The first tissue-engineered airway transplantation: 5-year follow-up results

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For the **quality of life questionnaire** used see http://www.cdc.gov/hrqol/spanish.htm

Summary

Background In 2008, the first transplantation of a tissue-engineered trachea in a human being was done to replace an end-staged left main bronchus with malacia in a 30-year-old woman. We report 5 year follow-up results.

Methods The patient was followed up approximately every 3 months with multidetector CT scan and bronchoscopic assessment. We obtained mucosal biopsy samples every 6 months for histological, immunohistochemical, and electron microscopy assessment. We also assessed quality of life, respiratory function, cough reflex test, and production and specificity of recipient antibodies against donor human leucocyte antigen.

Findings By 12 months after transplantation, a progressive cicatricial stenosis had developed in the native trachea close to the tissue-engineered trachea anastomosis, which needed repeated endoluminal stenting. However, the tissue-engineered trachea itself remained open over its entire length, well vascularised, completely re-cellularised with respiratory epithelium, and had normal ciliary function and mucus clearance. Lung function and cough reflex were normal. No stem-cell-related teratoma formed and no anti-donor antibodies developed. Aside from intermittent bronchoscopic interventions, the patient had a normal social and working life.

Interpretation These clinical results provide evidence that a tissue-engineering strategy including decellularisation of a human trachea, autologous epithelial and stem-cell culture and differentiation, and cell-scaffold seeding with a bioreactor is safe and promising.

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Introduction

In 2008, the first completely tissue-engineered airway replacement in a human being was successfully done by implantation of a bioengineered human trachea to restore lung function of a patient with end-stage left-main bronchus malacia.[1] This novel strategy—using an airway from a deceased human donor—was based on knowledge of how to bioengineer a human decellularised matrix that was structurally and mechanically similar to a native trachea, with chemotactic and proangiogenic properties.[2] The matrix was re-seeded in a specially designed bioreactor with in vitro expanded and differentiated autologous epithelial cells and chondrocytes of mesenchymal-stem-cell origin, and then implanted. 4 months after surgery, the patient was well, active, with normal lung function, and did not require immunosuppressive treatment.[1] Despite the clinical success, several points remained unaddressed: (1) the feasibility of obtaining a viable, re-cellularised, and functional engineered airway, and its maintenance once implanted, (2) long-term stability of the detergent–enzyme decellularised natural matrix, and (3) fate and tumorigenic risks associated with the implanted stem cells.[3] To answer these questions, we report the 5 year follow-up results.

Methods

The recipient

In 2008, the native complete malacic left main bronchus of a 30-year-old woman was replaced with a tissue-engineered trachea seeded with autologous epithelial cells and chondrocytes of mesenchymal stem-cell-origin.[1] During the first year after transplantation, the patient had clinical assessments as necessary. Since then, the patient was followed up every 3 months including assessment of graft properties and morphology. The patient provided written informed consent for each follow-up, and post-transplantation investigations were required by the ethics commissions of the Centro Nazionale Trapianti (Italian National Transplant Service), University Hospital Careggi (Florence, Italy), and Consiglio Superiore Sanità (Italian National Health Council).

Follow-up assessments

We assessed quality of life with the Centers for Disease Control four-item Health-Related Quality of Life Healthy Days Core Module (HRQoL–4). The questionnaire was completed while in hospital or by telephone between follow up visits.

During follow-up, the patient had routine lung function tests and cough reflex assessments every 2 years. Plethysmographic lung function testing included measurements of forced expiratory volume in 1 s (FEV_1), forced vital capacity (FVC), total lung capacity and residual volume, diffusion capacity for carbon monoxide and carbon monoxide transfer coefficient, airway resistance (Raw) and specific airway conductance

(SGaw). Maximum voluntary cough efforts were obtained before the cough reflex was induced. Reflex cough was induced by inhalation of progressively increasing fog concentrations ranging from 0·08 to 4·45 mL/min, produced by a Mist-O2-Gen EN143A ultrasonic nebuliser (Medical Equipment Services, Fulton, IL, USA).[4] We assessed intensity of voluntary and reflex cough by evaluation of the peak integrated electromyographic activity of the abdominal muscles. Cough threshold—an index of sensitivity of the cough reflex—was recorded as the lowest nebuliser output capable of evoking at least one cough effort during two distinct challenges, 30 min apart.[5] We assessed the urge to cough during cough challenge by a 0·1 m visual analogue scale.[6]

From the first year after transplantation, follow-up clinical evaluation to assess the graft was done every 3 months, with multidetector CT scan and bronchoscopy if necessary. CT was done with a 64-row scanner (LightSpeed VCT; GE Healthcare, Waukesha, WI, USA) with the following parameters: detector collimation 0·6 mm×64, reconstruction increment 0·6 mm. No contrast medium was used. We assessed the length of the graft with multiplanar volume rendering reformation, 3D reconstructions, and virtual bronchoscopic images. We used paired end-inspiratory and dynamic expiratory virtual bronchoscopy images to assess a graft malacia that developed. We did flexible bronchoscopy to assess the transplanted airway and take biopsy samples to assess the regenerated bronchial mucosa. The graft lumen was assessed with the Cotton-Myer scale.[7] We did rigid bronchoscopy every time an intervention—eg, taking biopsy samples, dilatation, or stenting—was necessary or when the patient did not tolerate the flexible bronchoscopy.

