STATE OF THE ART-A POSITION STATEMENT


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Abstract
There has been significant and exciting recent progress in the development of bioengineering approaches for generating tracheal tissue that can be used for congenital and acquired tracheal diseases. This includes a growing clinical experience in both pediatric and adult patients with life-threatening tracheal diseases. However, not all of these attempts have been successful, and there is ongoing discussion and debate about the optimal approaches to be used. These include considerations of optimal materials, particularly use of synthetic versus biologic scaffolds, appropriate cellularization of the scaffolds, optimal surgical approaches and optimal measure of both clinical and biologic outcomes. To address these issues, the International Society of Cell Therapy convened a first-ever meeting of the leading clinicians and tracheal biologists, along with experts in regulatory and ethical affairs, to discuss and debate the issues. A series of recommendations are presented for how to best move the field ahead.

Key Words: consensus, trachea, tissue-engineering

Introduction
End-stage tracheal disease necessitating replacement of diseased or damaged tissue is a rare but devastating situation. Despite recent advances in surgical techniques and ex vivo tracheal engineering, replacement continues to present major scientific and technical challenges [1–4]. As such, bioengineering this seemingly uncomplicated yet enigmatic organ has become emblematic of progress and also the challenges in regenerative medicine. Reasons for this include the alluringly straightforward demands of replacing a “simple” tube, the fact that pioneers have emerged from specialties focusing on the airway and cardiothoracic surgery, but most importantly because end-stage disease presents the most serious and sometimes acute threat to life. Thus, tracheal bioengineering has lent itself to compassionate use applications in a way that would be hard to justify for many other potential organ replacement targets. This has led not only to important leaps but also exposed important gaps in both scientific and clinical understanding [1–3]. Thus, progress in this “niche” area remains of high interest to the world’s regenerative medicine community (Figures 1–4).

One of the corollaries of this path has been that a number of groups in Europe and North America have taken different routes to the clinic. The various approaches have included the use of allografts, preserved homografts and of ex vivo tissue-engineered tracheas that are based on both biologic (decellularized) and...
synthetic scaffolds populated with a range of different cell types [1–4]. These approaches have yielded varying degrees of success, but, as yet, there is no clear optimal technique, and each has pros and cons for use. In particular, whether any one approach can result in an implanted graft with the full range of appropriate cellular and physiologic functions remains unclear and has provoked significant, sometimes acrimonious, debate and discussion [5,6]. A variety of growth factors and other agents have been used as adjunct treatments along with surgical implantation. These include both treatment of the graft either before or during implantation as well as systemic administration to the recipient. However, the utility, and in some instances the rationale, of these approaches is not clear and may be based on only limited pre-clinical data. Furthermore, clinical reports are sometimes lacking in key data that can help gauge the success or highlight the problems with current implantation approaches.

In parallel, discovery and translational scientists and the emerging regenerative medicine industry have made important recent breakthroughs that will significantly improve the underlying science. This is especially true of tracheal epithelial and stromal biology and upper-airway progenitor cells and will have implications for the design and application of bioengineered tracheas over the next decade [7–9]. These are rapidly progressing “cutting edge” areas of research favorably looked on by the National Institutes of Health and other relevant funding agencies.

To date, most of these international efforts have occurred in relative isolation, with little sharing of ideas and methodologies between the basic/translational scientific communities and the clinical and commercial efforts. Furthermore, more extensive communication must occur between the different clinical and commercial efforts. For example, clinical inclusion criteria, techniques used and reported outcome measures, particularly assessments of graft recellularization and optimal physiologic functions, vary widely. Some of this may have been necessary, given the nature of compassionate applications of tracheal grafts and the extraordinary speed of change in regenerative medicine science. Nonetheless, this confounds comparative assessments of the different approaches used, impedes more rapid coordinated progress and promotes unconstructive argument.

Thus, the Pulmonary Committee of the International Society for Cell Therapy convened a meeting to bring together the leading experts in clinical applications of bioengineered tracheas, leading scientists studying tracheal biology and industry leaders. The specific goal was to discuss and debate the current state of knowledge and to devise effective means of combining efforts to most effectively move the field of tracheal repair forward. These discussions were accompanied by presentations on regulatory and ethical issues with the goal of contributing to
efforts to move the field ahead. A synopsis of the presentations followed by a compilation of current key questions and recommendations were developed and are listed below. The full program of the meeting and list of participants is available in the on-line Supplementary Data for this article.

