Devices and Methods on Analysis of Biomechanical Properties of Laryngeal Tissue and Substitute Materials

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Abstract: For understanding the phonatory process in human voice production, physical as well as numerical models have been suggested. Material properties within these models are crucial for achieving vocal fold dynamics being close to *in vivo* human laryngeal dynamics. Hence, different approaches have been suggested to gain insight into human laryngeal tissue, evaluate clinical treatment, as well as to analyze and verify parameters within synthetically built vocal folds.

Purpose of Review: The authors want to give an overview of approaches on receiving material parameters being important in voice research. For the different devices and methods being applied for different set-ups, we will present the functionality and applicability. Hence, for future work, this review shall give an indication, what kind of measurement techniques are suitable for the intended study, advantages or disadvantages of the approaches, and what parameters can be obtained from them.

Recent Findings: For *in vivo* experiments, Color Doppler Imaging was found to be suitable for receiving vocal fold stiffness properties. Applying rheological measurements, the elastic modulus and the dynamic viscosity can be determined. In combination with histological analysis it is possible to objectively evaluate clinical treatment. Optical Coherence Tomography enabled to detect tissue boundaries for in vitro vocal folds. A pipette aspiration setup allowed to identifying spacially resolved mechanical properties of synthetic vocal folds. Numerical biomechanical models like finite element models have shown to be suitable to identify isotropic elastic material parameters

Keywords: Vocal folds, rheometer, viscosity, material parameters.

1. INTRODUCTION

Biomechanical properties of the vocal folds are important to characterize because they play a crucial role in the vibratory function of phonation. When the viscosity or elasticity of the vocal fold deviates from normal, the quality of voice production suffers greatly. Hence, many efforts to better understand biomechanical properties of the vocal folds have been proposed.

In vivo analysis of the viscoelastic properties of the vocal folds is essential to understanding the mechanisms that underlie vocal fold disorders. *In vivo* characterizations of the vocal fold tissue are important for effective treatment design because restoring function to the vocal folds after injury or trauma requires restoration of normal biomechanical properties. Evaluation of treatment also relies heavily on the ability to analyze biomechanical properties *in vivo* accurately and noninvasively in order for the biomechanical characterization to be clinically feasible. As treatments of vocal disorders continue to evolve, accurate *in vivo* methods of analyzing biomechanical properties for treatment design and evaluation are critical for continued progress.

Another approach of measuring material parameters is using excised human as well as animal larynges, i.e. ***in vitro*** experiments. *In vitro* analysis of vocal fold tissue requires an apparatus that is capable of measuring two properties, the applied force that causes stress, and the displacement of the tissue due to the resultant strain. The ability to apply stress to materials, and measure strain is not a new engineering discipline, and many companies manufacture standard laboratory apparatus that will achieve this objective. Collectively known as rheometers, these devices will be found in research and test laboratories throughout the world.

Synthetic Physical Vocal Fold Models: For simulating vocal fold dynamics, self oscillating synthetic vocal fold models are studied. There, the vocal folds consist of silicone or polyurethane mixtures. As these models should exhibit similar behavior to human vocal folds, measuring and comparing material parameters also has to be performed for these models. Hence, several methods have been developed to investigate the mechanical properties of both, biological tissues of vocal folds and artificial materials. These methods can be roughly divided in three different categories: (1) Rheometer based techniques (e.g. [1]), (2) Transmission of

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mechanical vibrations (e.g. [2]), and (3) Pipette aspiration techniques (e.g. [3]).

Apart from the direct or experimental measurement methods described so far, there are several **numerical models** used for inverse approaches. There, certain parameters of a numerical model are optimized in order to achieve the best possible agreement between the answers provided by the numerical model and measured data obtained from physical experiments. The motivation of such an approach is either the identification of physical parameters which cannot be easily measured or the calibration of a constitutive model, which is used to mimic the mechanical behavior of laryngeal tissue.

