Background and Methodology

In preparation for hosting a laryngeal tissue engineering meeting (*Strategic Directions in Laryngeal Tissue Engineering*) in July 2014 in Madison, WI, we decided to systematically assess the perspective of thought leaders in the area. We obtained Institutional Review Board approval and identified potential survey participants – individuals with an experienced perspective in laryngeal tissue engineering – using three sources: (i) senior authors of recently published peer-reviewed articles on laryngeal or tracheal tissue engineering found in the NCBI/NLM PubMed database (http://www.ncbi.nlm.nih.gov/), (ii) principal investigators on NIH-funded research grants on laryngeal or tracheal tissue engineering found in the NIH RePORTER database (https://projectreporter.nih.gov), and (iii) senior authors on podium presentations at the abovementioned academic meeting. We included investigators working on tracheal engineering as this larynx-related area has received substantial research attention and achieved early translational success, and because a number of researchers study both the larynx and trachea.

For NCBI/NLM PubMed, the search algorithm was: ((((((((((((vocal fold OR larynx OR trachea[Title])) AND "English"[Language]))) AND ("2009/05/01"[Date - Publication] : "2014/05/01"[Date - Publication]))) AND (tissue engineering or biological engineering or medicine, regenerative or nerve regeneration[MeSH Terms])))))); for NIH RePORTER, the search algorithm was: (vocal fold OR larynx OR trachea) AND ("tissue engineering" OR regeneration OR "nerve regeneration" OR "regenerative medicine") (Advanced), Search in: Projects Limit to: Project Title, Project Terms, Fiscal Year: Active Projects.

Once identified, potential participants were contacted via email through the University of Wisconsin-Madison Qualtrics Survey Hosting Service. Participants were asked to identify at least 10 critical steps needed to make the following statement a reality: “The field of laryngeal tissue engineering will exhibit broad clinical success by the year 2025.” Participants were also asked to provide their age, sex, number of years in the field, number of peer reviewed publications relevant to laryngeal or tracheal tissue engineering, number of extramural grants relevant to laryngeal or tracheal tissue engineering, and the mechanism of each relevant grant.

Ninety senior authors and principal investigators were identified and invited to participate; 20 individuals completed the survey. They were 63% male, had a mean age of 46.3 years (range, 32-65 years), had worked in the field for a mean duration of 9.3 years (range, 3-24 years), reported a mean of 13.4 relevant peer-reviewed publications (range, 1-80), and reported receiving a mean of 2.4 relevant grants (range, 0-14). They were geographically based in China, Japan, the United Kingdom, and the United States. Their research programs were funded by the NIH F31, R01, R03, R21, R31, SBIR mechanisms, the VA Career Development award program, the California Institute for Regenerative Medicine, non-United States government agencies (Japan Society for the Promotion of Science, National Science Foundation of China, United Kingdom Medical Research Council), and various charitable foundations.

We analyzed responses to the primary survey question using the Hoshin facilitation process, a well-established strategic planning technique that has been described in detail elsewhere.[1,2] Briefly, raw ideas were collected and sorted into distinct concepts, based upon their similarity. For each concept, the total number of relationships (*R*) and the total number of outgoing dominant relationships (*O*) were counted. Next, we each independently estimated the field’s progress-to-date for each concept, using a 10-point scale (0, no progress; 10, complete progress). Values were averaged and the mean estimate of progress (*P*) for each concept was recorded. Finally, each concept’s number of outgoing dominant relationships (*O*) was divided by its mean progress (*P*): the resulting quotient, normalized concept dominance (*O/P*), was used to rank each concept based on its potential importance to advancing the field.

Results

The survey question generated 149 raw ideas, which were grouped into 17 concept categories (**Table SI**). Hoshin data for each concept are summarized in **Table SII**.

References

1. Tennant C, Roberts PA. Hoshin Kanri: a technique for strategic quality management. *Qual Assur*. 2000; 8:77-90.
2. Johnson PC, Mikos AG, Fisher JP, Jansen JA. Strategic directions in tissue engineering. *Tissue Eng*. 2007;13:2827–2837.

