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

Alessandro Gonfiotti, Massimo O Jaus, Daniel Barale, Silvia Baiguera, Camilla Comin, Federico Lavorini, Giovanni Fontana, Oriol Sibila, Giovanni Rombolà, Philipp Jungebluth, Paolo Macchiarini

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.**

Funding **European Commission, Knut and Alice Wallenberg Foundation, Swedish Research Council, ALF Medicine.**

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

Published **Online** October 23, 2013 http://dx.doi.org/10.1016/S0140-6736(13)62033-4

See **Online/Comment** http://dx.doi.org/10.1016/S0140-6736(13)62110-8

Department of General Thoracic and Regenerative Surgery (A Gonfiotti MD, M O Jaus MD, D Barale MD), **Department of Biomedicine** (G Rombolà MD), **University Hospital Careggi, Florence, Italy; Advanced Center for Translational Regenerative Medicine, Division of Ear, Nose, and Throat, Karolinska Institutet, Huddinge, Stockholm, Sweden** (S Baiguera PhD, P Jungebluth MD, Prof P Macchiarini MD); **Division of Pathological Anatomy, Department of Critical Care Medicine and Surgery** (C Comin MD), **Department of Experimental and Clinical Medicine** (F Lavorini PhD, G Fontana FRCP), **University of Florence, Florence, Italy; and Department of Respiratory Medicine, Hospital de la Santa Creu i Sant Pau, Institut d´Investigació Biomèdica Sant Pau, Universitat Autonoma de Barcelona, Barcelona, Spain** (O Sibila MD)

Correspondence to: Prof Paolo Macchiarini, Advanced Center for Translational Regenerative Medicine, Division of Ear, Nose, and Throat, Karolinska Institutet, Alfred Nobels Allé 8, Huddinge, SE-141 86 Stockholm, Sweden **paolo.macchiarini@ki.se**

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

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.

	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**

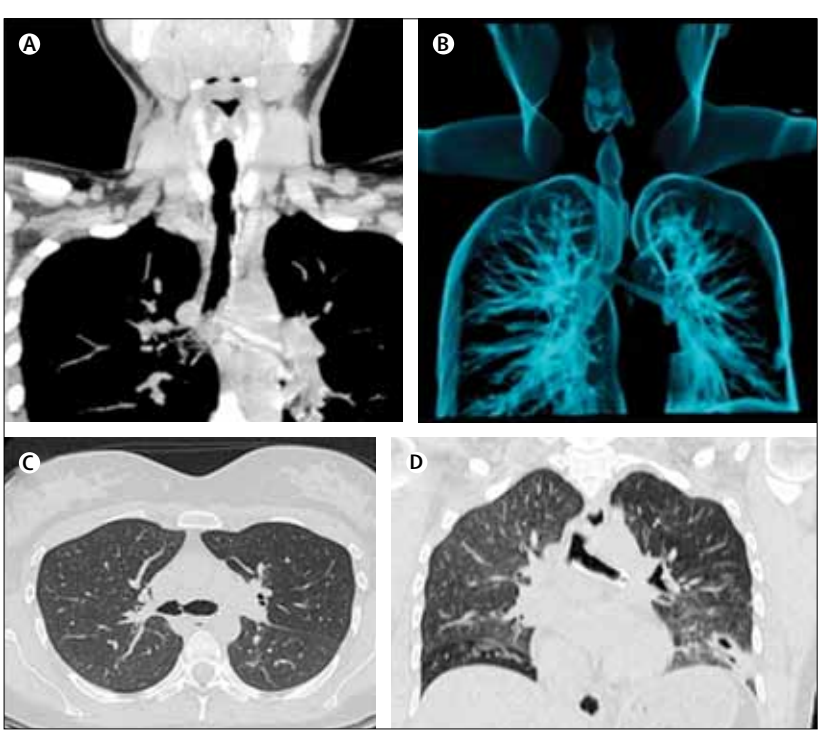

Figure 1: **Imaging findings at 3 years after transplantation**
(A) Multidetector CT scan (June 2011) showing a subtotal cicatricial stenosis of the origin of the left main bronchus, at the level of the proximal anastomosis. (B) 3D reconstruction of the whole graft; distal to the proximal anastomosis, 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.

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 cacodylate buffer solution (pH 7·2). The tissue was further processed and embedded in epoxy resin. We mounted ultrathin serial microtome sections on Formvar-coated (Assing; Rome, Italy) copper/rhodium grids, stained them with uranyl acetate and

	Subglottic treatment	Proximal anastomosis*	Distal anastomosis*	Graft body*	Stent type	Stent size (mm)	Interval between prostheses (months)
December 2008	..	II	..	..	Dumon	12×40	6
June 2009	..	II	..	..	Dumon	12×40	7
November 2010	..	I (granulation tissue)	..	Granulation tissue	Dumon	12×30	18
January 2011	..	II	..	..	Polydioxanone	13×25	2
April 2011	..	I	..	..	Polydioxanone	13×25	3
June 2011	..	III	..	..	Polydioxanone	13×25	2
September 2011	..	III	..	..	Silmet	12×40	3
November 2011	..	III	Granulation tissue	Granulation tissue	Ultraflex	14×40	2
November 2011	..	..	..	..	..	..	..
December 2011	1	I	Granulation tissue	..	Polydioxanone	14×23	1
February 2012	2 (dilatation)	..	..	..	..	..	..
March 2012	1	I (granulation tissue)	..	..	Polydioxanone	14×23	3
June 2012	1	II	..	..	Polydioxanone	14×23	3
September 2012	2 (dilatation)	I	..	..	Polydioxanone	14×20	3
December 2012	2 (dilatation)	..	..	..	Polydioxanone	14×23	3
March 2013	1	I	..	..	Polydioxanone	14×20	3

*According to the Cotton-Myer grading system.[7]

Table 2: **Sequence of bronchoscopic treatments with the corresponding placed stent, condition of the transplanted airway, and subglottic stenoses**

citrate, and observed them with a Philips 410 LS transmission electron microscope.