2. *IN VIVO* EXPERIMENTS

2.1. Imaging Techniques

Current imaging techniques provide a valuable source of information for characterization of *in vivo* biomechanical properties of the vocal folds. *In vivo* analysis is important for the clinical applications of diagnosis and treatment evaluation since many vocal fold disorders are characterized by a change in biomechanical properties. Most of the imaging techniques discussed here characterize mucosal wave parameters during phonation. The body-cover model proposed by Hirano suggests that the vocal folds be treated as a two mass model. In this model the body consists of the vocalis muscle and elastic conus while the cover consists of the lamina propria [4]. The body is responsible for the lateral motion of the vocal folds while the mucosal wave is seen in the loose connective tissue of the lamina propria or “cover” [5]. The mucosal wave is initiated by subglottal pressure (SGP) against the lower lips of the vocal folds. The lower vocal fold lips are split open by the SGP and this deformation continues upward until the upper lips separate [6]. The mucosal wave is directly affected by the viscoelastic biomechanical properties of the vocal folds, especially those of the vocal fold cover. The parameters of amplitude, phase difference and velocity will change as a result of altered biomechanical properties such as increased stiffness. Photoglottography (PGG) and electroglottography (EGG) are methods of mucosal wave characterization that can determine the opening and closing phases of the glottal cycle. Using light transmittance, as in PGG, or electrical impedance, as in EGG, the glottal area can be tracked in real time [6]. However, these methods are limited by the inability to determine mucosal wave amplitude or asymmetries of the vocal folds. More information on mucosal wave parameters is revealed by imaging modalities; both indirect and direct visualization methods such as color Doppler imaging (CDI), stroboscopy and high speed digital imaging (HSDI). The value of these techniques lies in that they provide options for *in vivo* analysis, however each technique has limitations. As these limitations are addressed, clinical applications for diagnosis and treatment evaluation will become more feasible. Further study into the relationship between changes in the mucosal wave and changes in biomechanical properties is necessary for reliable *in vivo* biomechanical analysis.

2.1.1. Indirect Imaging

Indirect imaging methods use biosignals to generate an image for analysis. Currently, ultrasound, magnetic resonance imaging (MRI) and compute tomography (CT) are used to image the vocal folds [7]. MRI and CT are limited by resolution, especially when imaging vocal fold vibration. The low cost, high resolution and noninvasive nature of ultrasound have made this imaging modality useful for diagnosing diseases of the vocal folds as well as other analysis applications [7]. Of particular interest for biomechanical characterization is color Doppler imaging (CDI).

CDI is an indirect imaging technique used to measure mucosal wave velocity and its relationship to fundamental frequency and vocal fold stiffness. A color contour of the vocal fold vibration is generated, allowing vocal fold length to be determined. From length frequency measurements, vocal fold tension, stress, strain and Young’s modulus can be identified. This method has proven to be a feasible way of quantifying elastic properties of the vocal folds [8]. CDI has the potential to be a valuable clinical tool if used to characterize elasticity in human vocal folds for diagnostics and evaluation of treatment effectiveness. Results from a study by Hsiao *et al.* [8] yielded results similar to those from an elastic model by Alipout-Haghighi and Titze in 1991 [9]. However, further study into the reliability of the relationship between mucosal wave velocity and vocal fold stiffness is warranted before the widespread application of this technique.

2.1.2. Mucosal Wave Visualization

Stroboscopy is the most commonly used visualization method due to its low cost, quickness and utility. It has become standard practice to first evaluate a disordered voice with stroboscopy [10]. While improvements in light source and sensitivity have made stroboscopy clinically valuable, it is limited by the conditions it can evaluate. Stroboscopy captures 30 frames per second creating an averaged composition image of the mucosal wave as seen in Fig. (**1**). The camera is set at a frequency slightly different from the fundamental frequency of phonation in order to capture the mucosal wave at different time points of the glottal cycle. In order to reliably image the mucosal wave, the subject must phonate at a nearly constant fundamental frequency throughout the recording. Because of these requirements, stroboscopy is limited to the evaluation of vocal folds with periodic vibration at constant frequencies [6]. Since many vocal fold disorders result in aperiodic vibration of the vocal folds and difficulty maintaining a constant fundamental frequency, stroboscopy can miss subtle changes in mucosal wave dynamics.

