Final Progress Report

Research Toward Occupational Safety in Vocalization

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Principal Investigator Ingo R. Titze
National Center for Voice and Speech
University of Utah
Salt Lake City, UT

Summary from the original Research Plan

This research addresses an important public need, the protection of an estimated 10 million workers in the U.S. who rely heavily on their voice as a primary tool of trade. Evidence has been growing that occupational voice users, such as teachers, telephone workers, ministers, counselors, interviewers, are at risk for vocal injury because they get inadequate recovery times from prolonged speaking. The underlying hypothesis is that there is a limited vibration dose that vocal fold tissues can withstand. As for hand-transmitted vibration in power tools use, a safe dose is governed by frequency, amplitude, and duration of vibration exposure. Microphone use solves the overdose problem related to vocal loudness, but does not address the problem of excessive duration. A voice dosimeter has been designed, tested, and is currently in use by teachers in the Denver area public school system to measure vocal dose. The teachers also self-monitor their vocal fatigue levels. In this renewal application, the primary focus will be on the molecular underpinnings of tissue response to excessive vibration. Specific aims are to (1) determine the ideal geometric and viscoelastic properties of the lamina propria, (2) quantify voice recovery times and relate them to auto-perceptive ratings, (3) culture distinct cell types at various states of differentiation, (4) determine the relative merits of co-culture for in vitro monolayer systems, (5) engineer a 3D in vitro model of the lamina propria in a bioreactor, (6) identify and functionally characterize candidate vibration-responsive genes, (7) develop a theoretical model of economic voice production, and (8) explore two types of economy-based vocal therapy. The ultimate goal is to provided heavy voice users with safety criteria based on genetic disposition to vocal injury, degree of training in economic voice use, accumulated dose of vibration in a typical work day, and the amount of recovery available at night and on weekends. A multi-disciplinary research team has been assembled to address this public health concern at physical, biochemical, molecular, and behavioral levels of investigation.

Specific Aims from the original Research Plan

The long-term goal of this research is to protect workers who use their voice as a primary tool of trade. We will develop a self-monitoring strategy for safe voice use in occupations with high vocal demand. This strategy will be useful to professional speakers such as teachers, telephone workers, salespeople, agents, ministers, trial lawyers, dispatchers, counselors, and public speakers who are engaged in three or more hours of dialogue or monologue in a typical workday. From recently conducted surveys, it appears that significant work loss and early abandonment of careers in occupations with high vocal demand can be linked to ineffective voice use and, more importantly, inadequate attention paid to daily vocal doses and appropriate recovery periods. Our research is based on the premise that *vocal dose* (as measured by speech output) is closely related to *vibration dose* (the absorbed vibration energy in the vocal folds, which is difficult to measure). The difference is vocal economy, a quantity that we are defining and exploring as a vocal output-to-cost ratio. In addition, there may also be a genetic predisposition to vocal injury, a tender larynx condition, which makes even a normal vibration dose excessive for some individuals.

We are using teachers as our primary subject base because they are (a) at great risk, (b) represent a sizeable sector (4%) of the US workforce, (c) have extended vacation time for baseline measurements, and (d) can accommodate wearable devices on the job for field testing. But, the benefits are obviously not restricted to teachers. The following specific aims are targeted for a five-year granting period:

1) To determine ideal geometric and viscoelastic properties of the lamina propria for normal and exceptional ranges of pitch and intensity.
2) To quantify voice recovery times (short term, intermediate term, and long term) and relate them to auto-perceptive ratings of *inability to produce soft voice*, for which vibration is confined to the lamina propria, which means “layers in motion.”
3) To culture distinct cell types at various states of differentiation (human mesenchymal stem cells, laryngeal fibroblasts, and vocal fold stellate cells) that appropriately model and regulate extracellular matrix (ECM) changes under conditions of vibration.
4) To determine the relative merits of co-culture of the above cell types for an *in vitro* monolayer system that appropriately models and regulates ECM changes under conditions of vibration.
5) To engineer a 3D *in vitro* model of the lamina propria in a bioreactor, using data collected from aims 3 and 4, that allows for specific vibration loads, measurement of viscoelastic properties, and observation of changes in ECM modeling.
6) To identify and functionally characterize candidate vibration-responsive genes and genetic pathways that regulate ECM modeling under various vibration loads over time.

7) To develop a theoretical model of economic voice production by computing an output-to-cost ratio for a large number of glottal source and vocal tract filter conditions.

To explore two types of economy-based voice therapy, one based on the use of semi-occluded vocal tracts for a high degree of source-system interaction, and one based on adductory training to produce a higher glottal efficiency.

The specific aims are built upon the hypothesis that there is a limited dose of vibration that laryngeal tissues can withstand. This dose is speaker-specific and may have a genetic, tissue environmental, and behavioral component. When high vocal demand is placed on an individual, safe doses can be exceeded and recovery times are often inadequate. The vibration dose can be lowered by: 1) electronic amplification, 2) increasing recovery periods (vocal rest), or 3) acquiring more economic voice production (through therapy).

Figure 1 shows a flow chart of the studies to be carried out. There will be multiple levels of analysis. Computer simulation of voice biomechanics, which is one of the well-established tools available to this research team, will be used to first determine the ideal tissue properties of the lamina propria (from a self-oscillating point of view). This ideal state will include the layer thickness, viscous properties, and elastic properties as target inputs for *in vitro* modeling. The vibration loads (dosimetry) of a sizeable group of teachers are already available from the previous granting period as a second guiding input in generating an appropriate *in vitro* model.

The bulk of the laboratory work will entail tissue engineering and cell culture optimization combined with rheology and molecular analyses. Rheology will be used to determine if the engineered tissues match the ideal tissue properties, gene expression analysis will be used to determine which candidate genes are differentially expressed under vibrational stress, and western immunoblot combined with gelatinase/elastase zymography will be used to determine the effects of ECM-related proteins that alter viscoelasticity under vibrational stress.

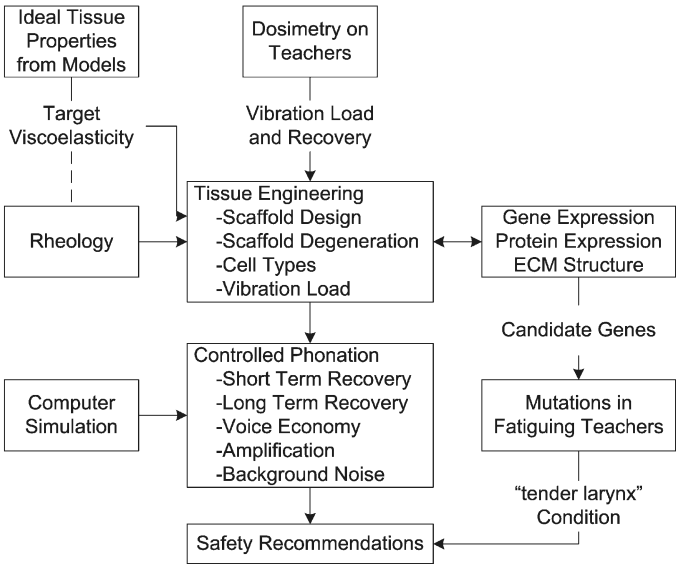

Figure 1. Flow chart of studies.

Vibration responsive genes that will be identified from genome-wide expression profiling experiments proposed in this grant, can be used in future studies to screen for genetic polymorphisms in teachers who are known to fatigue vocally. This may establish a genetic predisposition to a tender larynx condition for which special safety recommendations can be prescribed in terms of vibration exposure. The complete list of recommendations and self-pacing strategies for vocalization will include: voice economy training, means for dealing with competing noises, and amplification. Computer simulation will once again be used to explore the therapy techniques for economic voice use.

Complementing the specific aims of the previous grant period, which focused on the establishment of vocal dose and auto-perception of vocal fatigue, the present specific aims focus on the molecular underpinnings for tissue weakening under vibrational stress. Given that there is almost no literature available on the micromechanical effects of tissue exposed to phonation frequencies (100-1000 Hz), a large percentage of the effort will involve the methodological development of *in vitro* studies of vocal fold tissues.

Progress made to the above Specific Aims

(1) To determine ideal geometric and viscoelastic properties of the lamina propria for normal and exceptional ranges of pitch and intensity.