Translational Science—Philipp Jungebluth, MD, Karolinska Institute, Solna, Sweden: Synthetic and biologic-based tissue-engineered tracheas

The Macchiarini group has pursued “first-in-human” clinical applications of both biologic and synthetic scaffold-based approaches to tracheal replacement [10–12]. This group has also been active in investigating a wide range of adjunct approaches that might improve the success of graft implantations [13–16]. Dr Jungebluth presented results from an as-yet partly published series of patients receiving biologic scaffolds composed of decellularized cadaveric tracheas or synthetic-based scaffolds seeded with autologous bone marrow–derived mesenchymal stem (stromal) cell (MSC)-derived chondrocytes and nasal epithelial cells [2]. These have yielded varying clinical outcomes to date with significant mortality. Broadly, when replacement sections replaced were short, patients have done relatively well, whereas those requiring long-segment or total replacements have required stenting after a few months in situ. In some patients, the tracheal epithelium appeared to regenerate as evidenced by serial brush biopsy sampling. However, only partial data is available from some but not all patients treated to date. Furthermore, detailed assessment of the types of epithelial cells and the mechanisms and kinetics of re-epithelialization has not yet been performed. The mechanical/structural components of the grafts, assumed to be cartilage but without clear biopsy confirmation, given practical reasons, have apparently regenerated poorly (or incompletely). The reasons for this are not clear and currently under study.

As a result, the efforts of this group have turned in large part to synthetic scaffolds. More recently, several polymers including polyhedral oligomeric silsesquioxane (POSS), have been used clinically. While these retained excellent mechanical strength, there were complications with effective vascularization and epithelialization, bacterial and fungal contamination and development of granulation tissue around the synthetic replacement bronchial joins which necessitated subsequent stenting. More recently, this group has explored and clinically applied electrospun–devised synthetic scaffolds (based on polyethylene terephthalate), but to what stage seeded cells participate in the scaffold regeneration is unknown at present, and investigations are ongoing. However, animal data suggest the necessity of cell seeding before implantation [13,17,18].

Cell seeding techniques for the synthetic scaffolds have used ex vivo seeding with autologous MSCs and nasal epithelial cells at the time of graft implantation [12,13]. However, further pre-clinical studies are necessary and ongoing to understand the mechanisms by which effective seeding and development of fully reconstituted stromal and epithelial cell layers occurs. Other current activities of this group include a clinical trial of synthetic tracheal replacements ongoing in Russia.

Overall, the experience of this group has been that a number of deaths have occurred with both biologic
and synthetic implants (8 of 18 patients have died). Many of these patients were seriously ill at the outset, including some with tracheal malignancies who had undergone previous treatment attempts. As such, most of the implants have been “compassionate use” in difficult clinical situations. These deaths may present a biased unfavorable view of the field, therefore formal clinical trials and common outcome measures between groups are critical going forward. However, some patients also benefit from the transplantation [11]. Furthermore, more detailed work with adjunct treatments intended to improve survival and quality of both decellularized and synthetic grafts is a critically important area that must be pursued.

Alex Seifalian, PhD, University College London, London, United Kingdom: Nanocomposite tracheas

Professor Seifalian’s group has extensive experience with nanocomposite materials in preclinical and clinical studies, primarily of vascular replacement, but also other settings such as heart valves and lacrimal ducts. On the basis of this work, in collaboration with Drs Macchiarini and Birchall, they fabricated the tracheal and carinal replacement for one of the first patients to receive a synthetic graft recellularized with autologous stromal and nasal epithelial cells [12]. This patient, who was implanted after a cancer resection, survived for 3 years with reportedly a good quality of life, a record for a synthetic trachea. On the basis of this experience, plus that of a second graft implanted for palliative purposes in a UK patient (unpublished), this group, with others, has obtained European Union and United Kingdom peer-reviewed funding to optimize the material for this and other bioengineering applications.

The nanocomposite family of polymers under particular study is silicone-based POSS with non-biodegradable controllably porous (coagulated poly [carbonate-urea] urethane, PCU), solid (casted PCU) and bioabsorbable (polycaprolactone polyurethane) forms. POSS-PCU, which has a novel nano-cage structure, is now manufactured to Good Manufacturing Practice grade and is “clinic ready.” It is biocompatible, and viscoelastic various peptides, such as arginylglycylaspartic acid or antibodies, can be incorporated to enhance attachment of specific cell types. Silver nanoparticles are antibacterial and can be incorporated into the material to reduce biofilm problems. The material can be bioprinted with the use of 3-dimensional (3D) bioprinters to customize shape and size. Work on biocompatibility of the absorbable polycaprolactone polyurethane forms is ongoing.

To date, as with the experience of Drs Jungebluth and Macchiarini, epithelialization, local bio-integration, especially at junctions with native trachea and bronchus, and infection have limited clinical application and long-term success in the 2 patients implanted to date. However, strategies to overcome these challenges are being developed, including the possible use of hybridization with biological materials, in collaboration with investigators in a number of European Union countries.