High-speed digital imaging (HSDI) is considered the “gold standard” of visualization techniques with a high frame rate of at least 2000 frames per second that allows for the capture of several images from each mucosal wave cycle, as can be seen in Fig. (**2**). This is an improvement over the estimate provided by stroboscopy. Since many images are acquired from a single glottal cycle, differences between cycles due to aperiodic motion or changes in frequency can be seen. The advancement over stroboscopy has been demonstrated in various comparative studies, showing that HSDI is able to differentiate between normal and disordered voices better than stroboscopy [11,12]. This makes HSDI the most valuable tool for evaluation of disordered voice [6]. The use of HSDI is, however, limited by cost since imaging

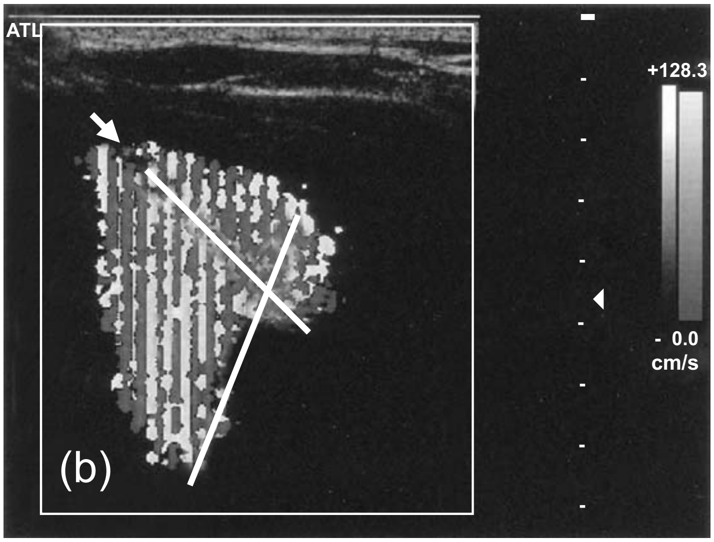

Fig. (1). A compilation of stroboscopic images depicting the glottal cycle. [6].

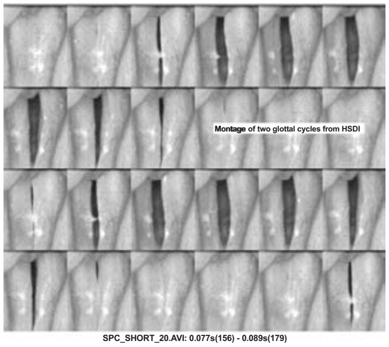

Fig. (2). Compilation of images from two glottal cycles using HDSI. [6].

equipment and data storage space can be prohibitively expensive for some clinics.

The direct imaging techniques of stroboscopy and high speed digital imaging (HSDI) allow for visualization of the muscosal wave from a supraglottal position. Qualitative information about the movement of the vocal folds can be obtained by viewing videos; however, quantitative methods are necessary for characterization of the mucosal wave. Several methods have been developed to quantitatively characterize mucosal wave properties of the vocal folds using high speed videos. These methods include digital kymography (DKG) and phonovibrograms (PVG).

DKG and PVG were both developed as objective and quantitative ways of evaluating the vocal folds from HSDI videos. DKG does this by generating a kymogram that depicts the open and closed phases, periodicity, left-right symmetry, phase difference and amplitude, allowing for detailed characterization of the mucosal wave [6]. This is accomplished by plotting distance from the midline of the left and right vocal fold lips along a single pixel line, generating an image that looks somewhat like a sine wave. Curve fitting can be used on the kymograms to generate quantitative information on each of these parameters. While DKG provides valuable information on the many mucosal wave parameters, it is limited to the analysis of a single pixel line of the video, rendering it unable to determine anterior-posterior asymmetry of the vocal folds. To address this limitation, PVG analysis was developed [13]. The distances of the right and left vocal folds from the glottal midline are computed and visually displayed. PVG allows for visualization of vocal fold dynamics from the anterior to the posterior ends, allowing visualization of anterior-posterior asymmetry of the vocal folds [14]. Since changes in the mucosal wave reflect biomechanical changes to the vocal fold, these mucosal wave visualization methods offer a valuable *in vivo* method of characterizing biomechanical properties. These methods have been used primarily for research purposes, but as they become more reliable could have clinical applications as a way to quantify vocal fold changes.