Studies suggest that occupational voice users have a greater incidence of vocal issues than the general population. Women have been found to experience vocal health problems more frequently than men, regardless of their occupation. Traditionally, it has been assumed that differences in the laryngeal system are the cause of this disproportion. We hypothesized that other potential gender distinctions which may make women more vulnerable to voice disorders. A search of the literature was conducted for gender-specific characteristics that might impact the vocal health of women. This search can be used by health care

practitioners to help female patients avoid serious vocal health injuries, as well as to treat better those women who already suffer from such vocal health issues. Specifically discussed were intrinsic physiological differences which may be included in our simulation models. These results were published in a paper entitled, "**Gender differences affecting vocal health of women in vocally demanding careers**" (Hunter et al., 2011).

A finite-element model of the viscoelastic properties and the geometric shape of the vocal folds were used to determine the optimal structure for vocal intensity, vocal efficiency, phonation threshold pressure, and periodicity of self-sustained oscillation. Results showed that tissue viscosity, and geometric shape of the medial surface of the vocal folds (upper and lower adduction and medial surface bulging) are critical for phonation threshold pressure. The ratio of longitudinal to transverse shear modulus of the lamina propria tissue (a measure of anisotropy) is critical for periodicity in vibration. A paper entitled "**Simulation of Developmental Changes in Vocal Fold Morphology**" by Titze and Palaparthi is in preparation for the Journal of the Acoustical Society of America

(2) To quantify voice recovery times (short term, intermediate term, and long term) and relate them to auto-perceptive ratings of inability to produce soft voice, for which vibration is confined to the lamina propria, which means "layers in motion."

Twenty voice dosimeters have been designed and tested using a rigid engineering approach. Major issues were the dynamic range, frequency response, storage capacity, continuous operation on battery power (at least 14 hours), accelerometer attachment to skin, flexibility of cables under clothing, and security of stored data from accidental disconnects or incorrect operation. By utilizing the features of a Pocket PC, the design task was greatly simplified with respect to building a durable and changeable custom device. These features include a powerful processor, a large amount of storage capacity, an operating system based on the familiar Microsoft® Windows, an existing file-transfer protocol for moving data to a laptop or desktop computer, a touch-screen user interface, built-in digital sound capture hardware, and a Microsoft®-supported software developer's toolkit for writing application programs. The latter feature allowed for inexpensive and rapid means of altering the dosimeter software as needed during the development and test phases. A paper entitled "**Adaptation of a pocket PC for use as a wearable voice dosimeter**" by Popolo, Švec, and Titze was printed in review in the *Journal of Speech, Language and Hearing Research* (Popolo et al., 2005).

To measure the exposure to self-induced tissue vibration in speech, three vocal doses were defined and described: *distance dose*, which accumulates the distance that vocal fold tissue particles travel in an oscillatory trajectory; *energy dissipation dose*, which accumulates the total amount of heat dissipated over a unit volume of vocal fold tissue; and *time dose*, which accumulates the total phonation time. These doses were compared to a previously used vocal dose measure, the *vocal loading index (Rantala and Vilkman, 1999, J of Voice)*, which accumulates the number of vibration cycles of the vocal folds. The vibration doses were compared to the industrial limits for hand-transmitted vibration (Griffin, 1990, Handbook of human vibration), for which a safe distance dose would be about 500 m. (This would be the total safe distance vocal fold tissue particles could travel in an oscillatory trajectory if hands and vocal folds could withstand equal exposure). A corresponding maximum time dose would be about 17 min of continuous vocalization, or about 35 min of continuous reading with normal breathing and unvoiced segments. The voicing pauses in normal speech and dialogue effectively prolong the safe time dose, however. Safety limits for vocalization will therefore require refinement based on a more detailed knowledge of the differences between hand and vocal fold tissue morphology, response to vibrational stress, and the effect of recovery of the vocal fold tissue during voicing pauses. Detailed results have been published in "**Vocal dose measures: quantifying accumulated vibration exposure in vocal fold tissues**" (Titze et al., 2003) and in "**Measurement of vocal dose in speech: experimental procedure and signal processing**" (Svec et al., 2003).

In a study of vocal dose and recovery, we collected objective vibration dose data on singers and relate them to subjective measurements of vocal fatigue. Seven subjects completed a 2-week study period. The National Center for Voice and Speech dosimeter recorded vocal load, soft phonation tasks, and subjective soft voice ratings. Three vocal doses (time, distance, and cycle) were measured in classical singers' larynges during an intensive practice period. Spikes in vocal load are reflected as harsher subjective ratings on the same day as well as 24-72 hours later. When at least 48 hours of vocal rest occurred before a vocal load, improved subjective evaluations were seen after the load. A paper entitled, "**Objective measurement of vocal fatigue in classical singers: a vocal dosimetry pilot study**" was published (Carroll et al., 2006).

Gathering large amounts of data on teachers in the workplace poses a number of administrative and logistical challenges. We have responded to these challenges by gathering baseline data on teachers during the summer break when they are not teaching; by working in collaboration with teachers unions, school district research boards, school principals and a comprehensive research integrity board to recruit a gender and ethnically representative population of teachers; and by insuring that all necessary human subject research protections are followed. Many large urban school districts in the U.S. (and specifically in the Denver area) have multiple layers of research approval bureaucracy. It was necessary to coordinate with state, district and local school officials as well as labor unions. Long delays in data gathering and extra expenses were encountered. An article entitled "**Protocol challenges for on-the-job dosimetry of teachers**" was published (Nix et al., 2007).

A report was created which discussed voice accumulation and dosimetry devices which are used for unobtrusive monitoring of voice use. While numerous studies have used these devices to examine how individuals use their voices, little attention has been paid to how subjects respond to them. Therefore, the purpose of this short communication is to begin to explore two questions: 1) How do voice monitoring devices affect daily communication? and 2) How do participants feel about the physical design and function of these types of voice monitoring devices? One key finding is that most of the subjects remain aware of the dosimeter while wearing it, which may impact the data collected. Further, most subjects have difficulty with the accelerometer and/or the data storage device. A paper entitled, "**Teacher response to ambulatory monitoring of voice**", was published (Hunter, 2012).

A study was conducted to determine how accurately sound pressure levels (SPLs) of speech can be estimated from skin acceleration levels (SALs) on the neck. Measurements using an accelerometer were carried out on 27 subjects (10 males and 17 females) who read the Rainbow and Marvin Williams passages in soft, comfortable and loud voice while SALs and SPLs were simultaneously registered and analyzed every 30 ms. The results indicate that the long-term mean SPL of speech can be estimated from SAL with accuracy better than $\pm$2.8 dB in 95% of the cases when the subjects are individually calibrated. This makes the accelerometer a useful sensor for SPL measurement of speech when microphones are problematic to use (e.g. in noisy environments or under clothing). A publication entitled "**Estimation of Sound Pressure Levels of Speech from Skin Vibration of the Neck**," was published (Svec et al., 2005).

In a study to compare how normal vocalist would produce a range of sounds differently than trained vocalists, Frequency and intensity ranges (in true decibel sound pressure level, 20 microPa at 1 m) of voice production in trained and untrained vocalists were compared with the perceived dynamic range (phons) and units of loudness (sones) of the ear. Results were reported in terms of standard voice range profiles (VRPs), perceived VRPs (as predicted by accepted measures of auditory sensitivities), and a new metric labeled as an overall perceptual level construct. Trained classical singers made use of the most sensitive part of the hearing range (around 3-4 kHz) through the use of the singer's formant. When mapped onto the contours of equal loudness (depicting nonuniform spectral and dynamic sensitivities of the auditory system), the formant is perceived at an even higher sound level, as measured in phons, than a flat or A-weighted spectrum would indicate. The contributions of effects like the singer's formant and the sensitivities of the auditory system helped the trained singers produce 20% to 40% more units of loudness, as measured in sones, than the untrained singers. Trained male vocalists had a maximum overall perceptual level construct that was 40% higher than the untrained male vocalists. Although the A-weighted spectrum (commonly used in VRP measurement) is a reasonable first-order approximation of auditory sensitivities, it misrepresents the most salient part of the sensitivities (where the singer's formant is found) by nearly 10 dB. These results were published in a paper entitled, "**Comparison of the produced and perceived voice range profiles in untrained and trained classical singers**" (Hunter et al., 2006).