Emmanuel Martinod, MD, PhD, APHP, Hôpitaux Universitaires Paris Seine-Saint-Denis, Sorbonne Paris Cité, Paris 13 University and Laboratory for Biosurgical Research, Paris Descartes University, Paris, France: Airway replacement with the use of aortic homografts

Historically, Herberhold developed preserved aorta-derived homograft tracheas for use in non-circumferential repair of critically stenosed tracheas in infants [19]. Changes in tissue regulations and the advent of slide tracheoplasty made the technique redundant in the 1990s [20]. However, recently, Dr Martinod and colleagues proposed the use of a more widely available aortic homograft to provide circumferential replacements. In preclinical animal models, such replacement resulted in excellent biomechanics strength and epithelialization and even the appearance of new cartilage in the graft wall [21–29]. First clinical applications were performed in patients with extensive tracheal or lung cancer, with encouraging results [30–32]. However, all patients required stenting for biomechanical purposes, and this in turn limited epithelial regeneration and mucus clearance. The group recently removed a stent from one of the current new series of patients and was observing to see if long-term stability could be maintained. Professor Martinod suggests that an ideal application for this technology is the replacement of the main stem bronchus to avoid pneumonectomy in patients with proximal lung cancer. This would be a significant advance for such patients because it would preserve substantial pulmonary function. The group continues to increase their clinical experience (TRACHEO-BRONC-ART study, NCT01331863) and will report this shortly. Parallel studies of the kinetics and biology of epithelial regeneration in the homografts are also being pursued.

Tom Waddell, MD, PhD, Toronto General Hospital and University of Toronto, Toronto, Canada: Decellularized tracheal scaffolds

The Waddell group has focused on developing approaches for using cadaveric human trachea-based grafts. Particular interest has been in developing appropriate decellularization and recellularization techniques, including optimization of detergent-based decellularization regimens in cadaveric donor
tracheas from animal and human sources [33]. One important observation has been that current approaches do not remove all major histocompatibility complex expression from submucosal glands, thus opening up questions as to the true allo-/xenogenicity of grafts that are based on this technology [34]. Interestingly, decellularization delays leukocyte infiltration, but eventually the cartilage is degraded nonetheless in animal tracheal allograft studies. There are no good assays for chondrocyte viability in such studies, and it is currently unknown if chondrocytes, or their remnants, actually get rejected. Furthermore, whether lymphocytes can and do actually infiltrate the chondrocyte-containing cartilage lacunae remains unknown. These are critical questions in understanding and thereby improving the mechanisms of survival and remodeling of biologic scaffold-based tracheal implants.

In experimental pre-clinical models in rats, after recellularization with MSCs and tracheal epithelial cells, a decrease in CD4/CD8 T-cell proliferation and induction of regulatory phenotype T cells was observed after allografting [34]. This may support a more constructive tissue remodeling response in the bioengineered trachea. The group hypothesizes that the source (allogenic versus autologous) of cells may not be as critical as is the short-term activity of the implanted cells because neither source appears to persist for longer than a few weeks in vivo.

Dr Waddell also emphasized the need to understand the process of epithelialization, including how this can be accelerated, how the new epithelium may be oriented, functionally ciliated and organized in a manner reflecting native epithelium. His team has started to investigate whether there is a role for airflow/shear in re-epithelialization, as is well-recognized for endothelialization in blood vessels. His team is further developing a novel and efficient perfusion seeding technique for tracheal scaffolds but has found that even continuous perfusion for 72 hours is not enough to achieve complete, functional epithelial coverage of the grafts [35].

Martin Birchall, MD, Royal National Throat Nose and Ear Hospital, London, United Kingdom: Biologic scaffold and autologous cell-based tracheal replacement in adults and children

Professor Birchall presented preclinical and clinical work on tracheal bioengineering performed in Bristol and London, United Kingdom, initially in collaboration with Dr Macchiarini and others. The group’s initial efforts were directed at culture of human tracheal epithelial cells in an effort to reduce rejection in laryngeal transplants. They combined this with the Hollander (Bristol, United Kingdom) group’s work on production of chondrocytes from MSC and the Conconi (Milan, Italy) group’s work on decellularization and, on the basis of encouraging results from Jungebluth and Macchiarini’s pre-clinical studies in pig models, collaborated with the latter to produce a bronchial/carinal replacement in a young woman [10], a graft that although requiring intermittent stenting, remains patent and functional 5 years later [11].