2.1.3. Histological Visualization

Another visualization technique, to be discussed further in a later chapter, is optical coherence tomography (OCT) which can help discern the layered microstructure of the vocal folds. In a study by Burns *et al.* [15], real time OCT measurements were used to track the presence of subepithelial implants in phonatory mucosa in an *in vivo* canine model. Results of this study showed that OCT has sufficient resolution to provide confirmation of hydrogel implant placement in the vocal fold. Optical frequency domain imaging (OFDI) (also called swept source OCT) utilizes a mechanism similar to OCT but has an increased signal-to-noise ratio (SNR). Results from a study by Boudoux *et al.* 2009 [16] showed that OFDI allowed visualization of the vocal fold architecture deep within the tissue, from the superficial mucosa to the vocalis muscle. It demonstrated the ability to differentiate between the epithelial layer and the lamina propria. OCT and OFDI provide *in vivo* options for determining the histology of the vocal folds, potentially allowing rheological properties to be predicted based on results from histological and rheological investigations. A thorough understanding of the relationship between histological and rheological properties of the vocal fold tissue is essential for OCT or OFDI to be viable options for *in vivo* analysis.

2.2. Histological and Rheological Techniques

Histological and rheological analyses of the vocal folds are important to understanding biomechanical properties of the vocal folds. Components of the extracellular matrix (ECM) play an important role in vocal fold biomechanics; however the contribution of individual components and their role in scarred vocal folds has yet to be established, making histological analysis of the vocal folds an important part of biomechanical research. Collagen, procollagen, elastin, and hyaluronic acid are frequently studied components of the ECM. Histological analysis is especially important when studying changes in viscoelastic properties of the vocal fold due to scarring, remodeling from injury or trauma, or benign lesions that alter vibratory function. Further understanding of the ECM components' influence on biomechanical properties would allow for better characterization of scarred vocal fold tissue. When histological analysis is paired with rheological analysis, the effect of ECM components on vocal fold biomechanics can be studied. Using rheological analysis, the elastic modulus, which describes tissue stiffness, and the dynamic viscosity, which describes resistance to flow, can be determined of the vocal fold tissue. Both elasticity and viscosity are important characterizations for diagnostics, as well as treatment development and evaluation. As the relationship between histology and rheology of the vocal fold tissue is better understood, it becomes more feasible to use *in vivo* histology imaging techniques, such as OCT to predict the rheological properties of elastic modulus and dynamic viscosity.

2.2.1. Histology

Using *in vivo* animal models, it has been demonstrated that when the vocal folds are injured scar tissue that forms during wound repair can alter the biomechanical properties of the vocal folds [17-19]. Characterization of normal and pathological histology is crucial to understanding biomechanical changes. Chronic vocal fold scarring has been characterized in animal models, both rabbit and canine at six months postoperatively [17,18]. A limitation that must be taken into account when reviewing animal studies is that since animals vocalize less than humans the loading and stress on their vocal folds is lower. Studies have shown that increased stress or loading over time can alter ECM expression and biomechanical properties [19]. While this limitation does not invalidate the studies, it should be considered when predicting the kind of changes that might be seen in human vocal folds during wound healing. These studies found that compared with control samples, scarred tissue samples had more fragmented and disorganized elastin fibers and significantly increased collagen organized into thick bundles.

The histological ultrastructure and rheological properties of the scarred vocal fold lamina propria in a rabbit model 60 days postoperatively have also been studied [19]. Differences were seen between ECM composition at 60 days and 6 months, particularly in procollagen content, collagen content and organization and elastin content. Differences in content of the vocal folds at 60 days and 6 months postoperatively indicate that at 60 days, wound healing has not reached maturity. Further characterization of the histology of the vocal folds throughout the wound healing process would give better insight into the changes that occur as a result of wound healing and remodeling and indicate the nature of biomechanical changes throughout the process. OCT has the potential for *in vivo* histological analysis. However, the *in vivo* use of OCT has not been widely established. It has been used in an *in vivo* canine model [15] and suggested for *in vivo* use based on preliminary ex vivo findings [16]. Future study of *in vivo* animal models would benefit from improved *in vivo* testing so that wound healing can be evaluated on a single specimen. The use of OCT could provide a method of characterizing the histology of laryngeal tissues throughout the wound healing process after injury.

2.2.2. Rheology

Elasticity and viscosity of the vocal folds have a direct effect on laryngeal resistance to vibration and therefore play a significant role in determining the ease and quality of phonation. Histological results can be correlated to changes in the rheological properties of elastic modulus and dynamic viscosity. The studies noted above also characterized the rheological properties of the scarred vocal folds and all studies found that the majority of samples had both increased elastic shear modulus and increased dynamic viscosity [17-19]. These changes indicate that the resistance to vibration increased, making the tissues less biomechanically efficient. There have been consistent results across studies on the histological and rheological changes to the vocal fold resulting from injury and scarring. CDI has been used to determine the elastic modulus of the vocal folds and has the potential to be a valuable tool for *in vivo* rheological analysis [8]. Further study into the *in vivo* use of CDI to determine its accuracy in characterizing rheological properties of laryngeal tissues is the next step toward widespread application of CDI. In addition, further study in the correlation between the histology and rheology of the vocal folds is necessary to better understand biomechanical changes during the wound healing process, and develop treatments to help minimize these changes.