Our data bank on voice dosimetry was used to study the distributions of continuous voicing periods and silence periods in 31 teachers over the duration of two weeks. Recordings were made during all awake hours of the day. Voicing periods were grouped into half-decades, ranging from 0.0316 – 0.10 s for the shortest periods of phonation to 31.6 – 100 s for the longest periods of phonation. Silence periods were grouped into similar half-decades, but ranged up to periods of several hours. On average, the teachers had 1,800 occurrences of voicing (onset followed by offset) per hour at work and 1,200 occurrences per hour while not at work. Voicing occurred 23% of the total time at work, dropping to 13% during off-work hours and 12% on weekends. The greatest accumulation of voicing occurred in the 0.316 – 1.0 s voicing periods, whereas the greatest accumulation of silence occurred in the 3 – 10 s silence periods. The study begins to lay the

groundwork for understanding vocal fatigue in terms of repetitive motion and collision of tissue, as well as recovery from such mechanical stress. A paper entitled "**Voicing and Silence Periods In Daily and Weekly Vocalizations Of Teachers**" was published (Titze et al., 2007).

A study was done to illustrate a more concise picture of the vocal demands placed on teachers by comparing occupational voice use with non-occupational voice use. The National Center for Voice and Speech voice dosimetry databank was used to calculate phonation time dose, as well as average dB SPL and F0. Occupational voice use (9am-3pm) and non-occupational voice use (4pm-10pm, weekends) were compared from 57 subjects (two weeks each, 8400+ hours). Five key findings were uncovered: [1] previous findings of occupational (~30%) and non-occupational (14%) voicing were substantiated; [2] teachers experienced a wide range of occupational voicing percentages (33%, SD ±11%); [3] the occupational voice was on average only about 1 dB SPL louder than the non-occupational voice and remained constant throughout the day; [4] the occupational voice exhibited an increased pitch, trending upward throughout the day; [5] apparent gender differences in voicing percentages, as well as changes in dB SPL and F0, were shown. Data regarding voicing percentages, F0 and dB SPL provide critical insight into teachers' vocal health. Further, because non-occupational voice use is added to an already vocally overloaded voice, it may add key insights into recovery patterns, and should be the focus of future studies. An article entitled, "**Variations in intensity, fundamental frequency, and voicing for teachers in occupational versus non-occupational settings**" was published in the Journal of Speech Language and Hearing Research (Hunter and Titze, 2010).

In another study, we sought to determine whether full-cycle glottal width measurements could be obtained with a quantitative laryngeal imaging system using videostroboscopy, and whether glottal width and vocal fold length measurements were repeatable and reliable. Synthetic vocal folds were phonated on a laboratory bench, and dynamic images were obtained in repeated trials by use of videostroboscopy and videokymography (VKG) with an imaging system equipped with a 2-point laser projection device for measuring absolute dimensions. Video images were also obtained with an industrial videoscope system with a built-in laser measurement capability. Maximum glottal width and vocal fold length were compared among these 3 methods. The average variation in maximum glottal width measurements between stroboscopic data and VKG data was 3.10%. The average variations in width measurements between the clinical system and the industrial system were 1.93% (stroboscopy) and 3.49% (VKG). The variations in vocal fold length were similarly small. The standard deviations across trials were 0.29 mm for width and 0.48 mm for length (stroboscopy), 0.18 mm for width (VKG), and 0.25 mm for width and 0.84 mm for length (industrial). For stable, periodic vibration, the full extent of the glottal width can be reliably measured with the quantitative videostroboscopy system. An article entitled, "**Qualification of a Quantitative Laryngeal Imaging System Using Videostroboscopy and Videokymography**" was published (Popolo and Titze, 2008).

The NCVS dosimeter's role was expanded to a successful use in a pilot study of a child's voice. A healthy male child (5 years, 7 months) was evaluated. The child completed four voice tasks used in a previous study of the influence of task type on F0 values: (1) sustaining the vowel /a/; (2) sustaining the vowel, /a/, embedded in a word at the end of a phrase; (3) repeating a sentence; and (4) counting from 1 to 10. The child also wore the voice dosimeter during all activities for 34 hours over 4 days. Throughout a set of structured vocal tasks within the clinical environment, the child's F_0, as measured by both the dosimeter and acoustic analysis of microphone data, was similar for all four tasks, with the counting task the most dissimilar. The mean F0 (~257 Hz) matched very closely to the average task results in the literature given for the child's age group. However, the child's mean fundamental frequency during the unstructured activities was significantly higher (~376 Hz). Finally, the mode and median of the structured vocal tasks were respectively 260 Hz and 259 Hz (both near the mean), while the unstructured mode and median were respectively 290 Hz and 355 Hz. The results of this study suggest that children may produce a notably different voice pattern during clinical observations compared to routine daily activities. In addition, the child's long-term F_0 distribution is not normal. If this distribution is consistent in long-term, unstructured natural vocalization patterns of children, statistical mean would not be a valid measure. Mode and median are suggested as two parameters which convey more accurate information about typical F0 usage. An article entitled, "**A comparison of a child's fundamental frequencies in structured elicited vocalizations versus unstructured natural vocalizations: A case study**" was published (Hunter, 2009). A follow up study tracked how the child changed his voice use in various communication environments. When the child was primarily with adults, his F_0 patterns were most similar to the range found in literature (mode,

258.4 Hz; median, 312.2 Hz; mean, 334.4 Hz). In the three other environments, distinct F_0 patterns were found. At a birthday party with peers, the child's F_0 patterns were much higher (mode, 366.1 Hz; median, 409.1 Hz; mean, 423.4 Hz). Falling between these two were the F_0 values for time in an organized classroom setting (mode, 279.9 Hz; median, 376.8 Hz; mean, 396.3 Hz) and time at home with his parents and younger siblings (mode, 290.7 Hz; median, 355.3 Hz; mean, 378.7 Hz). This study suggests that children produce significantly different acoustic patterns dependent on type of activity and conversational partners, with children in a more controlled communication environment not using as wide of range as they would otherwise. An article entitled, "**Impact of four nonclinical speaking environments on a child's fundamental frequency and voice level: a preliminary case study**," was published (Hunter et al., 2012).

Vocal fatigue was quantified using the *Inability to Produce Soft Voice* (IPSV) in 86 adult participants during a two hour reading task. Subjects also rated their IPSV after the 2 hour reading and over the next two days. Short-term recovery was apparent with 90% recovery within 4-6 hours and full recovery at 12-18 hours. Recovery was shown as a vocal recovery trajectory (exponential time constant of 3.7 hours), similar to a wound healing trajectory. The vocal recovery trajectory was shown to be similar to chronic wound healing trajectory rather than an acute wound healing trajectory, suggesting that vocal fatigue could be thought of as a chronic injury. Thus, with daily use of the voice, there is continual damage and the healing mechanism is in a state of constant repair. In addition, there is likely a vocal fatigue threshold which the level of tissue damage would shift the healing trajectory from chronic to acute. An article entitled, "**Quantifying Vocal Fatigue Recovery: Dynamic vocal recovery trajectories after a vocal loading task**", was published (Hunter and Titze, 2009).

In conclusion of this aim, a final paper is being prepared based on just concluding analysis. The vocal non-pathologic recovery trajectories of vocal fatigue like symptoms found previously seem to have a short-term and long-term component. Using these components, perceived vocal fatigue was predicted using the NCVS vocal dose database as an input (~8400 hrs of observational data). A vibration dose exposure and recovery model which has been developed (recovery being based on two aspects of dermal wound healing). This paper presents how such a model of perceived vocal fatigue and recovery can give insights on optimal vocal vibration exposure and vocal rest periods. An article entitled, "**Using Inability to produce soft voice to quantify vocal fold tissue fatigue and recovery**" is in preparation.

(3) To culture distinct cell types at various states of differentiation (human mesenchymal stem cells, laryngeal fibroblasts, and vocal fold stellate cells) that appropriately model and regulate extracellular matrix (ECM) changes under conditions of vibration.