For microsatellite analysis, we extracted DNA from the patient's blood, the decellularised matrix, and the implanted airway. Tracheal specimens were disintegrated and homogenised in 1 mL denaturing solution with TissueLYser (Qiagen; Milan, Italy; four cycles at maximum speed for 2 min). We mixed the lysate with chloroform and centrifuged it at 15 000 revolutions per minute for 15 min. After phenol/chloroform extraction, the DNA was precipitated from the aqueous phase with isopropanol, washed with 75% ethanol, and air-dried. We then dissolved the pellet in ribonuclease-free water and stored it at 4°C. We did PCR amplifications of 15 short tandem repeat loci with the AmpFLSTR Identifiler kit (Applied Biosystems), according to the manufacturer's instructions. We analysed amplified PCR products by capillary electrophoresis with an ABI PRISM 3100 Genetic Analyzer (PE Applied Biosystem; Foster City, CA, USA).

Each year, the patient was screened for production and specificity of anti-donor human leucocyte antigen antibody. Serum was screened by complement-dependent cytotoxicity according to a National Institutes of Health technique[8] compared with a 56-cell panel (Lymphoscreen; Biorad; Dreiech, Germany). We also used a highly sensitive solid phase Luminex beads technique (Luminex Mix and SA I and II; One-Lambda, Canoga Park, CA, USA) for detection and identification of human leucocyte antigen class I and II antibodies, according to manufacturer's instruction (cutoffs: ratio=2·5 for screening, median fluorescence intensity=1000 for identification).[9]

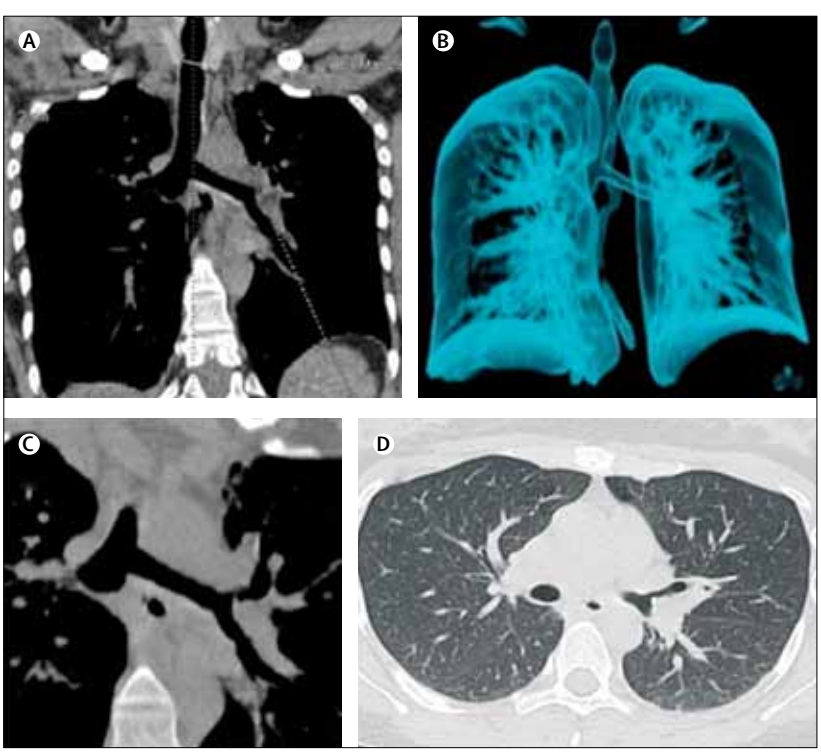

Figure 2: **Imaging findings at 5 years after transplantation**
Multidetector CT scan (March, 2013) showing the entire graft with a normal proximal anastomosis; (A) coronal view, (B) 3D reconstruction. Multidetector CT scan (March, 2013) showing a normal distal anastomosis (C) with a normal inflated lung (D).

Role of the funding source

The sponsor of the study had no role in study design, data collection, data analysis, data interpretation, or