2.3. Applications for Treatment Evaluation

Treatment design and evaluation are critical applications of *in vivo* biomechanical characterization. Since biomechanical properties directly affect vocal quality, they provide a good indication of overall vocal health [20]. The relationship between biomechanical properties and vocal quality has been established through many studies [20-25]. As *in vivo* biomechanical characterization methods are further refined, various treatment options designed to restore biomechanical function can be compared. The ability to characterize *in vivo* biomechanical properties is crucial to future treatment development. Treatments designed to restore biomechanical function include stem cell injection for tissue regeneration after injury, synthetic ECM (sECM) injection for tissue regeneration after injury, and vocal fold augmentation and medialization for vocal fold paralysis. Further discussed below are applications of biomechanical characterization methods as applied to current treatment research and development.

2.3.1. Stem Cell Injection

Injection of stem cells into injured vocal folds to improve the histological and rheological properties has been studied as a treatment for scarred vocal folds in rabbits and rats [26-30]. Analysis of the survival and effects of these cells has been performed in order to evaluate this treatment option. Histological and rheological analysis of the vocal folds after stem cell injection is currently used to determine if the treatment had a restorative effect. Using *in vivo* animal models, mesenchymal stem cells (MSC) have been shown to improve biomechanical properties by reducing the elastic modulus and dynamic viscosity as compared to untreated controls [26,28]. These rheological changes are reflected in the reduced lamina propria thickness and collagen type I content. These are promising results, however, this study is limited by the inability to accurately characterize histological and rheological properties *in vivo*. As *in vivo* imaging techniques improve, *in vivo* analysis of the stem cell injected will be more feasible and reliable. CDI has the potential to be a valuable tool for investigating changes in the elastic modulus of the tissue after treatment, while OCT has the potential to yield important information about the histological properties of the tissue before and after treatment. In addition, HDSI and the analysis techniques of DKG and PVG may be used to quantify how the histological and rheological changes post treatment have affected mucosal wave propagation efficiency in order to quantify improvements to the voice.

2.3.2. Synthetic Extracellular Matrix for Vocal Fold Regeneration

The extracellular matrix (ECM) is composed of fibrous proteins such as collagen and elastin and interstitial proteins and other components such as fibromodulin, fibronectin and hyaluronic acid (HA). The biomechanical properties of the ECM are determined by the content of each of the proteins in the ECM. The effects of ECM components have been widely studied in relation to vocal fold biomechanics [21,23,31,32], but since there is so much interaction between the components, it is difficult to isolate the role that each plays [33]. When vocal folds are scarred, changes to the ECM cause changes in biomechanical properties, which can greatly reduce functionality [17-19]. In order to restore normal function, biomechanical properties of the ECM need to be restored. For this reason, *in vivo* biomechanical analysis is essential to the study of treatments intended to restore normal biomechanical function.

Synthetic extracellular matrices (sECM) have been a widely popular topic of research as a treatment for scarred vocal folds. The ability of certain materials to promote tissue regeneration in the vocal folds has been investigated by many studies. Studies of *in vivo* animal models have demonstrated that the presence of an sECM has resulted in increased wound healing [34] and decreased scarring, providing improved biomechanical properties when injected into a scarred rabbit vocal fold during wound healing [28,34-38]. The analysis used for these studies has the same limitation as that done for the stem cell treatments in that the analysis is done ex vivo. The use of visualization imaging techniques offers the potential to characterize the tissues *in vivo*. CDI, OCT and HDSI have the potential to characterize the biomechanical properties of the tissues *in vivo*, however future studies are necessary to further establish the reliability and accuracy of these methods.