The maculae flavae of the human vocal folds include dense extracellular matrices and compacted cells with a stellate morphology. These vocal-fold stellate cells are thought to participate in the metabolism of extracellular matrices essential in maintaining vocal-fold viscoelasticity required for phonation. We have isolated and cultured these new cells and have tested the hypothesis that they maintain a distinct cellular and biochemical phenotype. Our results reveal that these cells are capable of responding to exogenous all-trans retinol in culture. Exposure to this synthetic co-factor causes deactivation characterized by decreased proliferation, loss of the activated stellate cell marker, alpha-smooth muscle actin, and restoration of intracellular lipid and vitamin A metabolite storage. These data establish a new and distinct cellular target for future investigations of the viscoelastic properties of the vocal-fold mucosa during normal phonation, aging, vocal-fold scarring, laryngeal fibrosis, and myofibroblastoma. A paper entitled "**Transdifferentiation of vocal-fold stellate cells and all-trans retinol-induced deactivation**" was published in Cell Tissue Research (Fuja et al., 2005).

The regulation of extracellular matrix (ECM) constituency is critical in maintaining vocal cord biomechanical viscoelasticity required for phonation. We have shown that vocal fold stellate cells (VFSC) undergo transdifferentiation that is partially reversed by exposure to all-trans retinol (ATROH). This is the first report on the expression of various ECM components, MMPs, TIMPs, pro-fibrogenic cytokines, and other ECM modulators in transdifferentiated and deactivated VFSCs. These cells maintain an ECM expression pattern similar to laryngeal cancer and scars but distinct from tracheal fibroblasts. Exposure to ATROH differentially affects the VFSC expression of ECM components, matrix-regulating enzymes, and fibrogenic factors suggesting that the inhibitory effects of this synthetic cofactor should be studied further in laryngeal fibrosis and scarring. Increased exposure to retinol induces sequential reorganization of the actin cytoskeleton in activated VFSCs. These cells are capable of regulating vocal fold ECM constituency

important throughout normal laryngeal development. VFSC activation may also be implicated in ECM misregulation which is a hallmark of several laryngeal pathologies. A paper entitled "**Changes in expression of extracellular matrix genes, fibrogenic factors, and actin cytoskeletal organization in retinol treated and untreated vocal fold stellate cells**" has been published (Fuja et al., 2006b).

A study was conducted to better understand cellular mechanisms of vocal fold macula flava stellate cells. Our other studies showed that porcine VFSCs had characteristics very different than tracheal fibroblast cells (TFs). VFSCs are vitamin A storing, have the ability to transdifferentiate in vitro, and have elevated mRNA expression of relative to TFs for the following transcripts: collagen 1a1, connective tissue growth factor (CTGF), platelet derived growth factor (PDGF), transforming growth factor b1 (TGFb1), and peroxisome proliferator-activated receptor b/g (PPAR b/g), all presented in "**Changes in expression of extracellular matrix genes, fibrogenic factors, and actin cytoskeletal organization in retinol treated and untreated vocal fold stellate cells**" has been published in Matrix Biology (Fuja et al., 2006b). These elevated markers are key constituents in wound healing and fibrosis processes. To better understand the role VFSCs may have in vocal fold lamina propria repair, we first investigated the VFSC ability to generate TGFb in control conditions, measured with an ELISA test. Then TGFb production was measured again with cells immersed in chemical environments, allowing us to better identify cellular mechanisms involved with TGFb production. Our results show VFSCs plated on fibronectin produce TGFb in as little as 24h after initial seeding. Based on a serial dilution line fit, VFSC TGFb production increased three-fold in 48 h (100 pg/ml at 24h and 300 pg/ml at 72h). TGFb production 24h after VFSC exposure to DJP or SB was significantly reduced (two-tailed student t-test with $p < 0.05$) relative to the control but returned to near-control amounts at 72 h. These two treatments evaluate involvement of known pathways along which TGFb operates, namely the SMAD3 pathway (blocked by DJP) and the p38 MAPK pathway (blocked by SB). It appears as though both pathways inhibit TGFb production in the short term but not in the long term. TGFb production is high in fibrotic tissues and is known to stimulate collagen synthesis. When blocking the SMAD3 pathway in VFSCs, collagen mRNA production was unaffected but when blocking the p38 MAPK pathway, even though TGFb production returned to near normal levels, collagen Ia1 production was significantly reduced relative to control. 15d-Deoxy $\Delta^{12\ 14}$–prostaglandin J2 (abbreviated DJP) is a naturally occurring prostaglandin and ligand to PPARg. When bound to PPARg, the SMAD3 signaling process is inhibited, thus reducing propensity for ECM production.(Fu et al., 2001) Because the inhibitor DJP did not diminish collagen 1a1 expression, this suggests a regulatory involvement of the p38 MAPK pathway and not the SMAD3 pathway and is consistent with the low PPARy expression shown in the Fuja et al, 2006 publication. Additional genes involved with cell adhesion were studied. The integrin b3 is a transmembrane protein essential for cell migration, adhesion, and proliferation. It is also a stress transducing protein, known to be sensitive to mechanics of extracellular surroundings as well as externally applied forces. INTGB3 expression was not significantly different than controls when VFSCs were treated with ATROH (vitamin A), Dexamethasone (synthetic anti-inflammatory drug), or ATRA (derivative of vitamin A). It was, however, significantly diminished when treated with PDGFbb ($p=0.02$) and trended downward when treated with DJB or SB. A manuscript is currently in preparation summarizing these results.

Another study compared vocal fold cells originating from the midmembrane to those originating from the macula flava. In this study, no attempt was made to preserve the stellate phenotype via cell sorting as was done in the 2005-2006 studies. Rather, cells were extracted using generic isolation methods. We tested the hypothesis that the fibroblast cells from the middle portion and the differentiated stellate cells from maculae flavae portion are two different populations of cells. The fibroblasts from middle portion of lamina propria are in a more proliferative state than those from the maculae flavae portion. We hypothesized that the maculae flava cells will be activated under certain conditions to replenish those exhausted fibroblasts in the middle portion of the vocal fold. Our results showed that the midmembranous fibroblast cells (MID) double more rapidly and macula flava fibroblasts (MF), suggesting that the MID cells age faster. The 2-day doubling time of MF cells was two to three times slower than for the MID cells. The result was confirmed with flow cytometry data showing a lag in MF cells going into the S-phase of cell proliferation. We further tested the fibroblasts molecular markers CD44, a-SMA, Vimentin, Desmin, and GFAP, markers thought to uniquely identify stellate from fibroblast cells. Results showed the presence of these markers was nearly the same between the two populations. For both MID and MF cells, desmin presence was nearly zero and vimentin was present but in limited quantities. GFAP and a-SMA presence were very prominent in both cells types as was CD-44. A final

qualitative marker, that of CD36, claimed to be present in hepatic stellate cells but not neighboring fibroblast cells, was also determined using immunohistochemistry. Surprisingly, CD36 was clearly present in the MID cells but minimally present in the MF cultures. These results support the hypothesis that young stellate cells from the maculae flavae might be the source to replenish the old exhausted fibroblasts in the middle portion, but that they are likely of similar phenotype to cells in the midmembranous vocal fold. A manuscript is currently in preparation and entitled, "**Comparison of cells originating from the midmembranous vocal fold mucosa and macula flava**."

We have now cultured four different cell lines, (1) laryngeal fibroblasts from primary tissue cultures, (2) stellate cells and midmembranous fibroblast cells harvested from pig primary cultures, (3) tracheal fibroblast cells obtained from The University of Utah, and (4) two immortal fibroblast cell lines, 21 y.o. male and 59 y.o. female, obtained from Dr. Susan Thibeault at the University of Wisconsin. Laryngeal fibroblasts were harvested from human larynx within 48 hours postmortem (69 y.o. male). The anterior and posterior macula flava and the lamina propria were resected, minced and grown on a fibronectin coated T-flask. After 1-2 weeks, cells were digested, centrifuged and replated. After expanding the cells, they were preserved in liquid nitrogen. Cell growth rates were monitored using dye exclusion and hemocytometry after being brought out of frozen storage were then tracked for 13 passages. Growth rates were high for the first 3 passages, after which changes from passage to passage were near zero through passage 11. Beyond passage 11 growth rate declined rapidly. This cell line has been used for monolayer studies using passages 3 – 5 in order to assure minimal aging or genetic change. Ongoing studies will make use of the immortalized cell lines and the tracheal fibroblast cells, all of which have already undergone genetic and karyotype analysis. The immortalized cells have been expanded and divided into multiple aliquots for future studies.