Table SI. Concepts and their supporting raw ideas.

Increased collaboration and sharing
(15 ideas; 10.1%)

- Interdisciplinary research
- Collaborative projects rather than single lab projects
- Learning form mistakes of others rather than repeating them again and again
- Partnership between clinicians and scientists
- Unifying laryngeal academic centers of excellence to work together, recruit patients, increase study cohorts
- Sharing of findings into database
- Increase interdisciplinary team – engage folks from outside the world of otolaryngology
- Sharing results from different labs
- Apply ideas and concepts used in cardiovascular tissue engineering to vocal folds
- Increased availability of healthy tissue samples
- Availability of primary human cells
- Sharing of cell lines – used throughout
- Create cell lines for key cell types present in the vocal fold
- Development of cell lines – of all relevant types – nothing is available commercially and this is a huge bottle neck
- Storing the cells in a cell bank accessible to various research groups would be most helpful

Attention to regulatory issues
(11 ideas; 7.4%)

- Attention to GMP and clinical-grade manufacturing
- Clear understanding of what it takes to get something to the clinic – regulatory specialists role
- Identify regulatory strategy
- Increased efforts to move promising therapies through the regulatory pathway
- Conduct GMP validation to meet FDA/EMA approval
- Conduct first-in-man safety and potential efficacy studies
- Conduct phase 2 trial
- Identifying methods for capturing safety
- Identifying methods for capturing efficacy
- Assessing safety
- Assessing efficacy

Standardization of protocols and outcome measures
(9 ideas; 6.0%)

- Generating clinical protocols
- Consistent, standardized, and rigorous methodologies used across the field
- Improve ways to share protocols and methods to try to avoid duplication and promote better coordination
- Take advantage of advanced imaging methods to better understand reconstruction process
- Test whether treatments improve vocal function in the long term
- Demonstration of long-term benefits (1-2 years or more)
- Determine primary and secondary endpoints for clinical trials
  Develop criteria to monitor first patient populations for 5-10 years, evaluating long-term clinical improvement, stability, off-target effects, and biointegration
- Functional assessment of engineered laryngeal tissues

Definition of patient characteristics and suitability for therapies
(6 ideas; 4.0%)

- Identify the clinical needs with precision
- Define a common clinical problem/pathway that will benefit from laryngeal tissue engineering
- Identifying appropriate patients for laryngeal tissue engineering
- Identify patient characteristics
- Identify appropriate patients (diagnoses, comorbidities, exclusions, failure of other therapies)
- Enhance understanding of how patient characteristics interconnect with clinical outcome

Identification of best cell source
(11 ideas; 7.4%)

- Identify ideal cell source: iPS versus autologous non-iPS, versus. embryonic/fetal stem cells
- Clarifying a standardized (stem) cell source
- Identifying the ideal cell type
- A patient derived cell source that does not require vocal fold biopsy
- Development of specific cell therapy to prevent vocal fold scarring
- Development of specific cell therapy to treat chronic vocal fold scarring
- Demonstrate that the cells are functional (e.g., fibroblasts synthesize collagen)
- Use stem cells that can differentiate into vocal fold fibroblast cells or other types of cells
- The induction of these stem cells to form the important extracellular matrix mimicking the viscoelastic properties of the original vocal folds
- What is the role of donor MSCs in tissue engineering? Do they become the new tissue or just guide and direct regeneration?
- Are autologous MSCs required or could they be pooled donor allogenic MSCs, or even xenograft MSCs?