Material selection is also critical in the design of implant materials for sECM, since the mechanical properties of the material should be well matched to the biomechanical properties of the vocal fold tissue so that function can be restored. Elastic modulus, dynamic viscosity and degradation rate are important to characterize and match to the laryngeal tissue. The ability to tune mechanical properties and degradation rates has been studied in order to optimize treatment effectiveness. It has been shown that the degradation rate of hyaluronic acid (HA)-based microgel networks can be tuned to match the tissue regeneration rate without significantly altering the mechanical properties of the material by varying the amount of cross-linking among the micro gel particles [36]. Several research studies have focused on HA and its derivatives. HA has been implicated as an important component in the wound healing process, although the precise role has yet to be fully understood [17-19]. Injectable HA hydrogels have been shown to significantly improve tissue elasticity and viscosity of the vocal fold cover based on histological and rheological results [28,34,35]. Biomechanical analysis methods allow accurate mechanical properties of the injection materials to be chosen. As the *in vivo* analysis methods discussed improve, *in vivo* effectiveness of sECMs can be predicted and evaluated to better study the effects of sECM materials. CDI, OCT and HDSI have the potential to be valuable tools in evaluating the effect of sECMs as further study improves their accuracy and reliability.

2.3.3. Augmentation

When the vocal folds cannot be adequately medialized due to injury or paralysis, the larynx cannot efficiently transform aerodynamic energy into acoustic energy. This results in a breathy and weak voice. Surgical medialization of the vocal folds is an effective treatment for dysphonia and is accomplished by injection of an augmentative material lateral to the vocal fold muscle [39]. Different from sECM used for tissue regeneration, augmentative materials are intended to remain intact in the vocalis muscle, or body of the vocal folds, and if they degrade, must be replaced. In addition, augmentative materials are injected into the vocalis muscle in order to medialize the vocal folds rather than being applied to the location of injury. Mechanical properties are an especially important consideration when looking at potential implant materials. The mechanical properties such as elasticity and viscosity of the implant should be well matched to the biomechanical properties of the surrounding body tissue in order to optimize efficiency of the vocal folds. Injection using many materials such as Teflon, autologous fat, silicone suspension and hydroxyapatite cement has been performed [40], but limitations in the ability to match the mechanical properties of these materials to the biomechanical properties of the vocal folds have prompted research of new materials for use as augmentative materials.

In addition to use as an sECM component, HA has been studied for injection augmentation due to its biocompatibility and desirable mechanical properties., *In vivo* animal studies have demonstrated that HA-based implant materials do not alter the viscoelastic properties of the vocal folds, which helps maintain normal vibratory capacity after treatment [39,41]. *In vivo* analysis of biomechanical properties of the vocal folds is required for further study into HA and other materials as augmentative materials for vocal fold medialization. OCT has been studied in an *in vivo* animal model to track implant placement [15] and has the potential to be used to verify implant position over time. CDI offers the option to measure the elastic modulus of the vocal folds after injection while HDSI provides the option of mucosal wave analysis to determine if functionality has been restored. As these methods are improved, the ability to evaluate augmentative materials will greatly improve.

3. *IN VITRO* EXPERIMENTS

Analysis of excised vocal fold tissue requires a so called rheometer, an apparatus that is capable of measuring an applied force that causes stress, and the displacement of the tissue due to the resultant strain. Amongst the better known manufacturers of rheometers are Instron [www.instron.co.uk], and Bohlin [www.bohlin.com].

These devices operate in one of two modes; they either apply a linear stress or a rotational stress. In both cases it is possible to derive the shear modulus of the material under test as long as the geometry of the test setup is controlled and measurable. The technical difficulty that arises when measuring the vocal folds is that the tissue is very small, and exhibits a large deformation when even a small force is applied. Typically it is possible to measure shear deformations of a few mm when as little as 1g force is applied. As commercial devices such as those available from Instron & Bohlin are designed for measuring larger and stiffer samples, the design of rheometers for the vocal fold is an engineering discipline in its own right.

There are two viable alternatives to applying a shear stress. One is to stretch a sample of tissue in order to derive its Young's Modulus. The other is to use an indentor. The use of extension can be found widely in the published material. However even though indentors are used in related areas of biomechanical research, they do not seem to have found favor for vocal fold analysis. *In vitro* analysis of vocal fold tissue has progressed substantially in recent years as more precise and miniaturized force and displacement sensors have come on to the market. This is a brief summary of some of these devices, presented chronologically.