(4) To determine the relative merits of co-culture of the above cell types for an in vitro monolayer system that appropriately models and regulates ECM changes under conditions of vibration.

We have developed the experimental set-up for exposing the monolayers to shear stresses and inertial stresses. In one study, vocal fold fibroblast cells were isolated from human larynges and cover slips were seeded with the cells for approximately 18-22 hours. Vibration was exerted in the bioreactor by immersing the cover slip in DMEM with 2% methyl cellulose. H & E were used to stain the cells after exposure to mechanical stress. Phase contrast observations of cell morphology were made using a Light Microscope. In a recent study we examined cell adhesion to a surface under vibrational forces approximating those of phonation. Methods: A monolayer of human fibroblast cells was seeded on a fibronectin-coated glass coverslip, which was attached to either the rotating part or the stationary part of a rheometer-bioreactor. The temperature, humidity, carbon dioxide level, nutrients, and cell seeding density were controlled. The cell density was on the order of 1,000 to 5,000 cells per square millimeter. Target stresses above 1 kPa at an oscillatory frequency of 100 Hz were chosen to reflect conditions of vocal fold tissue vibration. Results: Fibronectin coating provided enough adhesion to support at least 2 kPa of oscillating stress, but only about 0.1 kPa of steady rotational shear. For stresses exceeding those limits, the cells were not able to adhere to the thin film of fibronectin. Conclusions: Cells will adhere to a planar surface under stresses typical of phonation, which provide a more stringent test than adherence in a 3-dimensional matrix. The density of cell seeding on the coverslip played a role in cell-extracellular matrix adhesion, in that the cells adhered to each other more than to the fibronectin coating when the cells were nearly confluent. An article entitled, **"Adhesion of a monolayer of fibroblast cells to fibronectin under sonic vibrations in a bioreactor," was** published in Annals of Otology, Rhinology, and Laryngology (Titze et al., 2012).

Investigations were made for developing a biomaterial is that its viscoelastic properties correspond to native tissues. This criterion is particularly important for vocal fold tissues. Collagen-based injectables are routinely used to repair paralyzed or scarred vocal folds and restore vocalization, yet reported viscoelastic properties are inconsistent. The present study evaluated the effects of pre-shearing samples on their viscoelastic measurements. Non-crosslinked bovine collagen and micronized dermal tissue were presheared by extruding directly through a mechanically operated syringe with different needle sizes. Pre-shear rates ranged from 31 to 5600 /sec for orifice radii of 1.21 to 0.0955 mm, respectively. Sandpaper-coated, parallel plate attachments on a stress-controlled rheometer were used to measure elastic moduli and dynamic viscosities from 0.01 to 10 Hz. A 3-10 fold reduction in elastic modulus and a 2-4 fold reduction in dynamic viscosity occurred for pre-shear rates exceeding 1000 /sec compared to rates of 31 and 131 /sec. These

results suggest a change in the molecular structure and the viscoelastic compatibility of vocal fold injectables when sufficiently pre-sheared. The results from the two previous studies have been presented at a conference.

(5) To engineer a 3D in vitro model of the lamina propria in a bioreactor, using data collected from aims 3 and 4, that allows for specific vibration loads, measurement of viscoelastic properties, and observation of changes in ECM modeling.

Effects of vibration on human vocal fold extracellular matrix composition and the resultant tissue viscoelastic properties are difficult to study *in vivo*. An *in vitro* bioreactor, simulating the *in vivo* physiological environment, was explored. A stress-controlled rheometer was used to administer shear vibrations to living tissues at stresses and frequencies corresponding to male phonation, while simultaneously measuring tissue viscoelastic properties. Tissue environment was evaluated and adjustments made in order to sustain cell life for short term experimentation up to 6 hours. Cell culture medium evaporation, osmolality, pH, and cell viability of cells grown in three dimensional synthetic scaffolds were quantified under comparably challenging environments to the rheometer bioreactor for 4 or 6 hours. The functionality of the rheometer-bioreactor was demonstrated by applying three vibration regimes and quantifying strain to cell-seeded three dimensional substrates for 2 hours. Rheologic data and cell viability are reported for each condition, and future improvements are discussed. A manuscript entitled , "**Cell viability and viscoelastic measurements in a rheometer used to stress and engineer vocal fold tissues"**, was published (Klemuk et al., 2008).

Porous 2,3-dialdehydecellulose (2,3-DAC) membranes were investigated for use as a synthetic scaffold for engineering vocal fold-like tissues. Two criteria of this application are (i) the viscoelastic shear properties of the scaffold should be controllable in the range of vocal fold tissues and (ii) scaffolds should remain biomechanically stable to withstand vibrational stresses in a bioreactor. Porous 2,3-DAC membranes were fabricated from methylolcellulose by water-induced cellulose regeneration, with or without sodium chloride leaching, followed by periodate oxidation. Rheological studies were performed to investigate the effect of freeze-drying, porosity, degree of oxidation, sterilization, and incubation time on elastic and viscous shear moduli, G' and G'', respectively, for frequencies 0.01-10 Hz. Freeze drying increased G' and G'', while increased porosity and degree of oxidation reduced G' and G''. Sterilization had no effect on viscoelasticity. When incubated in Dulbecco's minimum essential medium at 37°C, membranes with 6-7% and 19-20% oxidation disintegrated after 7 and 3 days, respectively, while membranes with 3-4% oxidation showed little viscoelastic change over a period of 42 days. The upper frequency limit of rheologic measurement was a limitation of the study and should be addressed in future investigations. Published was, "**Effects of fabrication parameters on viscoelastic shear modulus of 2,3-dialdehydecellulose membranes-Potential scaffolds for vocal fold lamina propria tissue engineering"** (Roychowdhury et al., 2009).

In many of our studies, viscoelastic measurements made with a stress-controlled rheometer are affected by system inertia. Of all contributors to system inertia, motor inertia is the largest. Its value is usually determined empirically and precision is rarely if ever specified. Inertia uncertainty has negligible effects on rheologic measurements below the coupled motor/plate/sample resonant frequency. But above the resonant frequency, G' values of soft viscoelastic materials such as dispersions, gels, biomaterials, and non-Newtonian polymers, err quadratically due to inertia uncertainty. In the present investigation, valid rheologic measurements were achieved near and above the coupled resonant frequency for a non-Newtonian reference material. At these elevated frequencies, accuracy in motor inertia is critical. Here we compare two methods for determining motor-inertia accurately. For the first (commercially-used) phase method, frequency responses of standard fluids were measured. Phase between G' and G'' was analyzed at 5-70 Hz for motor inertia values of 50-150% of the manufacturer's nominal value. For a newly devised two-plate method (10 mm and 60 mm parallel plates), dynamic measurements of a non-Newtonian standard were collected. Using a linear equation of motion with inertia, viscosity, and elasticity coefficients, G' expressions for both plates were equated and motor inertia was determined to be accurate (by comparison to the phase method) with a precision of ± 3%. The newly developed two-plate method had advantages of expressly eliminating dependence on gap, was explicitly derived from basic principles, quantified the error, and required fewer experiments than the commercially used phase method. An article entitled, "**Determination of motor inertia of a stress-controlled rheometer",** was published in Journal of Rheology (Klemuk and Titze, 2009).

A review paper was written describing collaborative studies at the University of Iowa and the National Center for Voice and Speech aim to help the voices of teachers. Investigators study how cells and tissues

respond to vibration doses simulating typical vocalization patterns of teachers. A commercially manufactured instrument is uniquely modified to support cell and tissue growth, to subject tissues to vocalization-like forces, and to measure viscoelastic properties of tissues. Through this basic science approach, steps toward safety limits for vocalization and habilitating rest periods for professional voice users will be achieved. An article entitled, "**Rheometers, bioreactors, and vocalization forces: using basic science investigations to help the voices of teachers**", was published in the American Speech-Language Hearing Association Perspectives publication (Klemuk, 2008).