On the basis of this success and further laboratory work, a modified technique was adopted to treat a young boy whose tracheal stent had eroded into his aorta. A cadaveric donor trachea was decellularized and, on the basis of some limited animal data, the child was treated preoperatively with granulocyte colony-stimulating factor (G-CSF) and erythropoietin. During surgery, the MSC bone marrow fraction was isolated and poured over the scaffold, which was also injected with transforming growth factor-β to theoretically induce chondrogenesis. A bioabsorbable (poly L-glutamic acid) stent was placed, and the child continued to be empirically treated with erythropoetin and G-CSF for some days. His recovery was stormy and characterized by the secretion of a very thick DNA “net” into the tracheal lumen, hypothetically in response to excessive neutrophil accumulation caused by the G-CSF. The stent needed replacing twice, and although the child was discharged at 6 months, he needed a metal stent insertion at 1 year, which has remained in place since. At 4 years, he is alive and well, growing and at school.

The group recently performed a 3D computed tomography scan to compare with earlier scans; these templates were then used to bioprint replica tracheas for airflow studies, revealing that both ends of the graft had grown with the child, but that there was a highly localized central narrowing that had not grown. It is not possible to tell whether this represents failure of growth, in-stent stenosis or simple graft stenosis.

Subsequently, bioengineered tracheal production has been converted into a fully Good Manufacturing Practice—compliant process, and this was applied to a similar child in 2012. Although her immediate latency and quality of life was dramatically improved and she was well enough to go home after 2 weeks, she died of unknown complications 2 weeks after discharge, raising fresh questions about biomechanics in particular. One area of current investigation, in collaboration with Dr Sefalian, is to determine whether biomechanical improvements may result from hybridization with polymers, such as POSS, and/or external forms of stenting.

Presently, the group has been using human cells to seed porcine decellularized scaffolds in preclinical studies. The results have been promising and have provided evidence sufficient to obtain permission for 2 formal clinical trials of partial laryngeal and
tracheal replacement, which will commence recruiting in 2015. However, continuous improvement is clearly required and focuses on more efficient and earlier epithelialization, understanding angiogenesis and how far the technology can be translated into treatments for infants and younger children with stenosis and malacia not amenable to conventional treatments. Furthermore, the use of adjunct growth factors remains poorly clarified and is an area requiring more extensive study.

**David Williams, PhD, Wake Forest University, Winston Salem, North Carolina, USA: Biomaterials for bioengineering tracheal scaffolds**

Professor Williams discussed the options for biomaterials in tracheal bioengineering, including biologic, synthetic, and gels [36], and the constraints placed by the regulatory process. Currently, according to the US Food and Drug Administration, there is a need to test materials for tissue-engineering scaffolds as if they were components of a medical device, especially with the use of International Standards Organization standard tests [37]. However, Professor Williams argues that this is a restricted and wrong approach for tissue engineering when the science is moving rapidly and the safety data produced by these standards does not address the requirements of biological activity in such scaffolds. Further dialogue with the US Food and Drug Administration and other world-regulatory agencies such as the European Medicines Agency is required. Dr Williams further described a 2013 summit held in Xi’an, China, which brought together opinion leaders from all over the world in an effort to achieve consensus on streamlining the path to the clinic for synthetic grafts and implants. The Xi’an protocol is the subject of a statement to be published in *Science, Translational Medicine* [38]. The anticipation is that this will provide a powerful framework on a basic, translational-clinical and regulatory framework for moving ahead the application of biomaterials to a number of clinical problems, including tracheal grafts.

**Natalie Mount, PhD, Chief Clinical Officer, Cell Therapy Catapult, London, United Kingdom: Regulation of tissue-engineered products**

Dr Mount continued the theme of streamlining the route of tracheal grafts to the clinic. Particular focus was on the importance of carefully defining the requirements of the end product and use of knowledge of the applicable regulations to efficiently navigate through development. Careful consideration must be taken with the planning of the clinical and regulatory paths for the concept of a cell-based biologic therapy as opposed to a device implant. Dr Mount emphasized that combining tracheal scaffolds with cultured cells creates an Advanced Therapeutic Medicinal Product and demanded as high a level of regulatory scrutiny by European Union national competent authorities (such as the United Kingdom’s Medicines and Healthcare Products Regulatory Agency for clinical trial authorization in the United Kingdom and the European Union’s European Medicines Agency for marketing authorization as a new drug might require [39,40]. This approach is similar to that taken by the US FDA [39], providing the field with non-clinical, quality and clinical standards that must be met. One obvious future area for moving the field ahead is the conduct of carefully designed formal clinical trials along with having manufacturing and testing of cell and scaffold combinations accompanied by regular dialogue and joint development with regulatory agencies. Support in navigating this area efficiently can be offered in this emerging area by organizations such as the United Kingdom’s Cell Therapy Catapult, which is a forward-looking government-supported (Technology Strategy Board), not-for-profit company that provides translational expertise to support progression of cell therapy and bioengineering clinical investigations [41,42].