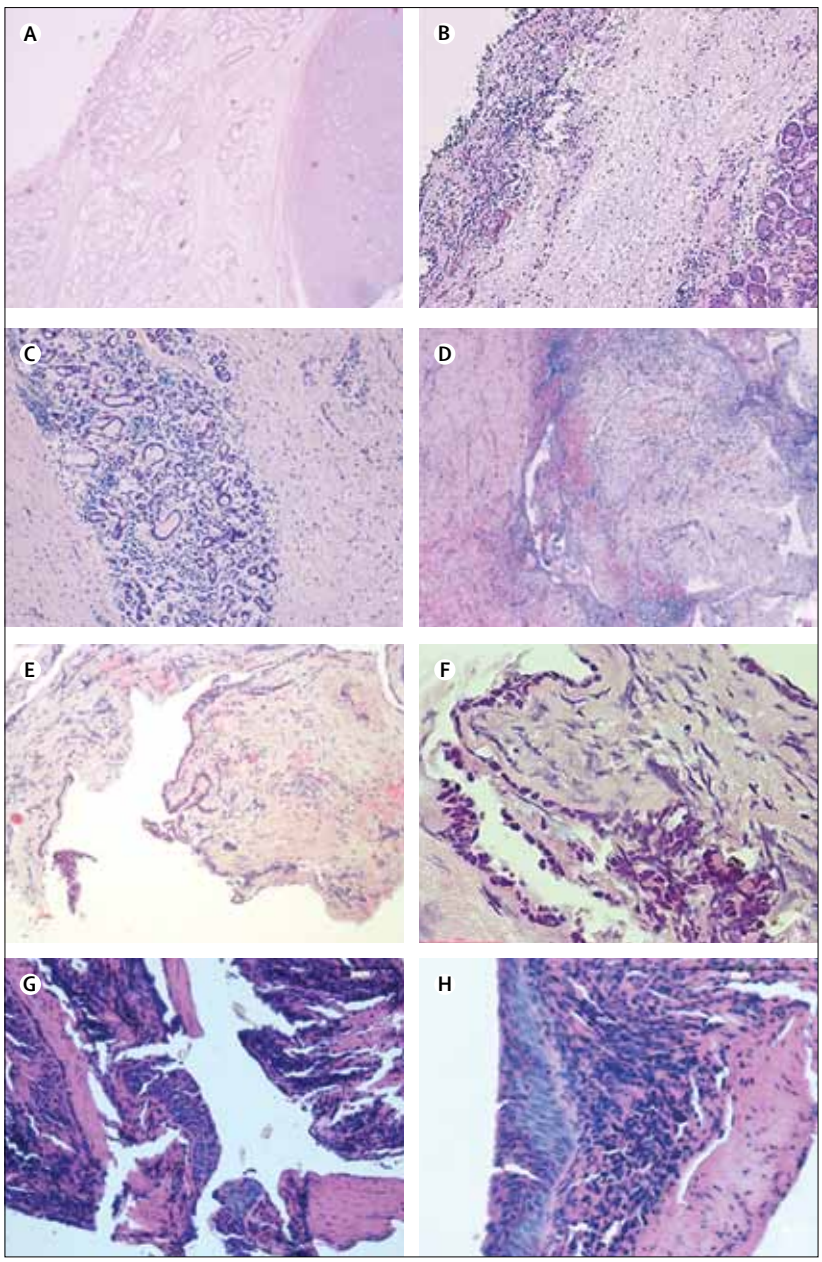

Figure 3: **Histological staining**
Haematoxylin and eosin stains of decellularised (A) and native (B) airway matrix, and of two parts of the implanted airway 1 year (C, D), 2 years (E, F), and 4 years (G, H) after the transplantation. Magnification ×10.

writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

See Online for video 1

Results

The CDC HRQOL-4 results were affected only by the need for endoscopies: the days in hospital for these procedures were reported as "not healthy days" even though the patient always defined her health as "excellent" or "very good" (question 1). During the past 5 years the patient has never been prevented from usual activities—eg, self care, work, recreation, or social relationships—except for days spent in hospital for follow-up.

FEV_1, FVC, static lung volumes, and diffusion capacity remained within normal limits until 44 months (February, 2012) after surgery (table 1). Subsequently, the patient had a worsening cough and wheezing associated with slight flattening of the expiratory portion of the flow-volume curve, with a normal vital capacity. Values of spirometric variables suggested an upper airway obstruction[10,11] but were within normal limits. Nonetheless, flexible bronchoscopy confirmed a sub-occlusive stenosis immediately below the vocal cords as well as at the anastomotic site of a subglottic resection (done in 2004) and primary end-to-end anastomosis. Stenosis required repeated endoluminal dilations without stenting. Spirometry, Raw, and SGaw values were normal (table 1). Total lung capacity was 4·53 L (98% of predicted value), residual volume was 1·28 L (90% of predicted value), diffusion capacity for carbon monoxide was 19·33 mL/mm Hg per min (79% of predicted value), and transfer coefficient was 4·64 mL/mm Hg per min (85% of predicted value). Air oxygen saturation was 98%. Cough reflex sensibility, urge to cough, and motor response were normal at each assessment (data not shown).

The graft behaved as expected until 6 months after surgery. Thereafter, the proximal (native to tissue-engineered trachea) anastomosis began to show a progressive cicatricial diaphragm-shaped scar.[12] The remaining tissue-engineered trachea and distal anastomosis were patent. Once the area of the scar reduced by more than 50% (Cotton-Myer grade II), the stenosis was bronchoscopically dilated and a Dumon standard bronchial stent (12×40 mm; Boston Medical Products; Novatech SA, France) placed. The patient had several stent obstructions caused by secretions, requiring flexible bronchoscopy, and began to have progressively worsening cough, which became intractable after 6 months (June 2009). Despite progression of cough, the patient was never restricted in daily social life and did not down-grade her quality of life according to HRQOL-4. Between November 2010, and January 2011, relapsing stenoses have been treated with several Dumon stents (12×30 mm), that were finally replaced because of intolerance with a custom-made absorbable polydioxanone self-expanding stent (Ella-Cs; Hradec Kralove, Czech Republic).[13] This stent can be placed across the stricture (14×23 mm or 14×20 mm; video 1) and was well tolerated.

