the construct was directly put into the pocket (Fig. 3B1). Then incision was closed in layers and the animal was allowed to breathe spontaneously and recover from anesthesia.

2.5. Reconstruction operation

The reconstructive operation of segmental tracheal defects was performed after 4 weeks of implantation. In experimental group, the same midline incision as

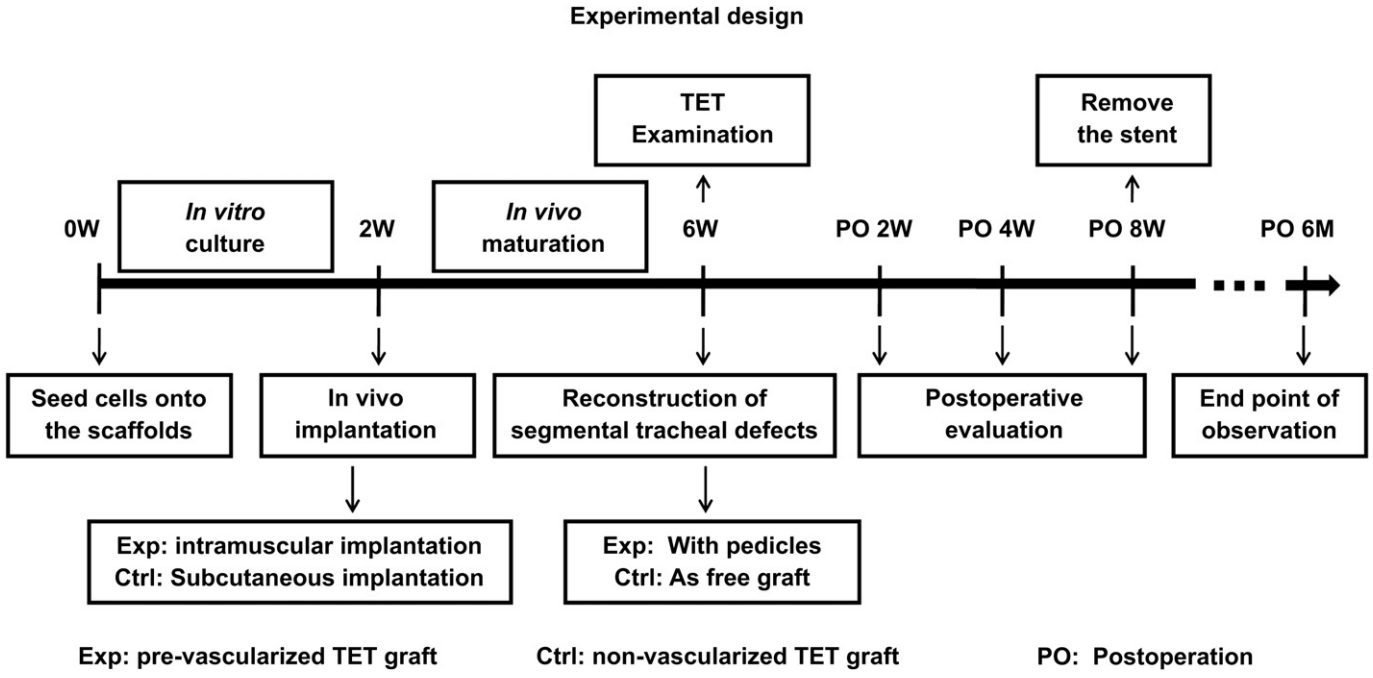

Fig. 1. The overview of total experimental design.