**Discovery Science—Dario Fauza, MD, Boston Children’s Hospital and Harvard University, Boston, Massachusetts, USA: Bioengineering the fetal and neonatal trachea**

Dr Fauza has studied bioengineering of the trachea and diaphragm for many years [43–63]. He discussed a particular need for tracheal bioengineering for babies with congenital tracheal anomalies, babies who are almost universally aborted or die at birth. Earlier and more accurate prenatal diagnosis, made possible because the obstructed fetal airway fills with fluid, giving characteristic ultrasound images, now provides at least a 3-month window in which planning for a replacement can be undertaken if technology is available. Dr Fauza’s group has focused in particular on the use of amniotic fluid MSCs (AFMSC) as the basis for neonatal/intra-uterine regenerative therapies, including for trachea [43–63]. AFMSCs proliferate much faster than those from cord blood or bone marrow, despite the highly hypoxic environment in amniotic fluid. They can also make a peculiar type of cartilage *in vitro*, richer in both glycosaminoglycans and β-elastin than cartilage engineered from other types of stem cells.

To create a functional tracheal replacement in preclinical sheep models, investigations have focused on both synthetic and decellularized tracheal scaffolds repopulated with AFMSCs to promote chondrogenesis
and other cell growth. Stable, revascularized grafts resulted in which re-epithelialization was observed by 3 weeks. However, epithelial regeneration was perhaps incomplete because no goblet cells were observed in the neo-epithelium. More complete characterization of the resulting epithelial layer is in progress. Because smooth muscle contraction/peristalsis is required for normal alveolar/pulmonary development, it is possible that affected children, unlike the animal models used here, have abnormal lungs also, and this may limit clinical outcomes. Nonetheless, the outlook is bleak for these children presently, and preparations for compassionate use application and clinical trials are well advanced in Boston. Further biologic characterization of the recellularized tracheas is necessary.

Dr Rawlins is a leader in study of upper-airway development and regeneration after injury [64—66]. Her work is also emblematic of a long line of comparable studies on upper-airway regeneration and repair in other laboratories including those of Brigid Hogan at Duke University and more recently Jason Rock at the University of California at San Francisco [67,68]. As such, the cumulative efforts from these investigators, other investigators participating in this meeting whose presentations are listed below and other investigators unable to participate in the meeting, including but not limited to Drs Ivan Bertoncello at the University of Melbourne, Hal Chapman at the University of California at San Francisco, Susan Reynolds at National Jewish Hospital in Denver and Barry Stripp at the Cedars Sinai Center for Lung Biology, have provided a vibrant and rapidly evolving understanding of the biology of tracheal regeneration. As such, this is an opportune time for closer collaborative efforts with the clinical investigators.

Most recently, Dr Rawlins has used a novel tracheal trauma preclinical model to study respiratory epithelial regeneration. Here, the application of sulfur dioxide kills all columnar and related cells but crucially leaves the basal cells and some deeper glandular secretory cells intact to participate in regeneration [65,69]. In practice, it was observed that most of the replicating cells are basal, with secretory cells having little stem cell capacity. The differentiation pathway between basal and secretory cells appears to be 2-way, with secretory cells having the capacity to de-differentiate into basal cells [70]. However, it is still unknown whether basal cells give rise to ciliated cells in vivo, with or without going through a secretory cell step. Notch signaling appears to allow exit from the basal layer into secretory cells, which may in turn become ciliated cells. Single-cell polymerase chain reaction that was based on 6 genes showed that basal and secretory cell populations both split into 2 distinct subtypes. Further characterization is ongoing, and it will be of high interest to seed the various progenitor populations into decellularized or synthetic tracheal scaffolds. Dr Rawlins also raised an important point regarding the ability to obtain sufficient human cells for study. Biopsy samples are generally small and autopsy samples are generally of limited use. A key area for future development will be devising means to obtain enough human materials for study.

John Engelhardt, PhD, University of Iowa, Ames, Iowa, USA: Are tracheal submucosal glands important for tracheal bioengineering and cell therapies?