Because of the polydioxanone stent's 30 day reabsorption time, the patient had bronchoscopic examinations every 3 months or when symptoms of bronchial obstruction returned. Restenting was considered at each follow-up visit to guarantee the patency of the anastomotic lumen and prevent a complete anastomosis obstruction—as measured by a multidetector CT scan in June 2011 (figure 1)—and consequent permanent graft damage. To

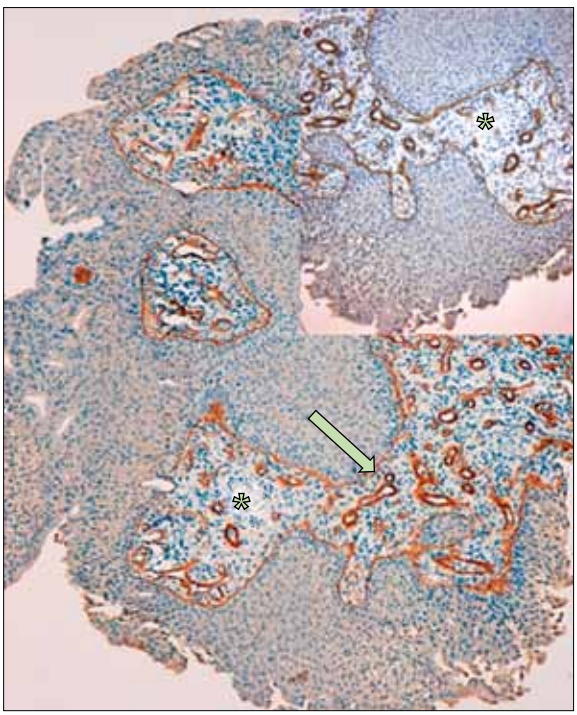

Figure 4: **Immumohistochemical staining at 4 years after transplantation**
Immunostaining of implanted airway showing strong immunoreactivity against anti-laminin. *Indicates vessels and arrows indicate the basement membrane.

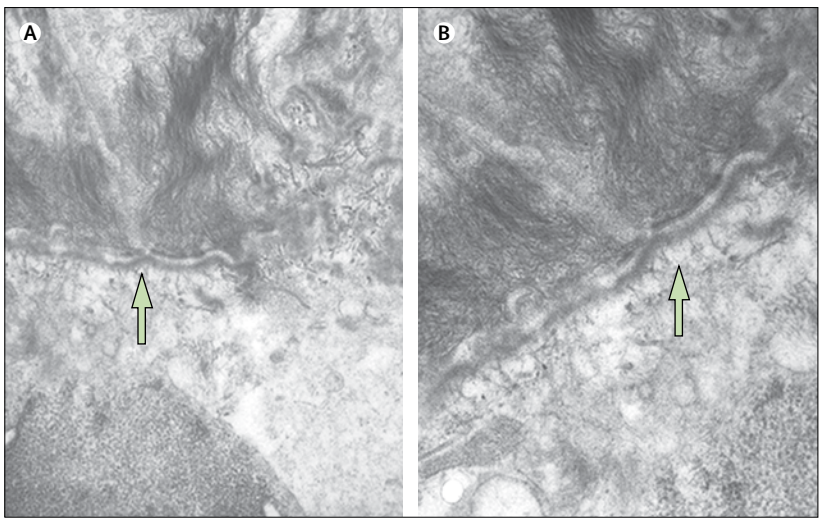

Figure 5: **Transmission electron microscopy of implanted airway at 4 years after transplantation**
Panel B shows detail of panel A. Arrows show basement membrane.

reduce the need for bronchoscopy, placement of a metallic stent was attempted twice: both the metallic stent (Silmet 12x40 mm; Boston Medical Products; Novatech SA, France) and a partly covered Ultraflex (14×40 mm; Micro-invasive, Boston Scientific, Watertown, MA, USA; figure 1C, D) were not well tolerated. By contrast, no stenotic formations developed at the distal bronchial anastomosis and the rest of the graft remained patent during the 5 years of follow-up (table 2 shows detailed follow-up schedule). To date, the patient has a patent tissue engineered graft with an asymptomatic subglottic stenosis (figure 2, video 2).

1 year after transplantation, biopsy samples showed complete re-cellularisation of the bioengineered airway, with the presence of seromucus glands (figure 3A, B). An inflammatory reaction was detected, mainly surrounding the seromucus glands (figure 3C). At this time, no ciliated epithelium was present (figure 3D). At 2 years, haematoxylin and eosin staining showed simple ciliated columnar epithelium with a basement membrane (figure 3E, F). The last biopsy sample (taken December 2012) showed a pseudostratified, ciliated, columnar-type epithelium with areas of immature metaplastic squamous epithelium (figure 3G, H). Laminin immunostaining confirmed the presence of a continuous layer of basal membrane and small blood vessels (figure 4).

Transmission electron microscopy also showed the presence of a basement membrane at the base of the ciliated epithelium (figure 5A, B). Scanning electron microscopy analysis provided microstructural and ultrastructural details of the regenerating airway matrix: 1 year after transplant, the morphology of the implanted airway appeared different from the acellular implanted matrix (figure 6C, D) and several cellular elements had repopulated the matrix (figure 6E, F). In June 2012, we detected a completely re-cellularised airway (figure 6G, H), with a morphology very similar to the native airway (figure 6A, B).