Improved understanding of basic biology and pathophysiology
(10 ideas; 6.7%)

- Study the role and differentiation of stem cells in human vocal fold
- Isolate each kind of human vocal fold cells and define their characteristics
- Study harvesting and culture of human vocal fold stem cells
- More knowledge about normal and scar fibroblasts
- Better understanding and characterization of the tissue itself
- Increased knowledge about wound healing with and without scarring
- More knowledge about general medical and behavioral treatments and prevention of chronic vocal fold damage
- Improving our understanding of the superficial lamina propria
- Better defining the wound healing inflammatory response
- Improve developmental biology knowledge of the larynx – very little is published – therefore often feel like there is no foundation upon which to work from

Focus on parameters that modulate cell/biomaterial performance
(7 ideas; 4.7%)

- Identifying if growth factors etc would enhance a therapy
- Investigate the potential of drug-loaded biomaterials
- What cytokines and pharmaceuticals can be used to manipulate tissue engineering to enhance regeneration and vascularization of graft?
- Standardized way of sterilizing tissue engineering grafts – method/dose – effects of this on graft biology and structural stability
- Study the relation and interaction of biomaterials and induced cells
- Study the relationship and interaction of each type of vocal fold cell
  Study the interaction of induced cells and local resident cells
- Study the relation and interaction of biomaterials and local cells

Identification of best scaffold
(10 ideas; 6.7%)

- Identify ideal scaffolds: naturally synthetic biomaterial versus biologic versus hybrid
- Development of novel materials
- Development of proper scaffold for cell implantation and transfer
- Maintaining the scaffold after implantation without absorption
- Identifying the ideal scaffold
- Use smarter synthetic composite biomaterials with features that promote cell proliferation and migration
- Use advanced manufacturing tools to design and build scaffolds and/or implants
- Develop more compatible and effective biomaterials
- Development of scaffolds to be used in treatment of vocal fold scarring
- What is the role of decellularization – removal of antigens? Change in ECM protein by altering glycosylation patterns? Other? Does the detergent used matter?

Study of market factors and healthcare payors
(6 ideas; 4.0%)

- The cost of the new materials/methods must be comparable to, or at least not dramatically exceed, the cost of existing treatment. A great treatment that is too expensive won't have "broad clinical success"
- Determine most critical unmet needs in this area, market size, and price point for needs; prioritize difficulty and price point before proceeding
- Achieve health system (e.g., VA, NHS) adoption
- Garner payor support (i.e., convince Medicare and private payors to cover these therapies)
- Develop affordable therapy/therapies
- Identify and confirm reimbursement strategies

Improved surgical strategies for implantation
(2 ideas; 1.3%)

- Medical intervention techniques that are straightforward (for surgeons and MDs)
- More development of surgical technique/injection/attachment/grafting approaches

Improved in vitro models
(10 ideas; 6.7%)

- Novel in vitro models
- Develop in vitro vocal fold model
- Bioreactor design improvement
- Increase use of in vitro bioreactors that can recreate human physiological conditions
- Bioreactor capable of creating air-liquid interface for a tube-like scaffold structure
- Develop reproducible in vitro models (engineered tissue) that mimic native vocal folds, including barrier function, to permit drug testing
- Create optimal materials for in vitro models (i.e., gels or scaffolds that support growth of various cells and demonstrate the appropriate biomechanical properties)
- Develop tissue that can be put in a bioreactor. Or develop a bioreactor that can accommodate gels or scaffolds with cells in them and on them
- Create disease models
- Create patient-specific models

Improved in vivo models
(11 ideas; 7.4%)

- Developing right animal models
- Conduct small animal studies
- Conduct large animal proof-of-concept studies
- Better animal models
- in vivo animal models for pre-clinical study, then move to clinical trial
- Conduct preclinical trials on animals that routinely vocalize in the audible range
- Developing and standardizing animal models for vocal fold and laryngotracheal scarring
- Increase the pace of animal studies in order to get more quickly to human studies
- Improve available animal models of laryngeal disease including assessment of laryngeal function.
- Test of novel materials in animal models
- Be able to put the engineered tissue in animals: the tissue should not create a significant immune response, should last over time, and should yield a structure and function that is appropriate for the animal model (e.g., permit phonation in a species-appropriate manner)

Focus on manufacturing and commercialization
(6 ideas; 4.0%)

- If market will support, achieve commercialization, assisted by post-adoption surveillance and audit…BINGO
- I wonder if commercialization of the new materials is a prerequisite to "broad clinical success"
- Gain industrial support
- Form industry partnerships for product development
- Increased engagement with industry and scaling up of engineering approaches
- Identify and confirm partnerships with cell companies, device companies, and physician organizations to

properly market devices and cell-device combinations

Initiation of human clinical trials
(8 ideas; 5.4%)