The study of biomaterials suitable for vocal fold implantation includes rigorous viscoelastic testing. The purpose of this study was to measure and compare biomechanical properties of commonly used vocal fold injectates Cymetra, Radiesse, Restylane, Hylaform, and one investigational injectate, Carbylan-GSX 5%, to determine suitability for mucosal injection. Oscillatory shear stress was applied to five samples of each injectate using a parallel plate controlled stress rheometer. Shear stress, shear strain, and strain rate associated with the oscillatory shear deformation were computed from the prescribed torque and measured angular velocity; viscoelastic data were obtained on the basis of these functions. Values calculated included elastic shear moduli, viscous moduli, and dynamic viscosity as a function of oscillatory frequency (0.01–150 Hz). *Results:* Elastic moduli for all samples increased as the frequency increased. Hyaluronan based materials were all comparable with each other and at least an order of magnitude lower than the stiffer and more viscous Cymetra and Radiesse. Carbylan-GSX 5% was found to have almost identical values to Hylaform with the exception of its mean viscosity, which was noticeably lower. *Conclusions:* Hyaluronan based biomaterials offer less resistance to flow and stiffness and may be better suited for injections into the mucosa, whereas Cymetra and Radiesse appear to be appropriate for injections into muscle. Viscoelastic properties of Hylaform and Carbylan-GSX 5% were found to most resemble that of the human vocal fold mucosa. An article entitled, **"Viscoelasticity of hyaluronan and nonhyaluronan based vocal fold injectables: implications for mucosal versus muscle use,"** was published in The Laryngoscope (Caton et al., 2007).

The next advance in viscoelastic characterization of vocal fold biomaterials is data acquisition at physiologic frequencies is paramount. Yet mechanical limitations have presented engineering challenges for decades. The Piezo-Rotary Vibrator is an attachment to the Malvern Gemini rotational rheometer, a device that has been beta-tested at the University of Iowa in partnership with Malvern instruments. It is capable of measuring viscoelastic properties of materials up to 2000 Hz. Viscoelastic properties of numerous vocal fold injectables have been reported but not at speaking frequencies. For materials intended for Reinke's space, ramifications of property values are of great concern because of their impact on ease of voice onset. Our objectives were: 1) to measure viscoelastic properties of a new nonresorbing carbomer and well-known vocal fold injectables at vocalization frequencies using established and new instrumentation, and 2) to predict phonation threshold pressures using a computer model with intended placement in Reinke's space. Study Design: Rheology and phonation threshold pressure calculations. Methods: Injectables were evaluated with a traditional rotational rheometer and a new piezo-rotary vibrator. Using these data at vocalization frequencies, phonation threshold pressures (PTP) were calculated for each biomaterial, assuming a low dimensional model with supraglottic coupling and adjusted vocal fold length and thickness at each frequency. Results were normalized to a nominal PTP value. Results: Viscoelastic data were acquired at vocalization frequencies as high as 363 to 1,400 Hz for six new carbomer hydrogels, Hylan B, and Extracel intended for vocal fold Reinke's space injection and for Cymetra (lateral injection). Reliability was confirmed with good data overlap when measuring with either rheometer. PTP predictions ranged from 0.001 to 16 times the nominal PTP value of 0.283 kPa. Conclusions: Accurate viscoelastic measurements of vocal fold injectables are now possible at physiologic frequencies. Hylan B, Extracel, and the new carbomer hydrogels should generate easy vocal onset and sustainable vocalization based on their rheologic properties if injected into Reinke's space. Applications may vary depending on desired longevity of implant. An article entitled, **"Phonation Threshold Pressure Predictions Using Viscoelastic Properties Up to 1,400 Hz of Injectables Intended for Reinke's Space",** was published in The Laryngoscope (Klemuk et al., 2010).

(6) To identify and functionally characterize candidate vibration-responsive genes and genetic pathways that regulate ECM modeling under various vibration loads over time.

Our monolayer model is ideal for identifying cellular mechanisms sensitive to vibration over short time periods. Prior to vibration exposure, it was essential to determine baseline adhesion knowledge pertaining to vocal fold fibroblast cells. To that end a study investigating adhesion of vocal fold stellate cells to various

extracellular matrix proteins. The human vocal folds are a complex layering of cells and extracellular matrix. Vocal fold extracellular matrix uniquely contributes to the biomechanical viscoelasticity required for human phonation. We investigated the adhesion of vocal fold stellate cells, a novel cell type first cultured by our laboratory, and fibroblasts to eight vocal fold extracellular matrix components: elastin, decorin, fibronectin, hyaluronic acid,
laminin and collagen types I, III and IV. Our data demonstrate that these cells adhere differentially to said substrates at 5 to 120 min. Cells were treated with hyaluronidase and Y-27632, a p160ROCK-specific inhibitor, to test the role of pericellular hyaluronan and Rho-ROCK activation in early and mature adhesion. Reduced adhesion resulted; greater inhibition of fibroblast adhesion was observed. We modulated the fibronectin affinity exhibited by both cell types using Nimesulide, an inhibitor of fibronectin integrin receptors α5β1 and αvβ3. Our results are important in understanding vocal fold pathologies, wound healing, scarring, and in developing an accurate organotypic model of the vocal folds. This research study entitled**, "Differential cell adhesion to vocal fold extracellular matrix constituents"**, was published in Matrix Biology (Fuja et al., 2006a).

Delivery of physiologic vibration to multiple samples was worked out. A multi-well disc is a new appliance for the torsional rheometer bioreactor (TRB) system accommodating up to twenty samples in partially fluid-filled wells for three different acceleration conditions in an aseptic environment. To mimic in vivo vibration of vocal fold cells, we studied the controllability and range of vibration parameters g-force, duration, and shear stress in a new bioreactor attachment. The custom multiwell disc appliance fits to a commercially built rheometer, together termed a torsional rheometer bioreactor (TRB). Previous attachments to the TRB were capable of 50-100 Hz vibrations at relatively high strains but were limited to single-sample experiments. The multiwell disc-TRB system accommodates twenty samples in partially fluid-filled wells in an aseptic environment delivering three different acceleration conditions to different samples simultaneously. Frequency and amplitude used to calculate g-force along with duration and shear stress were controllable and quantifiable using a combination of built-in rheometer sensors, manufacturer software, and smooth particle hydrodynamics (SPH) simulations. Computed shear stresses at the well bottom computed with SPH in two and three dimensions were verified with analytical approximations. Results demonstrate capabilities of the multiwell disc-TRB system that, when combined with computational modeling, provide quantifiable vibration parameters covering a 100-fold range. As a high throughput system, it is well-suited for studying cell function underlying vocal fold lamina propria homeostasis, inflammation, and wound healing under differential vibration conditions. The information has been presented at conferences and is in preparation for peer review submission, entitled, **"A multiwell disc appliance used to deliver quantifiable g-forces and shear stresses at sonic frequencies"** (Klemuk et al, in preparation).

(7) To develop a theoretical model of economic voice production by computing an output-to-cost ratio for a large number of glottal source and vocal tract filter conditions.

The Voice Range Profile (VRP) was obtained from each of eight professional actors with sustained tones and compared to Speech Range Profiles (SRPs). One speech profile was obtained during the dramatic reading of a scene in the laboratory and the other during a performance on stage in a professional theatre at the Denver Center for the Performing Arts. One question was whether the pitch and loudness ranges used by the actors in speech were greater than those for sustained tones. A second was whether the actors stayed within the center of the VRP, or whether they tended to drift toward the boundaries of intensity and frequency. A third question was whether the performance within the laboratory accurately reflects that of a stage performance. The results showed that some subjects tend to exceed the boundaries of the VRP during the acting. It is hypothesized that some of these actors, like teachers, may stress their vocal mechanism during performance and may become candidates for vocal injury. An article entitled "**Vocal fundamental frequency and intensity range in actors: a studio versus stage comparison**" was published in the *Journal of Voice* (Emerich et al., 2005).

Maximum flow declination rate (MFDR) in the glottis is known to correlate strongly with vocal intensity in voicing. This declination is in part attributable to the maximum area declination rate (MADR) and in part to the overall inertia of the air column of the vocal tract (lungs to lips). The purpose of this theoretical study was to show the possible contributions of air inertance and MADR to MFDR. A computational model of vocal fold movement was used to compute a glottal area function. We showed that MADR depends almost entirely on the ratio of vibrational amplitudes of the lower to upper margins of the vocal fold tissue.