DNA microsatellite analysis confirmed that cells on the decellularised donor matrix had no role in re-cellularisation—the implanted airway was repopulated with the patient's autologous cells only (appendix). Screening of the patient's serum showed no human leucocyte antigen antibody, particularly no donor-specific antibodies, confirming the low (if any) immunological effect of this kind of transplant (data not shown).

See Online for appendix

See Online for video 2

Discussion

Since Rose and colleagues reported the first allogeneic tracheal transplantation in a person in 1979,[14] several attempts have been made to overcome the difficulties associated with reconstructing long airway defects by finding an ideal tracheal substitute (panel).[15–19] The experimental and clinical failures of previous allograft or xenograft airway transplantation include immunological response with subsequent rejection and slow or inadequate revascularisation, causing necrosis with liquefaction or graft stenosis and fibrosis.[18–20] To date, no ideal treatment is available for patients with long segmental airway disorders. Tissue engineering might be the next promising therapeutic alternative for tracheal replacement.[15]

Our attempt to address these failures resulted in the first clinical transplantation of a completely tissue-engineered

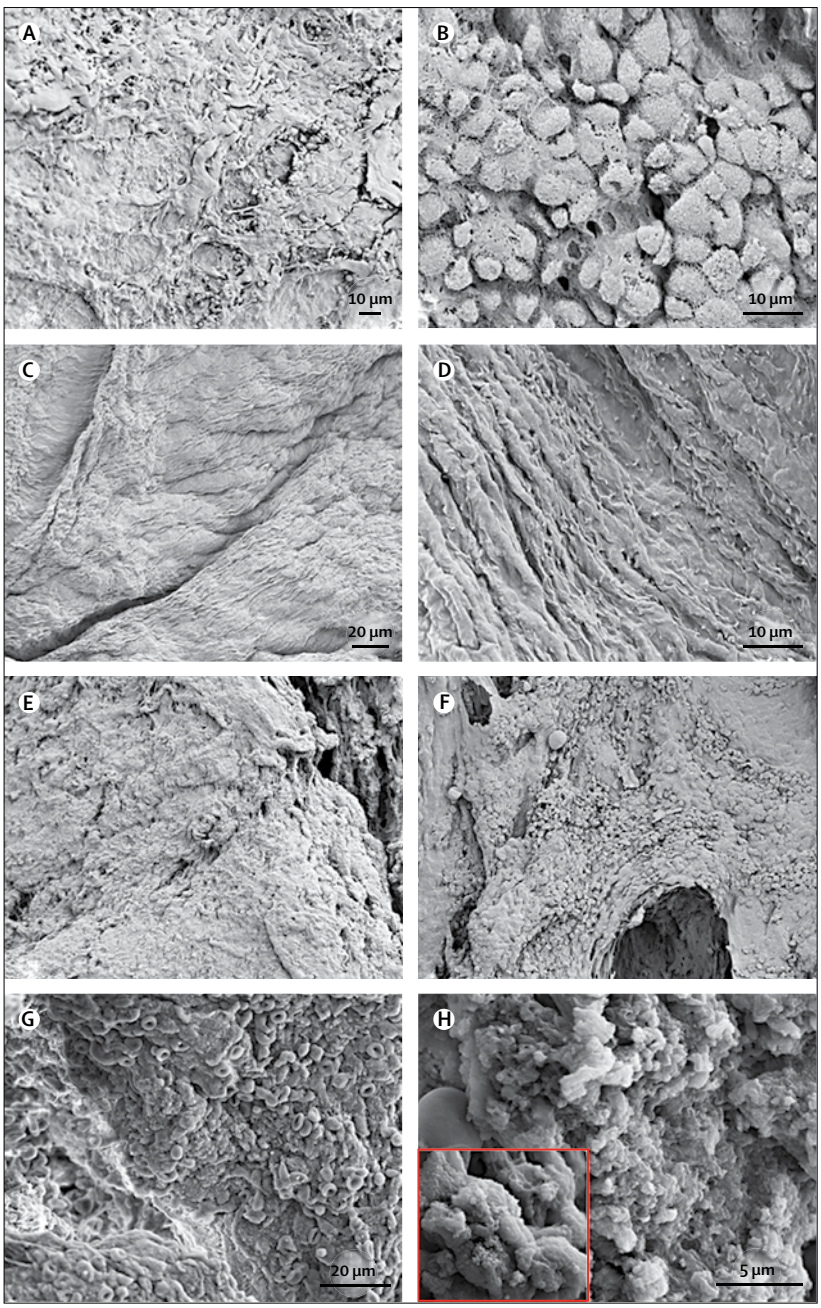

Figure 6: **Scanning electron microscopy of implanted airway**
Showing native (A, B) and decellularised (C, D) airway matrices and of implanted airway at 1 year (E, F), and 4 years (G, H) after transplantation. Showing external (A, C, E, G) and luminal (B, D, F, H) surfaces.

airway in 2008. 4-month follow-up showed no complications, no signs of antigenicity, and a normal quality of life.[1] However, despite these initial promising results, concerns remained about the long-term viability of this engineered airway, particularly the fate and tumorigenic risks associated with use of autologous in-vitro terminally differentiated stem cells, the immunological late response, maintenance of adequate neovascularisation and its ability to support the regenerated mucosa, the long-term retention of adequate biomechanical properties, and whether new chondrogenesis (or osteogenesis) from the seeded chondrocytes would occur.