- The advances in the lab must be moved into human trials. There is a lot of good research, but so far little translation
- Clinical trials that include physiologic, perceptual, and quality-of-life measures with appropriate controls
- Clinical trials – need more human trials of tissue engineered constructs
- Need support for clinical trials
- Perform multi-center human trials
- Focus the many potential therapies into those most likely to succeed, for clinical trials
- Perform clinical trials through the FDA and other relevant institutions
- Demonstrate improved outcomes over current therapies

Focus on advanced tissue properties
(17 ideas; 11.4%)

- Understanding the microbial factors involved with mucosal scarring
- Attention to vascularization and nutrient delivery/oxygenation of grafted materials
- How long does it take for a graft to develop a new blood supply?
- Angiogenic properties for the scaffold
- Immunogenicity
- Study the special immune response of vocal fold to any induced cells and materials and relation of vocal fold local immune response and body immune system
- Maintaining the structure after implantation to avoid being destroyed by the host immune systems
- More attention to in vivo tolerance, host response, rejection etc
- Attention to airway lumen barrier of engineered materials, interaction with 'biome etc
- Integration of multiple components together, muscle, nerve, mucosa etc
- Increased use of organotypic, multicellular, more complex 3D engineering approaches
- Improve approaches to promote innervation in transplanted larynges.
- Reinnvervation
- Reinnervation of the laryngeal muscles
- Conquer selective nerve control
- New methods to reinnervate larynx
- Possibilities to transplant or reconstruct a larynx with function for phonation and swallowing as well as breathing

Invest in further development of the field
(3 ideas; 2.0%)

- Improved rigor of the literature and publications – less salami-slicing of work
- More publication outside the field and more in basic science journals: this would increase the quality of the work
- Increase in NIH funds for this area, including through the Small Business Innovation Research (SBIR) mechanism

Focus on discrete laryngotracheal tissue subtypes
(7 ideas; 4.7%)

- New methods to construct or regenerate lamina propria
- New methods to construct or regenerate epithelium and lamina propria
- For more serious vocal fold damage, regeneration of an epithelium in addition to the lamina propria will be required
- Regeneration of epithelium
- Engineering of the laryngeal framework, cartilage engineering
- Cartilage
- Muscles, movement

Table SII. Analyzed Hoshin data, sorted by normalized concept dominance.

Concept	Ideas	*O*	*R*	*P*	*O/P*
Increased collaboration and sharing	15	9	9	2.5	3.6
Attention to regulatory issues	11	6	8	2	3
Standardization of protocols and outcome measures	9	9	9	3	3
Definition of patient characteristics and suitability for therapies	6	4	4	1.5	2.7
Identification of best cell source	11	7	9	4.5	1.6
Improved understanding of basic biology and pathophysiology	10	7	9	4.5	1.6
Focus on parameters that modulate cell/biomaterial performance	7	7	10	5	1.4
Identification of best scaffold	10	6	10	4.5	1.3
Study of market factors and healthcare payors	6	1	5	1	1
Improved surgical strategies for implantation	2	3	5	3.5	0.9
Improved *in vitro* models	10	1	5	5	0.2
Improved *in vivo* models	11	0	6	6	0
Focus on manufacturing and commercialization	6	0	6	1	0
Initiation of human clinical trials	8	0	9	2	0
Focus on advanced tissue properties	17	0	6	2.5	0
Invest in further development of the field	3	0	3	3.5	0
Focus on discrete laryngotracheal tissue subtypes	7	0	7	4	0
Mean	8.8	3.5	7.1	3.3	1.2
(SD)	(3.8)	(3.5)	(2.2)	(1.2)	(1.2)

Ideas, number of supporting raw ideas; *O*, number of outgoing dominant relationships; *R*, total number of relationships; *P*, mean estimate of progress*; O/P*, normalized concept dominance.