Dr Engelhardt is a leader in study of upper airway development and repair with particular focus on submucosal glands [71,72]. His group has long investigated the hypothesis that tracheal submucosal glands form a critical niche for stem cells in the proximal airway and a source of transient amplifying cells of for regeneration for of damaged and regenerating tracheal mucosa. In a naphthalene injury model in mice, bromo-deoxyuridine nucleotide labeling studies, a powerful technique for assessing cell replication in situ, demonstrated that submucosal gland cells have a critical role in repair and that this regeneration occurs from proximal to distal [71,73]. Furthermore, glandular regions of the trachea in mice contain larger number of highly proliferative airway progenitor cells in culture [73]. If confirmed in humans, these findings have major implications for clinical interventions after injury and as part of tracheal graft bioengineering strategies. Furthermore, a novel subset of glandular cells has been identified that cluster in the glandular ducts and tubules in the proximal trachea and remain slowly cycling after repeated injury. Wnt-β-catenin-mediated regulation of Lef-1 and SOX2 has a regulatory role for these cells’ primordial glandular stem cells, whose phenotype is characterized by the activation of Wnt reporters in transgenic mice β-galactosidase expression, along with some stem cell markers [71,74]. On the basis of these studies, a new model is proposed in which these clustered Wnt-active basal tubules within fully developed glands act as a niche for slowly cycling stem cells that differentiate into slow-cycling tubular cells, which have the capacity to regenerate tracheal mucosa. A parallel line of investigation has identified tracheal glands as critical for innate mucosal immunity [75], raising the question of mucosal health in tissue engineering models in which the glands are not reconstituted.
Finally, tracheal xenograft models show great utility as a means of delineating the contributions of various cell lineages before their selection as part of therapeutic strategies. Glandular cells and glandular restoration must be considered during tracheal bioengineering. Interestingly, these cells are more resilient to decellularization protocols than are other mucosal cell types. This may be another critical consideration for success or failure of regeneration in biologic-based tracheal implants.

Thomas Gilbert, PhD, ACell: On processing of tracheal extracellular matrix and the impact of functionality

Dr Gilbert is a leader in studies of tracheal regeneration, particularly with the use of decellularized tracheas. His work has provided some fundamental information on the protocols used for optimal tissue decellularization strategies and has extensively investigated the host response to decellularized scaffolds [76–82]. His work has also investigated fundamental questions on composition and role of different components of the remaining extracellular matrix (ECM) in decellularized tracheas [78]. This is a critically important area because there is still much to be learned about the role of different ECM proteins and glycoproteins (glycosaminoglycans) in subsequent behavior of cells seeded into the decellularized matrices. A fundamental unanswered question is, what constitutes an optimally decellularized matrix? Recent investigations have focused on comparative decellularization of tracheas with the use of different detergents with evaluation in an orthotopic rat tracheal reconstruction model. Other areas of active investigation include modulation of immune responses to implanted tracheal scaffolds and reconstitution of appropriate ciliogenesis in recellularizing scaffolds. Most recently, Dr Gilbert has been investigating hybrid mesh-based synthetic/ECM composites as alternative materials to use for the implants.

Sam Janes, University College London, London, United Kingdom: Autologous airway epithelium for tissue-engineered airways

Dr Janes has wide-based interests in pulmonary regenerative medicine. This includes both MSC-based therapies for respiratory diseases, with focus on lung cancers, as well as participating with Drs Birchall, Sefalian and others at University College of London to develop strategies for tracheal bioengineering [83,84]. As an example of work in tracheal engineering, Dr Janes presented data from a case of tracheal replacement performed in London. In addition to the clinical outcomes, Dr Janes highlighted the range of biologic outcomes that can and should be pursued. He further described some of the specific methodologic approaches that can be potentially used, including lineage tracing that is based on mitochondrial and other mutations. This presentation again highlights both the available techniques as well as the challenges in recapitulating normal cellular and organ functions in tracheal grafts of either biologic or synthetic origins.

Brigitte Gomperts, University of California at Los Angeles, Los Angeles, California, USA: Use of induced pluripotent stem cells for tracheal bioengineering

Dr Gomperts has been studying normal and aberrant regeneration and repair of the large airways for many years. The focus has been on delineating populations of endogenous upper-airway progenitor cells, and Dr Gomperts and colleagues have described a novel population of putative endogenous progenitor cells [85,86]. More recently, her lab published work demonstrating that flux of reactive oxygen species regulates the self-renewal of airway basal stem cells through signaling through Nrf2 and Notch [87]. This pathway is critical for basal stem cell homeostasis, and perturbation of the pathway has implications for lung carcinogenesis.