Our findings after 5 years allay these concerns. The recipient has shown neither an adverse immunological response nor serological signs of rejection, even without any immunosuppressive treatment.[21] Another concern was possible tumorigenic risk associated with both the in vitro terminal differentiation of the autologous stem cells and their ex vivo long-term seeding in the bioreactor. No evidence of cancer has been found. Microsatellite analysis showed that the patient's own cells re-populated the implanted airway matrix, eliminating any doubt about the potential presence or effect of the donor's residual cells.

Unfortunately, a recurrent cicatricial stenosis occurred at the native trachea closest to the transplanted trachea anastomosis. It was refractory to treatment and thwarted attempts to make the patient's life completely free of regular sequential bronchoscopy. Complications on the left main bronchus after surgical reconstruction has been reported previously,[22] and linked to reduced mucosal blood flow, particularly when associated with lung inflammatory or infectious diseases. The difficult and refractory cicatricial stricture and sudden restenosis formation developed where a tracheal resection was done 8 years previously, which might suggest the patient's tendency toward excessive and pathological scarring.

The patient did not tolerate any conventional metallic or silicone stent, requiring use of a short biodegradable polydioxanone stent.[13] The polydioxanone stent kept the bronchus mostly uncovered and reduced significant tension; however, its reabsorption time required several re-interventions to counteract the loss of radial forces and stabilise the stenosis. In situ graft epithelialisation was efficient and mechanical integrity was partly lost,

Panel: **Research in context**

Systematic review
We did a systematic search of PubMed and Ovid for publications, including clinical trials, meta-analyses, and reviews, published between Jan 1, 1963 and June 5, 2013 in English, Spanish, Italian, German, and French. Search terms were "trachea", "transplantation", "graft", "tissue engineering", "trachea replacement", "trachea reconstruction", "stem cells", and "follow up".

Interpretation
Despite several conventional therapeutic options, no proven treatments exist for patients with severe and end-stage airway diseases. Our study describes the 5-year follow-up of the first transplantation of a tissue-engineered tracheal graft in a human being, which suggests that such an approach might be safe and promising.

which resolved by use of a customised stent. We recorded an improved and preserved lung function and normal[4,6] cough sensitivity and expulsive force. These findings suggest no significant loss of airway nerve function 5 years after transplantation.

Our findings provide initial evidence that a tissue-engineering strategy, including decellularisation of a human trachea, autologous epithelial and stem cell culture and differentiation, and cell-scaffold seeding using a bioreactor, is safe and promising. Means to improve the biomechanical long-term stability of such grafts are under preclinical investigation[23] and the results of a first-in-man active clinical trial (Ministero della Salute DGPREV 0015677-P-28/06/2011) could soon provide definitive evidence before this technology can be translated into routine clinical practice.

Contributors

AG and MOJ did postoperative endoscopies, general medical care and follow-up, data collection, data interpretation, and wrote the article. DB collected data and provided medical care. SB did the molecular studies and stainings. PJ interpreted data and wrote the article. CC was responsible for all histological and pathological data production and interpretation. FL, GF, and OS did the respiratory function and cough tests and interpreted the corresponding data, and supervised specific respiratory medical care. GR supervised all immunological aspects during the postoperative period. PM was the leading surgeon in 2008, conceived the project, was principal investigator on the grant supporting the primary study, was responsible for the entire preoperative and postoperative period, and was lead author of the report.

Conflicts of interest

We declare that we have no conflicts of interest.

Acknowledgments

Supported by European Project FP7-NMP-2011-SMALL-5 (BIOtrachea, Bio-materials for Tracheal Replacement in Age-related Cancer via a Humanly Engineered Airway; number 280584e2), Knut and Alice Wallenberg Foundation, Swedish Research Council, ALF Medicine (Stockholm County Council; transplantation of bioengineered trachea in humans; number LS1101e0042.).

References

1 Macchiarini P, Jungebluth P, Go T, et al. Clinical transplantation of a tissue-engineered airway. *Lancet* 2008; **372:** 2023–30.

2 Baiguera S, Jungebluth P, Burns A, et al. Tissue engineered human tracheas for in vivo implantation. *Biomaterials* 2010; **31:** 8931–38.

3 Jandial R, Snyder EY. A safer stem cell: on guard against cancer. *Nat Med* 2009; **15:** 999–1001.

4 Fontana GA, Pantaleo T, Lavorini F, Boddi V, Panuccio P. A noninvasive electromyographic study on threshold and intensity of cough in humans. *Eur Respir J* 1997; **10:** 983–89.

5 Fontana GA, Pantaleo T, Lavorini F, Benvenuti F, Gangemi S. Defective motor control of coughing in Parkinson's disease. *Am J Respir Crit Care Med* 1998; **158:** 458–64.

6 Lavorini F, Fontana GA, Pantaleo T, et al. Fog-induced cough with impaired respiratory sensation in congenital central hypoventilation syndrome. *Am J Respir Crit Care Med* 2007; **176:** 825–32.

7 Myer CM 3rd, Cotton RT. Historical development of surgery for pediatric laryngeal stenosis. *Ear Nose Throat J* 1995; **74:** 560–62.

8 Pei R, Wang G, Tarsitani C, et al. Simultaneous HLA class I and class II antibody screening with flow cytometry. *Hum Immunol* 1998; **59:** 313–22.

