# Regenerated Airways – Opening Transplantation up to a Cancer Population

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There are limited treatments for advanced laryngeal cancer. While preserving an in situ larynx, chemoradiotherapy has many serious and unpleasant side-effects, whereas surgically-treated patients are left unable to protect their airway, swallow or speak. Improved reconstructive techniques would lower the threshold for surgical treatment, but laryngeal transplants have yet to enter clinical practice despite a largely successful demonstration of its feasibility. The need for immunosuppressive therapy for a 'quality of life' (QoL) procedure remains an ethical concern, and raises the fear of recurrence.

A solution to this problem may lie in the interdisciplinary field of Tissue Engineering. Successful tracheal replacement with tissue-engineered substitutes has already been clinically demonstrated by surgical teams around the world. A UCL team has now begun recruitment into a pioneering clinical trial of tissue-engineered laryngeal implantation.

## The scale of the problem

The most frequent cancer in the larynx is squamous cell carcinoma. Cigarette smoking and alcohol consumption remain its main risk factors, with a subset caused by the human papillomavirus. Despite government intervention and population education, smoking incidence and cessation rates may not reach an equilibrium for at least another 30 years [1].

Although overall five-year survival rates for laryngeal cancer have risen slightly over the last 40 years due to a number of factors [2] (Figure 1), the smaller subgroup of later-stage (WHO TNM Stage 3 or 4) cancers have much poorer outcomes with limited cures, leading to five-year survival rates of 25-60% in this patient cohort [3].

Future improvement in mortality rate is predicted by better risk stratification and personalisation of therapy. However, aside from the promise of immunotherapy in the prevention and treatment of HPV-provoked tumours, novel and less invasive curative treatments remain elusive for late-stage disease [4]. Around 1,000 oncological laryngectomies are performed every year in the UK, and after curative hemi or total laryngectomies, patients often have to come to terms with a potentially huge impairment in their QoL whilst living a normal lifespan.

## Drawbacks of current treatments

The current gold standard treatment is multimodal, according to TNM disease staging [5]. Localised laser or radiotherapy provides a high (85-95%) cure rate for smaller (T1 or T2) tumours, but larger or more invasive disease necessitates either wider radiotherapy or surgical removal via a hemi or total laryngectomy, combined with a neck dissection for nodal spread [6].

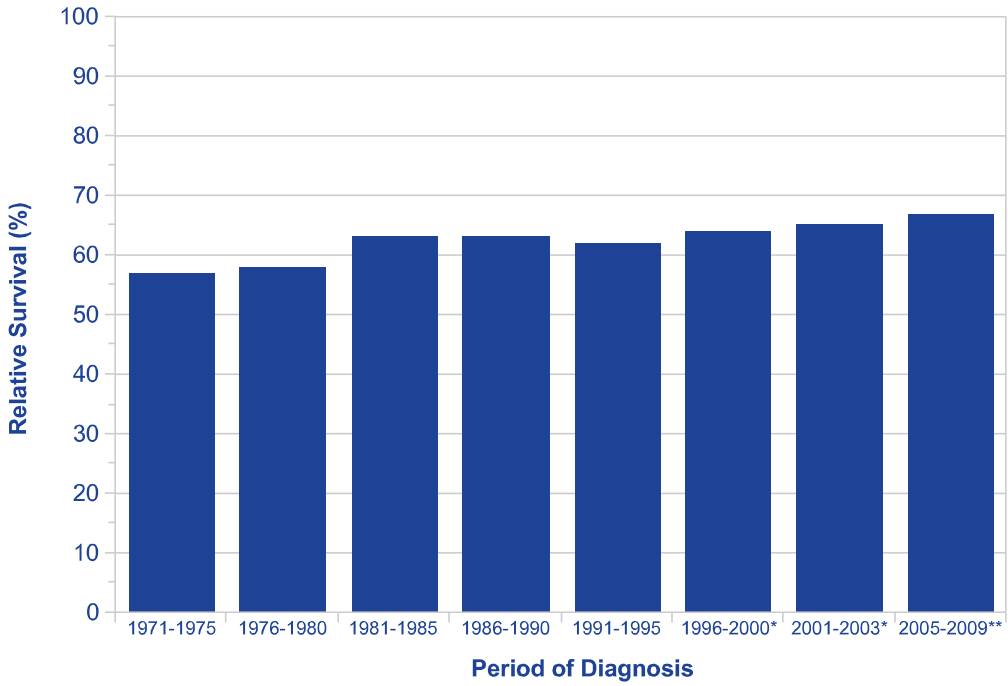



Figure 1: Overall age-standardised five-year survival rates for laryngeal cancer 1971-2009. Source: Cancer Research UK.