Recent focus has also been on the potential use of induced pluripotent stem (iPS) cells from which to derive appropriate populations of upper-airway cells for use in re-population of both decellularized and synthetic tracheal scaffolds. This approach offers the advantage of both the use of autologous cells as well as conceivably generating large numbers of differentiated epithelial and other cell types from the iPS cells. Dr Gomperts reviewed developmental pathways involved in tracheogenesis and discussed ways in which these pathways could be mimicked in culture to induce appropriate directed differentiation of the iPSC cells [88]. This strategy has been applied to a variety of other organs, including lung, and is thus a viable option for tracheal regeneration. Data were presented demonstrating that iPS cells cultured at the air-liquid interface can acquire morphologies and immunophenotypes representative of both ciliated epithelial cells as well as non-ciliated club cells [89–93]. Further data were presented demonstrating that the iPS cells can also be induced to acquire morphologies and immunophenotypes representative of vascular endothelial cells, smooth muscle, fibroblasts and cartilage [94–97]. Gene editing techniques can also be used to further modify the iPSC cells [98]. These are exciting findings that can be incorporated into clinical tracheal replacements. However, there remain a number of unanswered questions including whether the iPS-derived cells will all be appropriately functional and also non-immunogenic. The
intriguing possibility of incorporating the iPS cells and their derivatives into 3D bioprinting of synthetic tracheal scaffolds was also raised.

Industry perspectives

Sponsoring companies were offered an opportunity to present product information and design/manufacturing considerations relevant to tracheal regeneration. Stephen Minger, Chief Scientist in the Life Sciences Division of GE Healthcare, discussed efforts toward development of stem cell–based platforms for generation of cardiomyocytes, hepatocytes and neurons for drug testing and development of a range of toxicity models. Focus has been on industrialized expansion and differentiation of human embryonic stem cells. A number of industrial tools were highlighted, including the ability to optimize large-scale expansion in bioreactors with the use of constant perfusion to regulate glucose, lactate and other metabolic indicators.

A second presentation by Dr Gilbert highlighted the range of extracellular matrix–based wound care products developed by ACell. ACell has taken a leading position in development of a considered series of products that attempts to both understand the biology of the ECM and using this knowledge to best design patches and other matrices tailored to specific tissue indications. Similar considerations are being applied to the development of tracheal matrices.

Erik Woods of Cook Medical discussed efforts toward development of more optimal culture media as well as cell manufacturing and closed-system packaging for cell storage and delivery. One objective is to establish optimal techniques for long-term preservation of decellularized matrix. For example, freezing can induce changes in ECM scaffolds, notably opening holes in collagen networks. Viscoelastic properties can also change, depending on scaffold storage and handling.

Extensive discussion was held throughout this session and highlighted the advances as well as the gaps in industrialization of effective tracheal scaffolds.

**Table I. Key questions.**

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<th>Fundamental/basic</th>
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<tr>
<td>• What is the optimal material to use for a graft: decellularized native tissue or synthetic material? If synthetic, how should the optimal material be designed and evaluated? Will this be different in pediatric versus adult applications?</td>
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<td>• What cells need to be seeded into the tracheal grafts for optimal reconstitution of a function epithelium? When should they be administered: before implantation or during implantation? Is there any role for implanting unseeded grafts and letting in vivo recellularization occur?</td>
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<td>• What are the peculiarities and related mechanisms of the fetal environment for both cell procurement and implantation?</td>
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<td>• If pre-seeding and ex vivo culture of cells seeded onto grafts is to be used, is currently available bioreactor technology adequate?</td>
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<td>• Is there a role for use of endogenous tracheal airway progenitor cells or other stem/progenitor populations in lieu of or in combination with seeding with differentiated adult cells obtained from the eventual graft recipient?</td>
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<td>• How should optimal revascularization be achieved?</td>
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<td>• What are the functional biologic end points required from the graft? Is mucociliary clearance essential? Is structural integrity of the airway sufficient? How will these be measured?</td>
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<td>• Are key resources or mechanisms in place for data sharing?</td>
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<td><strong>Clinical</strong></td>
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<td>• Appreciating that compassionate use of tracheal grafts is unpredictable, is there an optimal population or populations that can be identified in both pediatric and adult patients for further investigation?</td>
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<td>• Is there an optimal surgical approach? This includes consideration of both direct and indirect allografting as well as homografting.</td>
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<td>• Is there a potential role for xenografting with decellularized pig tracheas? For example:</td>
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<td>• Is there a role for adjunct treatment with growth hormones or other biologic agents in the peri-transplant period?</td>
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<td>• What range of outcome measures should be used, and how should they best be assessed?</td>
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<td>• Is it feasible to obtain serial biologic samples, for example, serial biopsies of the graft mucosal lining, to study cellular and functional cellularization of the graft?</td>
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<td>• Can an international registry of patients be effectively created and appropriately maintained?</td>
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<td>• Is adequate infrastructure and organization in place to support rapid development and implementation of appropriate clinical investigations? Nationally? Internationally?</td>
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<tr>
<td>• Are the various potential infrastructure resources coordinated for most effective applications?</td>
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<tr>
<td>• Are there funding mechanisms in place that can be better used to support basic and clinical development of optimal tracheal grafts?</td>
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<tr>
<td>• Are there existing organizations and networks that might be effective partners for further development? Potential examples include the American, British and European Thoracic Societies.</td>
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<tr>
<td><strong>Industry</strong></td>
</tr>
<tr>
<td>• Have key pharmaceutical and biotechnology partners been identified?</td>
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<tr>
<td>• Is there an infrastructure in place to maximize productive collaborations between academic and industry partners?</td>
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<tr>
<td>• Will industry partners support full disclosure in a public international patient registry?</td>
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<tr>
<td>• Can appropriate partnerships be formed to facilitate optimal use of resources/technology in other biotech sectors, such as cell processing expertise from bone marrow transplant or other fields?</td>
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Table II. Recommendations: fundamental/basic-translational.