Adduction, vertical phase difference, and prephonatory convergence of the glottis have a lesser effect on MADR. A rule was developed that relates MFDR to a vibrational amplitude ratio and vocal tract inertance. We concluded that voice users have multiple options for control of intensity, some of which involve more source-filter interaction than others. The results were published entitled "**Theoretical analysis of maximum flow declination rate versus maximum area declination rate in phonation**" (Titze, 2006a).

Resonant voice has been identified by vocologists as easy, adequately loud, and vibrant in the facial tissues. It seems to be describable in terms of a heightened economy of acoustic output at the mouth in relation to respiratory or muscular effort expended at the source (the lung-larynx combination). An interactive source-filter simulation was used to test the dependence of vocal fold vibration on the vocal tract. The study utilized a biomechanical model of the vocal folds and a wave reflection model of the vocal tract based on MRI images. The degree of interaction was governed by the diameter of the Epi-Larynx, a narrow tube above the vocal folds which raises the entire vocal tract impedance to match the impedance of the glottis. The key component of the impedance was inertive reactance. Whenever there was inertive reactance, the vocal tract assisted the vocal fold in vibration. The amplitude of vibration and the glottal flow were more than doubled and the oral radiated power increased up to 10 dB. As F_0 approached F_1, the first formant frequency, the interactive source-filter system lost its advantage (because vocal tract impedance changed from inertive reactance to resistance and then to compliant reactance) and the non-interactive system produced greater vocal output. Thus, from a voice-training standpoint, there may be reasons to operate the source-filter system in either interactive or non-interactive modes, depending on what combination of fundamental frequency and vowel is required. In Aim 8, we will explore these two training approaches. An article entitled "**A theoretical study of F_0-F_1 interaction with application to resonant speaking and singing voice**" was published in the *Journal of Voice* (Titze, 2004).

A new theory of interaction between the source of sound in phonation and the vocal tract filter is developed. The degree of interaction is controlled largely by the cross sectional area of the laryngeal vestibule (epilarynx tube), which raises the inertive reactance of the vocal tract. Inertive reactance in turn enhances the driving pressures of the vocal folds and thereby increases the energy level at the source. Two levels of interaction are discussed, one that influences both glottal flow and vocal fold modes of vibration. Unlike in most musical instruments (e.g. woodwinds and brasses), a stable harmonic sources spectrum is not obtained by tuning harmonics to vocal tract resonances (formants), but rather by placing harmonics below the resonances. This allows for positive reinforcement of the harmonics by inertive reactance without the risk of instability. The traditional linear source-filter theory may be treated as a special case of this more general theory proposed here, but it is always encumbered with possible inconsistencies in the glottal flow spectrum. In addition, the linear theory does not predict the bifurcations in the dynamical behavior of vocal fold vibration due to acoustic loading by the vocal tract. A paper entitled "**Nonlinear source-filter coupling in phonation: theory**" was printed in the Journal of the Acoustical Society of America (Titze, 2008a).

In a study to more effectively understand economic voice use, experiments were conducted to determine if a semi-occluded vocal tract could be used to measure phonation threshold pressure. This is in contrast to the shutter technique, where an alternation between a fully occluded tract and an unoccluded tract is used. Five male and five female volunteers phonated through a thin straw held between the lips. Oral pressure behind the lips was measured. Mathematical predictions of phonation threshold pressures were compared to the measured ones over a range of frequencies. It was shown that, for a 2.5 mm diameter straw, phonation threshold pressures were obtainable over a two-octave range of fundamental frequency by all volunteers. In magnitude, the pressures agreed with the 0.2 – 0.5 kPa values obtained in previous investigations. Sensitivity to viscoelastic and geometric properties of the vocal folds was generally not compromised with greater oral impedance, but some differences were predicted theoretically in contrast to an open mouth configuration. Since phonation threshold pressure is always dependent on vocal tract interaction, it may be advantageous to choose an exact and fixed oral semi-occlusion for the measurement and interpret the results in light of the known acoustic load. An article entitled, "**Phonation threshold pressure measurement with a semi-occluded vocal tract"**, was published (Titze, 2009). A follow-up study was performed to see if the same advantage could be had by obtaining the metric Voice Range Profile also with the same acoustic impedance. An article entitled, "**Measurement of a voice range profile with a matched glottal acoustic impedance using a semi-occluded vocal tract**", was printed (Titze and Hunter, 2011).

Nonlinear source-filter theory is applied to explain some acoustic differences between two contrasting male singing productions at high pitches, operatic style versus jazz belt or theatre belt. Several stylized vocal tract shapes (caricatures) are discussed that form the bases of these styles. It is hypothesized that operatic singing uses vowels that are modified toward an inverted megaphone mouth shape for transitioning into the high pitch range, whereas belting uses vowels that are consistently modified toward the megaphone (trumpet-like) mouth shape. These shapes provide collective reinforcement to harmonics in the form of inertive supraglottal reactance and compliant subglottal reactance. Examples of lip openings from several well known artists are given, from which the vocal tract area functions and corresponding reactances are inferred. An article entitled, "**Modeling source-filter interaction in belting and high-pitched operatic male singing**," was printed (Titze and Worley, 2009).

In a summary report about how the vocal folds with the semi-occluded vocal tract aspects of the vocal system by describing that although the human vocal system is small, it manages to create sounds as varied and beautiful as those produced by a variety of musical instruments. All instruments have a sound source, a resonator that reinforces the basic sound and a radiator that transmits the sound to listeners. A human's sound source is the vibrating vocal folds of the larynx while the resonator is the sound-boosting airway above the larynx and the radiator is the opening at the mouth. The human voice can create such an impressive array of sounds because it relies on nonlinear effects, in which small inputs yield surprisingly large outputs. An article entitled, "**The human instrument**" was printed in Scientific American (Titze, 2008b).

(8) To explore two types of economy-based voice therapy, one based on the use of semi-occluded vocal tracts for a high degree of source-system interaction, and one based on adductory training to produce a higher glottal efficiency.

As a first step in a therapy approach, four public school teachers with a reported history of vocal fatigue were provided six sessions of a chant therapy, and six sessions of a placebo therapy. Before and after each of these therapies, the teachers were administered a two-hour fatiguing task, during which auto-perceptive measures of *vocal effort* and *voice quality* were obtained. We concluded that chant therapy positively affected the subject's responses to the fatiguing task, whereas the placebo therapy did not. Fatigue recovery was parsed into neuromuscular fatigue recovery and lamina propria fatigue recovery, the latter being much slower. An article entitled "**Chant therapy for treating vocal fatigue among public school teachers: a preliminary study**" was published in the *American Journal of Speech Language Pathology* (McCabe and Titze, 2002).

A study of the current literature involving voice therapy was conducted. Voice therapy has evolved considerably over the past decade. Our field has learned to draw from other disciplines to help facilitate the restoration of vocal function by implementing a more holistic approach and utilizing principles of motor learning to create our therapy programs. Clinicians have learned to recognize that the voice is more than just the larynx. Rather, it is a whole body system, and breakdowns in systems throughout the body can be responsible for vocal disturbances. This review will cover the nontraditional approaches that aid in treating certain voice disorders that often are not discussed in textbooks or classrooms. Facilitating techniques include principles from singing and acting voice production, Feldenkrais, Alexander technique, Qigong, and circumlaryngeal massage. A paper was published entitled, "**Nontraditional tools helpful in the treatment of certain types of voice disturbances**" (Emerich, 2003).

Voice therapy with a semi-occluded vocal tract (lip trills, tongue trills, bilabial fricatives, humming, and phonation into tubes or straws) has been hailed by clinicians, singing teachers, and voice coaches as efficacious for training and rehabilitation. Little has been done, however, to provide the scientific underpinnings. The purpose of the study was to investigate the underlying physical principles behind the training and therapy approaches that use semi-occluded vocal tract shapes. Computer simulation was used to elucidate source-filter interactions for lip and epilarynx tube semi-occlusions. It was found that a semi-occlusion in the front of the vocal tract (at the lips) heightens source-tract interaction by raising the mean supraglottal and intraglottal pressures. Impedance matching by vocal fold adduction and epilarynx tube narrowing can then make the voice more efficient and more economic (in terms of tissue collision). The efficacious effects of a lip semi-occlusion can also be realized for nonoccluded vocal tracts by a combination of vocal fold adduction and epilarynx tube adjustments. It is reasoned that therapy approaches are designed to match the glottal impedance to the input impedance of the vocal tract. A

paper ("**Voice training and therapy with a semi-occluded vocal tract: rationale and scientific underpinnings**") was published in Journal of Speech and Hearing Disorders (Titze, 2006b).