9 Tait BD, Süsal C, Gebel M, et al. Consensus guidelines on the testing and clinical management issues associated with HLA and non-HLA antibodies in transplantation. *Transplantation* 2013; **98:** 19–47.

10 Rotman HH, Liss HP, Weg JG. Diagnosis of upper airway obstruction by pulmonary function testing. *Chest* 1975; **68:** 796–99.

11 Ejnell H Bake B, Mansson I. Spirometric indices in the assessment of laryngeal obstruction. *Eur J Respir Dis* 1984; **65:** 600–10.

12 Freitag L, Ernst A, Unger M, Kovitz K, Marquette CH. A proposed classification system of central airway stenosis. *Eur Respir J* 2007; **30:** 7–12.

13 Vondrys D, Elliott MJ, McLaren CA, Noctor C, Roebuck DJ. First experience with biodegradable airway stents in children. *Ann Thorac Surg* 2011; **92:** 1870–74.

14 Rose K, Sesterhenn K, Wustrow F. Tracheal allotransplantation in man. *Lancet* 1979; **1:** 433.

15 Grillo HC. Tracheal replacement: a critical review. *Ann Thorac Surg* 2002; **73:** 1995–2004.

16 Steger V, Hampel M, Trick I, et al. Clinical tracheal replacement: transplantation, bioprosthesis and artificial grafts. *Expert Rev Med Devices* 2008; **5:** 605–12.

17 Delaere P, Vranckx J, Verleden G, De Leyn P, Van Raemdonck D; Leuven Tracheal Transplant Group. Tracheal allotransplantation after withdrawal of immunosuppressive therapy. *N Engl J Med* 2010; **362:** 138–45.

18 Kucera KA, Doss AE, Dunn SS, Clemson LA, Zwischenberger JB. Tracheal replacements: part 1. *ASAIO J* 2007; **53:** 497–505.

19 Doss AE, Dunn SS, Kucera KA, et al. Tracheal replacements: Part 2. *ASAIO J* 2007; **53:** 631–39.

20 Macchiarini P, Walles T, Biancosino C, Mertsching H. First human transplantation of a bioengineered airway tissue. *J Thorac Cardiovasc Surg* 2004; **128:** 638–41.

21 Terasaki PI, Ozawa M. Predicting kidney graft failure by HLA antibodies: a prospective trial. *Am J Transplant* 2004; **4:** 438–43.

22 Takao M, Namikawa S, Yuasa H, et al. Airway complications after lung transplantation: is there a left-sided predilection? *J Thorac Cardiovasc Surg* 1995; **109:** 815–17.

23 Baiguera S, Del Gaudio C, Jaus MO, et al. Long-term changes to in vitro preserved bioengineered human trachea and their implications for decellularized tissue. *Biomaterials* 2012; **33:** 3662–72.

The end of the beginning for tissue engineering

Innerspace Imaging/Science Photo Library

Published Online
October 23, 2013
http://dx.doi.org/10.1016/S0140-6736(13)62110-8

See Online/Articles
http://dx.doi.org/10.1016/S0140-6736(13)62033-4

For three decades we have heard about the hope of tissue engineering. Hyperbole has become routine, but amidst unreasonable expectations are serious scientists, like Paulo Macchiarini, who believe that by combining cells and degradable materials ex vivo they can develop organs to replace or repair diseased tissues. After many years of trying to build engineered tissues on a backbone of synthetic degradable polymers,[1] a growing body of evidence suggests that decellularised whole organs and tissues are clinically effective degradable scaffolds.[2] Until recently, decellularised tissues were used clinically without the addition of cells, and in many cases—eg, the decellularised porcine small intestine submucosa family of surgical implants—this approach was sufficient to generate a healing response. The idea of whole organ engineering—whereby organs are decellularised and then repopulated with desired mixtures of cells—seems to be a realistic path towards complex three-dimensional tissue engineering.

In 2008, Macchiarini's team announced that they had successfully grown a neo-trachea from a decellularised human trachea.[3] We were reminded of the pioneering clinical implantation of a partial neo-bladder in the 1990s,[4] and the sobering reality that an exciting first clinical experience can be separated by decades from even potential clinical implementation. In *The Lancet*, Alessandro Gonfiotti and coworkers report 5-year follow-up of the tracheal implantation,[5] and do not shy away from the harsh realities of a field that begs for even broad clinical implementation while researchers are still learning how to harness our understanding of the biology. Whole organ tissue engineering is akin to converting a Ford into a Ferrari while driving at top speed. The approach is elegant but fraught with challenges and opportunities for improvement; few medical advances have needed a complete biological understanding before implementation.

Tissue-engineered airway transplantation was achieved by seeding a decellularised graft with autologous stem-cell-derived epithelial cells and chondrocytes. Concerns that autologous stem cells might give rise to tumours have been allayed after careful follow-up.[5] Indeed, use of mesenchymal stem cells in clinical trials has shown that delivery of these cells (autologous or allogeneic) is safe.[6] Gonfiotti and colleagues present compelling evidence that the tracheal graft is now naturalised. More importantly, given the data for extracellular-matrix-derived restorative degradable materials and their use in airway and bladder neo-organ development[4,5] we can celebrate the end of the beginning for tissue engineering; the groundwork has been laid for clinical implementation in other specialties.