- Continue to encourage and fund new research that explores and defines fundamental pathways involved in tracheal/large-airway repair and regeneration. This includes focus on epithelial, stromal and vascular cell repair.
- Increased focus on appropriate regeneration of functional cartilage in airway grafts.
- Continue research toward the optimal means of tracheal decellularization and recellularization.
- Investigate the potential roles for xenogeneic tracheal grafts.
- Identify optimal biomaterial evaluation regimens that focus on the required biological activity of scaffolds rather than lack of toxicity.
- Develop optimal synthetic materials for use in tracheal grafts. Focus is on both mechanical properties as well as ability to support appropriate recellularization.
- Elucidate any fundamental differences between fetal, pediatric and adult tracheal regeneration and how this might affect design of optimal bioengineered tracheal grafts.
- Develop improved bioreactors for decellularization and recellularization/long-term culture of tracheal grafts before implantation.
- Careful and detailed study in pre-clinical models of the potential role of adjuvant growth factors and other biologic agents for use alongside tracheal recellularization approaches.
- Expand collaborations between individual basic and clinical investigators in both pre-clinical and clinical investigations.
- Explore wider ranges of funding opportunities for collaborative efforts between basic and clinical investigators.
- Foster inter-institutional, multi-disciplinary research collaborations and consortia as well as clinical/basic partnerships. Include a program of education on tracheal diseases and stem cell biology.
- Develop relevant training programs and opportunities for the most promising junior investigators.

Ethics

James Lawford Davies of the legal firm Lawford Davies Denoon has specialized in ethical and regulatory issues involving stem cells and cell therapies. The area of tracheal regeneration is a ripe area for these considerations, and Mr Lawford Davies highlighted a number of issues to be considered as the field develops. As with other areas of regenerative medicine, there are many gaps in current ethical approaches, with some of the more extreme cases involving sale of organs for profit. Careful consideration and oversight must be an integral part of tracheal bioengineering.

Summary of discussions

After extensive discussions after each presentation and in a final open discussion session, it is clear that there is great enthusiasm for combining resources to move the field of tracheal regeneration and clinical application ahead. However, there remains significant and, in some cases, vigorous scientific disagreement between groups. Within the clinical realm, important questions remain about appropriate patient populations, optimal surgical approaches and type of graft to be used. Further issues involve the need for comprehensive reporting of clinical outcomes. Critical underlying biologic questions focus on how to best recellularize the tracheal grafts to promote optimal cell and physiologic functioning to best mimic that of a native trachea. Included in these considerations are what types of cells to use, when to apply the cells and what type of adjunct growth factor and other treatments to consider. These are all areas ripe for investigation. In parallel, close, ongoing conversations with regulatory agencies and with industry partners will provide the dialogue necessary to move the best approaches most effectively toward clinical investigation and eventual use.

In addition to fostering the above collaborative efforts, strong sentiment was expressed for creation of an international patient registry. This would be administered by an independent agency and would collate all past and future clinical data including but not limited to clinical history and patient selection, surgical approaches, type of graft, adjunct treatments and the relevant detailed clinical outcomes. Importantly, this registry would also include information on biologic outcomes, with particular focus on evaluation of graft recellularization and physiologic function.

A list of key questions identified at the meeting is summarized in Table I. A list of suggested recommendations, including formation of an international patient registry, is detailed in Table II. These will provide a framework for ongoing discussion and planning.

Summary

The continued need for effective solutions to devastating tracheal diseases, both congenital and acquired, remains a paramount driving force for the rapid recent evolution of tracheal bioengineering. A coordinated effort between basic science, clinical, regulatory and industry efforts is both feasible and desirable and will help propel this field most effectively forward. The uniformly positive enthusiasm at this meeting affirms a commitment to these efforts. Future meetings dedicated to this rapidly evolving field will be a valuable resource for continued discussion and debate.

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References

Tracheal bioengineering: the next steps


Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.jcyt.2014.10.012.