These treatments are responsible for significant morbidity. Laryngectomy has changed very little since first performed in the 19th century, and whilst it provides a final cure for many life-threatening laryngeal diseases, the patient is left with a disfiguring neck stoma through which to breathe and is unable to swallow, strain or communicate freely with family and friends. Laryngectomised patients can become depressed and socially isolated [7]. Quality of life surveys of laryngectomees have shown that, although they are understandably worried about taking further treatment risks having already gone through gruelling primary treatments, many of whom would consider the surgical risks and long-term immunosuppression worthwhile to regain some of these lost functions [8].

The increasing number of patients treated with 'organ-preserving' chemoradiotherapy has led to a fall in laryngectomy rates and a modest survival increase, but this is not without its own problems [9]. The disappearance of the physical mass of a treated cancer will often leave behind ravaged laryngeal structures. Despite the physical presence of a larynx, radiotherapy can cause such extensive pain, oedema, scarring and vocal cord paralysis that the organ barely functions. Indeed, patients often report a worse QoL than laryngectomised patients [10].

## Laryngeal transplantation gets the green light

The world's first laryngeal transplant was done in 1998 by Strome and colleagues in Ohio, USA, on a laryngeal trauma patient [11]. Long-term follow-up data published in 2010 indicated that, despite remaining tracheostomised, the patient could swallow normally. He had been able to build a career as a professional speaker [12]. Possible chronic rejection unfortunately necessitated explantation in 2013, but the patient had remained well on low-dose immunosuppression.

Our multinational research group performed a laryngeal transplant with similar success (Figure 2): the transplant has restored the ability to smell, taste, speak and cough, though she still has a tracheostomy [13]. She has been immunosuppressed for her kidney-pancreas transplants, so the ethical concerns concerning another immunogenic transplant were less relevant. A number of transplants have also been carried out in Colombia, but formal outcomes are awaited [14].

Despite hopes that laryngeal transplants could lead to a radically improved QoL for patients, critical concerns remain to be addressed [15]. As an operation which would not necessarily confer a survival benefit, the need for subsequent lifelong immunosuppression is ethically controversial due to the risk of reactivation of high-grade or recurrent tumours. The ethical picture is clearer for patients who had laryngectomy following trauma, or oncology

Figure 2: Brenda Jensen, recipient of a laryngotracheal transplant.

patients with proven benign or low-grade tumours [15]. The requirement for rapid and intricate reinnervation for both sensory and motor function is also a major hurdle to success, as without detection and prevention of life-threatening aspiration, patients will remain tracheostomy-reliant.

Recent advances in composite organ grafting [16] have been immensely helpful in reviving optimism for laryngeal transplantation. Newer immunosuppressive medications, such as everolimus, have been suggested not only to have an improved side-effect profile, but may also act as tumour-suppressors [17], and tacrolimus can enhance the recovery of laryngeal muscle fibres following reinnervation [18]. Taking these considerations into account, the Royal College of Surgeons Working Party published guidance in 2011 approving the introduction of laryngeal transplantation to the UK [19].