Laboratory analyses

We obtained biopsy samples at every graft follow-up visit for histological and immunohistochemical analyses. Mucosal fragments were fixed for 24 h in 10% buffered formalin at room temperature. We sectioned paraffin-embedded mucosal fragments at a thickness of 5 µm and stained them with haematoxylin and eosin (Merck; Darmstadt, Germany) to assess morphological changes. We assessed the presence of laminin by immunohistochemistry. All tissue sections were placed on the automated stainer BenchMark XT ICH system (Ventana Medical Systems; Tuscon, AZ, USA) and then deparaffinised, rehydrated, and processed for blocking endogenous peroxidase and epitope retrieval. After pretreatment with protease 1 (Ventana Medical Systems), we incubated the slides with monoclonal mouse antibody to human laminin (clone 4C7; BioSystems, CA, USA; dilution 1:10) at 37°C for 32 min. We used ultraView Universal DAB Detection Kit (Ventana Medical Systems) for detection. Negative controls were made by substitution of the primary antibody with non-immune serum immunoglobulins (at the same concentration as the primary antibody). We used skin as the positive control, treated in parallel with the samples in the same run.

To qualitatively assess the graft's morphology, we fixed biopsy samples with 3% (v/v) glutaraldehyde (Merck) in a buffered solution of 0·1 M sodium cacodylate buffer (pH 7·2; Prolabo; Paris, France) and further processed them for scanning electron microscopy with a Leo Supra 35 microscope. We modified biopsy processing for transmission electron microscopy following standard procedures: biopsies were fixed in 2% (v/v) glutaraldehyde in 0·1 M sodium

	December, 2007 (before surgery)	September, 2008 (3 months after surgery)	February, 2012 (diagnosis of subglottic stenosis)	June, 2013
FVC (L; % of predicted value)	2·35 (62%)	3·86 (100%)	3·20 (98%)	3·45 (96%)
FEV_1 (L; % of predicted value)	1·75 (55%)	3·25 (100%)	2·42 (86%)	2·49 (84%)
FEV_1/FVC	0·74	0·84	0·76	0·72
Raw (kPa/L per s)	5·57	3·31	4·71	5·47
SGaw (kPa/s)	0·058	0·213	0·086	0·05

FVC=forced vital capacity. FEV_1=forced expiratory volume in 1 s. Raw=airway resistance. SGaw=specific airway conductance.

Table 1: **Lung function**

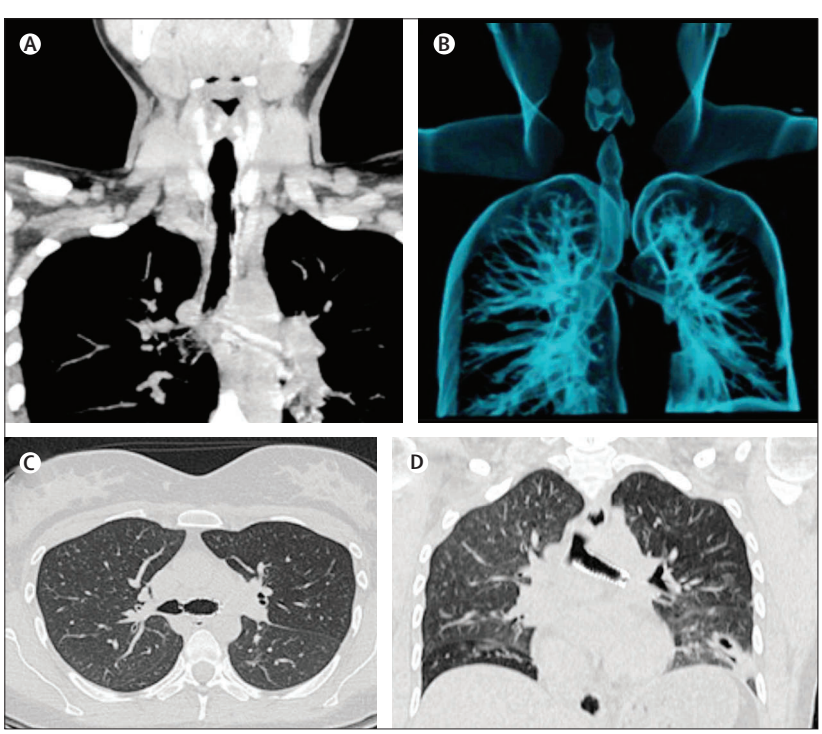

Figure 1: **Imaging findings at 3 years after transplantation**
(A) Multidetector CT scan (June 2011) showing a subtotal cicatricial stenosis of the origin of the left main bronchus, at the level of the proximal anastomosis. (B) 3D reconstruction of the whole graft; distal to the proximal anastomosis, the graft and the distal anastomosis are viable. Axial view (C) and coronal view (D) multidetector CT scan (November, 2011) showing the graft with a metallic Ultraflex stent.