Further, 18 persons participated in some the vocal economy training study. Since all of these individuals have normal, albeit fatigueable voices, the metrics used to monitor any improvement in their voice has been difficult develop. We are currently investigating two promising metrics, a vocal task based on the nonlinear vocal fold vocal tract interaction and an autopercptive measure of the inability of produce soft voice. To study the first task, was to pinpoint the proportion of source instabilities that are due to nonlinear source-tract interactions. Eighteen subjects (9 adult males and 9 adult females) performed three vocal exercises that represented a combination of various fundamental frequency and formant glides. Results indicated that the bifurcation occur more often in phonations with F0 – F1 crossovers, suggesting that nonlinear source-filter interaction are partly responsible for source instabilities. Furthermore it was observed that male subjects show more bifurcations in phonations with F0 – F1 crossovers, presumably because in normal speech they don't encounter these crossovers as much as females and hence have less practice in suppressing unwanted instabilities. A paper entitled "**Nonlinear source-filter coupling in phonation: vocal exercises**" was published in The Journal of the Acoustical Society of America (Titze et al., 2008).

In a study to investigate whether clinicians could detect voice changes reported by teachers, from self ratings teachers conducted of their inability to produce soft voice (IPSV), one of the metrics we use in therapy, ten teachers wore a vocal dosimeter and completed daily IPSV ratings approximately every 2 hours for 14 days. Following the 2 weeks of dosimetry, two speech clinicians specialized in voice rated the teachers' IPSV from dosimeter recordings. Teacher and clinician ratings were compared for each participant. Although teacher and clinician ratings were not significantly correlated, descriptive analyses demonstrated an average difference score of 1.7 (1.4) between teacher and clinician ratings. This study supports the potential usefulness of the IPSV as a simple tool to detect voice changes in oneself or others. [an article entitled, "**The inability to produce soft voice (IPSV): a tool to detect vocal change in school-teachers**" was published (Halpern et al., 2009).

In a related study to analyze nonlinear dynamic features of a series of high-pitched, soft phonation tasks performed by a single subject using self ratings, one hundred and ninety-seven phonations were analyzed to quantify the severity of instabilities in the voice attributed to nonlinear dynamic phenomena, including voice breaks, subharmonics, and frequency jumps. Instabilities were first counted; then a severity index was calculated for the instabilities in each phonation. The two quantities were compared to the subject's autoperceptual rating. Generally speaking, the measures derived from nonlinear dynamic analysis of the high-pitched, soft phonations followed the subject's own rating of inability to produce soft voice. These results are relevant toward formulating acoustic and autoperceptual measures for the fatiguing effects of prolonged speaking in vocally demanding professions. An article entitled, "**Towards a Self-Rating Tool of the Inability to Produce Soft Voice Based on Nonlinear Events: A Preliminary Study**", was published (Popolo et al., 2011).

In conclusion of this aim, two studies are being finalized in papers for submission.

The Inability to Produce Soft Voice (IPSV) was assessed for tracking vocal improvement in teachers with general voice complaints during and after voice therapy. Eleven grade school teachers received direct voice therapy ("Vocal Fold Adduction Training", VFAT) or Voice Amplification (VA) over six weeks, and rated their ability to produce soft phonation using the IPSV before, during, and after treatment. Baseline data were also collected during a two-week period prior to VFAT and VA. Teachers were actively teaching during all phases of the study. Pre- to post-therapy IPSV changes were calculated, and compared to Voice Handicap Index (VHI) scores and acoustic voice measures. IPSV ratings during the baseline period were stable and showed no learning effect. IPSV ratings improved throughout therapy for the VFAT but not the VA group. Improvements were significant and correlated somewhat with VHI improvements. Acoustic measures did not change significantly. The IPSV appears to sensitively track improvement in teachers with general voice complaints during a period of voice therapy, with no evidence of learning effect. Future research should replicate these findings with larger subject and control groups. The IPSV should also be tested for usefulness in tracking response to other voice treatments. A paper entitled, "**Measuring Improvement in Teachers with Minor Voice Complaints Using the Inability to Produce Soft Voice (IPSV): Preliminary Data"**, is in review.

A study was conducted that compares a therapy program based on phonation through flow-resistant straws (FRS) with Vocal Function Exercises (VFE), an established set of exercises that utilize semi-occlusion. Twenty subjects (16 female, 4 male) with dysphonia and/or vocal fatigue were randomly assigned to one of four treatment conditions: 1) immediate FRS therapy, 2) immediate VFE therapy, 3) delayed FRS therapy, and 4) delayed VFE therapy. Subjects receiving delayed therapy also served as a no-treatment control group. Results: Voice Handicap Index scores showed significant improvement for both treatment groups (VFE and FRS), and no significant change for the no-treatment group. (VHQ, CAPE-V, CSID, F0, economy of administration). A paper entitled, **Comparison of two semi-occluded vocal tract voice therapy protocols**, is in review.

SIGNIFICANT RESULTS

A highly significant result of our decade of research on occupational voice use is the quantification of damage-risk. Knowing the epidemiology of voice disorders among teachers, and knowing the exact vibration doses they accumulate in their workplaces, we have set a 30% damage-risk criterion for the combination of three variables shown: loudness, duration, and fundamental frequency. This parallels research on damage-risk for hearing loss (Kryter et al., 1966).

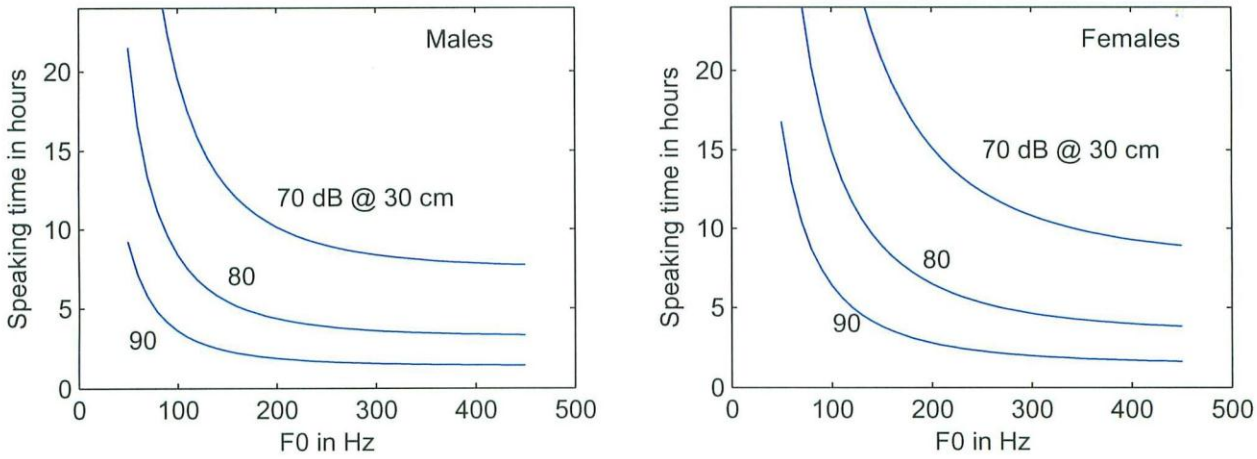

Figure 1. 30% damage risk criterion from speaking at various loudnesses, frequencies, and durations.

STATEMENT OF HOW RESEARCH PRODUCTS WILL BE MADE AVAILABLE

Significant findings are made available in the standard publications and on with our online technical notes: http://www.ncvs.org/research_techbriefs.html

More than 63 publications have come from the research. Additionally, there have been several dozens of presentations at scientific conferences. These are not listed below. Additionally, there were two disclosed inventions from the research.

PUBLICATIONS RESULTING FROM THE WORK (63)

Editor Reviewed Publications (10)

E. J. Hunter, J. Spielman, Starr A.C., and P. S. Popolo. Acoustic Voice Recording, "I am seeking recommendations for voice recording hardware...". Perspectives on Voice and Voice Disorders 17(1):7-14, 2007. http://div3perspectives.asha.org/content/17/3/7.full.pdf

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