Excitement about tracheal regenerative therapy might be muted by realisation that the patient in this study was not restored to full health. Although heroic in complying with the needs of a research study, the patient is suffering from ongoing complications from scarring at the proximal anastomotic site. There is nothing unusual about a tracheal stricture forming at a surgical site and, in fact, this patient had already had such a post-surgical stricture. Rather, the formation of a stricture shows that the remaining challenges for tissue engineering of thin hollow organs such as trachea, oesophagus, intestine, blood vessels, and bladder relate to how neo-tissues are incorporated into existing structures. Research is needed to understand how to minimise scarring at anastomotic sites, particularly for a graft that is itself degrading, releasing biological signals, and regenerating in vivo. Degradable inductive scaffolds are not biologically inert because they are replaced by native tissue, and how these scaffolds are attached and introduced to the vasculature will affect the degree to which inflammation-driven versus regeneration-driven healing takes place. For this patient, the reward of maintaining the airway and lung had to be balanced by undergoing multiple imaging studies, biopsies, and dilations. Perhaps as much can be learned from the absence of a distal stricture as from the presence of an intractable proximal stricture. Either way, the patient has enjoyed (as shown by quality of life data) 5 years of life, with less than 2% of the time in hospital being treated for the stricture.

Despite this glimpse of a clinically relevant future, several challenges need to be addressed. How long should a cell–material construct be incubated ex vivo compared with in vivo? Much of the clinical effect of extracellular matrices—which have been implanted in millions of patients as inductive wound healing scaffolds—has been derived without preseeding the matrix. Research is needed to understand what happens

when a graft that has been cell-seeded in a reactor is transplanted into the body. A deeper understanding of this process might also help reduce scarring at anastomotic sites.

How can we control the biomechanical properties of the construct during preparation, ex-vivo culturing, and post-implantation? Substantial effort is being made to understand the role of biomechanics in controlling cell growth and differentiation,[7] which will be essential to delineate how we can match the mechanical characteristics of a neo-organ or tissue to those of the recipient location. Minimising mismatch throughout the conversion of an implanted neo-tissue into natural tissue could also minimise susceptibility to scarring, restenosis, or graft failure. Another important area of biomechanical research will be adapting modern day rehabilitation science and practice to the needs of tissue engineering-based regenerative therapies.

How can we tailor the natural response to surgical transplantation so that scarring at anastomotic sites is controlled and tolerable? Biomaterials are excellent supports for targeted local drug delivery. Advances have been made in reducing surgical scars, and combinations of synthetic and natural degradable matrices might provide even greater benefit. For example, the development of effective degradable tissue glues and adhesives that create seamless junctions between graft and host will improve tissue integration.[8]

Finally, can we predict patient-specific responses to injury so that a graft can be optimised to an individual's biology as well as size? The patient described by Gonfiotti and colleagues seems to have a propensity for scarring. Most regenerative therapies will probably need to be customised to individuals or groups of individuals. Many drugs have greatest efficacy in a small proportion of the population and regenerative therapies will probably be no different. However, work to understand the genetic and epigenetic indicators that might predispose a patient to successful regenerative therapy is yet to be done. Answering these questions, and others, will open the flood gates for clinical tissue engineering, but will require the willingness of patients and doctors to take educated scientific risks together.

Alan J Russell
Disruptive Health Technology Institute, Carnegie Mellon University, Pittsburgh, PA 15213–3890, USA; and Allegheny Health Network, Pittsburgh, PA, USA
alanrussell@cmu.edu

AJR has received funding from Bayer, Pfizer, and Vertex Pharmaceuticals for work related to tissue engineering; has received honoraria and travel reimbursements for presentations from Vertex Pharmaceuticals and Greffex; and serves on the Board of Directors for Applicell and Greffex and on the Science Board of the US Food and Drug Administration.

1 Lee SJ, Atala A. Scaffold technologies for controlling cell behavior in tissue engineering. *Biomed Mater* 2013; **8:** 010201.
2 Badylak SF, Taylor D, Uygun K. Whole-organ tissue engineering: decellularization and recellularization of three-dimensional matrix scaffolds. *Annu Rev Biomed Eng* 2011; **13:** 27–53.
3 Macchiarini P, Jungebluth P, Go T, et al. Clinical transplantation of a tissue-engineered airway. *Lancet* 2008; **372:** 2023–30.
4 Atala A, Bauer SB, Soker S, Yoo JJ, Retik AB. Tissue-engineered autologous bladders for patients needing cystoplasty. *Lancet* 2006; **367:** 1241–46.
5 Gonfiotti A, Jaus MO, Barale D, et al. The first tissue-engineered airway transplantation: 5-year follow-up results. *Lancet* 2013; published online Oct 23. http://dx.doi.org/10.1016/S0140-6736(13)62033-4.
6 Parekkadan B, Jack M. Mesenchymal stem cells as therapeutics. *Annu Rev Biomed Eng* 2010; **12:** 87–117.
7 Butler DL, Goldstein SA, Guldberg RE, et al. The impact of biomechanics in tissue engineering and regenerative medicine. *Tissue Eng Part B Rev* 2009; **15:** 477–84.
8 Gilbert TW, Badylak SF, Beckman EJ, Clower DM, Rubin JP. Prevention of seroma formation with TissuGlu surgical adhesive in a canine abdominoplasty model: long term clinical and histologic studies. *J Plast Reconstr Aesthet Surg* 2013; **66:** 414–22.