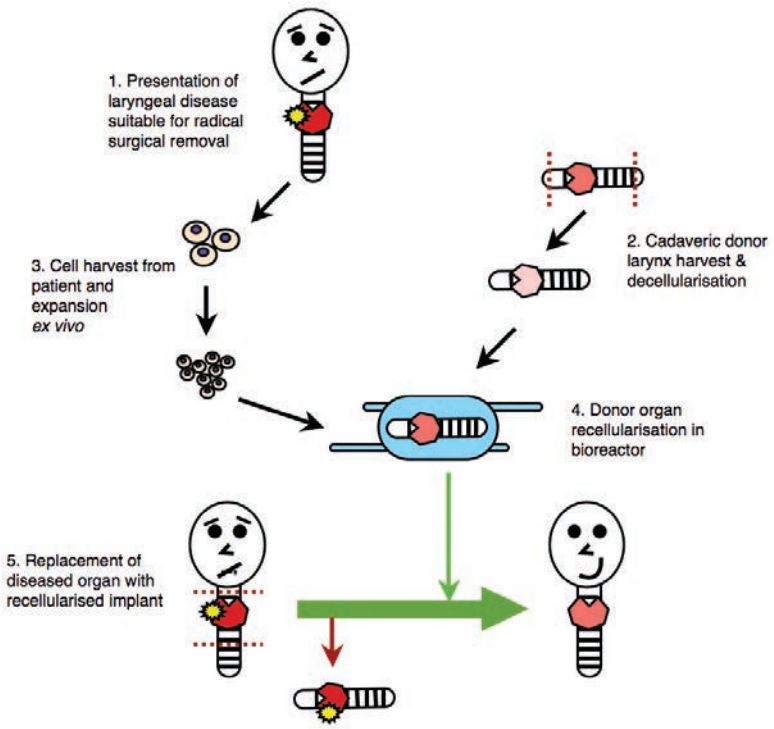
## Tissue-engineered solutions

The field of tissue engineering is an interdisciplinary middle-ground where engineering and biological sciences combine to create biological substitutes to restore, maintain or improve tissues [20]. One of the most common strategies is to treat a conventionally acquired donor organ with a decellularisation process and removal of immunogenic material, leaving the bare scaffold of extracellular matrix (ECM). This is seeded and cultured with a patient's autologous cells before implantation into the patient (Figure 3).

Decellularised ECM retains a myriad of site-specific molecular cues to support cell survival, ingrowth and differentiation into the correct cell types present in the normal larynx. Under the influence of multiple environmental cues from both the scaffold and the surrounding host tissues, endogenous and implanted cells eventually repopulate the scaffold to form a new organ.

This strategy obviates the need to sustain a patient with immunosuppression medication, and the reduction in tumour reactivation renders it potentially suitable for patients with higher-grade tumours than with conventional laryngeal transplantation. Removal of cells and major histocompatibility complex (MHC) material from the scaffolds also allows for a much wider application of transplantation, as there is no need to wait for close immunological matches. Immediate surgical reconstruction of the larynx could therefore be performed as part of the original procedure, thereby removing the need for two long operations

Figure 3: Schematic of the basic principle of tissue-engineered laryngeal replacement with a decellularised donor organ.



with separate anesthetic and post-operative recovery periods.

The enormous potential of this strategy for airway reconstruction has been clinically demonstrated by our group [21, 22].

## The RegenVOX Timeline

RegenVOX is a UCL-based multidisciplinary team of clinicians and scientists, led by Professor Martin Birchall, which seeks to refine a safe and effective strategy for tissue-engineered laryngeal implantation that can be routinely performed in the National Health Service [23], through funding from the MRC Biomedical Catalyst programme.

The first phase of the project successfully dealt with the preclinical work underpinning the creation of quality tissue-engineered larynxes for implantation (Figure 4) [24]. For Phase 2, recruitment has now commenced into a RegenVOX clinical trial to implant 10 patients with donor-derived decellularised hemilarynxes. Surgery is planned from March 2014, with patients under intensive follow-up for a minimum of two years.

Initially patients may belong to rare cohorts of benign laryngeal diseases where

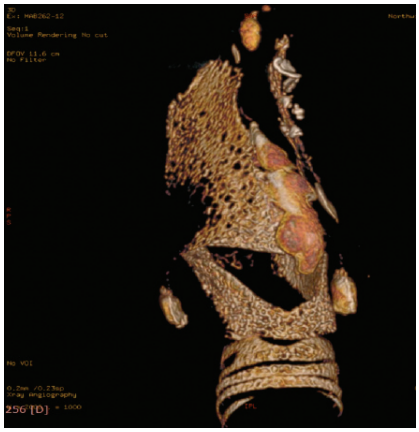


Figure 4

organ preservation is impossible, such as traumaor scarring from 'burnt-out' inflammatory conditions (eg relapsing polychondritis) [15]. As no immunosuppression is required for these implants because of the use of autologous cells and MHC-free scaffolds, it is theoretically possible that this technology can be used for primary reconstruction after tumour resection. In the first instance, this would be reserved for rare benign tumours or those of low grade malignancy, in line with conventional transplantation. There is some evidence that mesenchymal stem cells, one of the predominant cell types used for scaffold repopulation, might promote the development of metastatic disease through their pro-angiogenic and immunomodulatory effects [25], but decellularised scaffolds may abrogate a pro-inflammatory response [26]. Therefore, application of stem-cell based reconstruction after primary cancer treatment awaits safety testing in animals, as well as the outcomes of our present trials.

## Conclusion

Tissue-engineered airway replacements have enormous potential to alleviate, and even prevent, some of the long-term morbidity of advanced laryngeal cancer, both that associated with local destruction by cancer and that due to existing surgical treatments. Regenerative medicine-derived hemilarynx implants are to become a reality in the UK in 2014. If successful, this will be a significant and welcome addition to surgical oncology. ■

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