3.1. Linear Extension

3.1.1. The Ergometer

Ergometers, which may also be known as dynanometers, are widely used in tissue studies. Ergometers are capable of applying very low forces and displacements to materials under test, and the Dual Mode versions are capable of providing an instantaneous readout of the actual forces and displacements that occur during dynamic tests. Their operating principle is based on the use of rotary electro-magnetic coils. Mutual inductance relationship means that it is possible to apply a precision electrical current to the actuating coil such that it delivers a known force. Such an arrangement when established with a counter force can also be used to apply a precision displacement. This technology can be reversed such that an applied force will generate an electrical current change, which can be measured; these are known as Dual Mode Ergometers. Small displacements can also be measured by a variety of techniques, including the industry standard Linear Voltage Differential Transformer (LVDT) or a capacitive probe. An examination of the published literature will result in many researchers using ergometers, mainly to study the operation of muscle tissue.

Fariz Alipour and Ingo Titze are early pioneers of the design of specialist devices intended to measure the biomechanical properties of the vocal fold that are based on Dual Mode Servo Ergometers, that are still in use today. Examples are manufactured by Cambridge Instruments and Aurora Scientific [www.AuroraScientific.com]. One of the earliest published results obtained from an ergometer was a paper by Alipour & Titze in 1984.

In this study the excised canine larynx was suspended in an aerated Krebs solution, and stress was applied by the dual mode servo ergometer. Stress Strain relationships were successfully obtained, and the reader is invited to review the full publication for the physiological results, which contains some useful mathematical analysis derived from the original work by F C Fung [42].

The ergometer is a remarkably versatile device, as it is capable of both applying and measuring extremely low forces and displacement. The Iowa team continued to develop new experiments and are still today producing fascinating work. Of particular interest is the experiment constructed by Perlman & Titze in 1988 [43]. They published details of an advance on this device in which they used an external electrical stimulus to adduct the muscle. This device see Fig. (**3**) mounted canine vocal folds vertically in a chamber, using sutures that were attached to small sections of thyroid & arytenoid cartilage; the sample consisted of the underlying muscle, ligament and vocal fold cover. One suture was affixed to a Grass FT.03 force-displacement sensor, which was arranged via a rack & pinion to a micrometer screw. The contractions were achieved by applying electrical stimulation to the muscle using Grass S48 stimulator connected to platinum electrodes. Using this arrangement the team were able to apply nerve stimulations *in vitro* to the vocal fold, whilst simultaneously logging the extension. The results demonstrated that there was a direct relationship between nerve stimulation and an increase in vocal fold tension. The study examined the active & passive properties of the laryngeal posturing muscles, and concluded that twitch contraction time was 32 +/- 1.9 ms. While this finding is subjectively well known, this is the first recorded experiment that quantified this relationship.

Both these early devices operate at low speed, and were used to derive the Elastic of Young's Modulus of the vocal folds under varying simulated physiological conditions. Pioneering work is still continuing at Iowa using more modern variations of ergometers. One example is the experimental set-up reproduced in Fig. (**4**) [44].

Of interest to the engineer will be the fact that the underlying electro-mechanical principles of measurement and control are unchanged, but have been refined, improved and modernized. The particular value of an ergometer is its ability to both apply and to measure very low linear forces. The dual mode servo ergometer will continue to be an important tool for *in vitro* analysis of the vocal fold for many years to come.

3.1.2. The First In Vivo Device

Worthy of mention at this point is a device developed by Tran, Berke, Gerratt and Kreiman see Fig. (**5**). Whilst intended for *in vivo* measurements of the vocal fold, it represents an important and early advance in instruments designed specifically for this field [45].

This device applied the prevailing techniques for measuring linear deformation, and whilst intended for *in vivo* use nevertheless is equally applicable to *in vitro* studies. A small plate was inserted behind the vocal fold of the patient, while the other end was attached via a cantilever arrangement such that small quantifiable displacements were made with a micrometer screw. The resultant deformation causes a force that was recorded using a force gauge. Thus the team were able to publish the first ever *in vivo* measurements taken from a vocal fold. The *in vivo* data obtained by Tran *et al* offers a range of shear modulus from 2450 Pa to 29,4 kPa.

3.2. Parallel Plate Rheometry

The next stage in development of *in vitro* analysis was the successful application of Parallel Plate Rheometry. Whilst this technique is widely used, the bulk of the published literature and some of the leading results, have been produced by Roger Chan from Austin University. The most used device in the literature is the Bohlin Parallel Plate Rheometer [http://www.malvern.com] see Fig. (**6**).

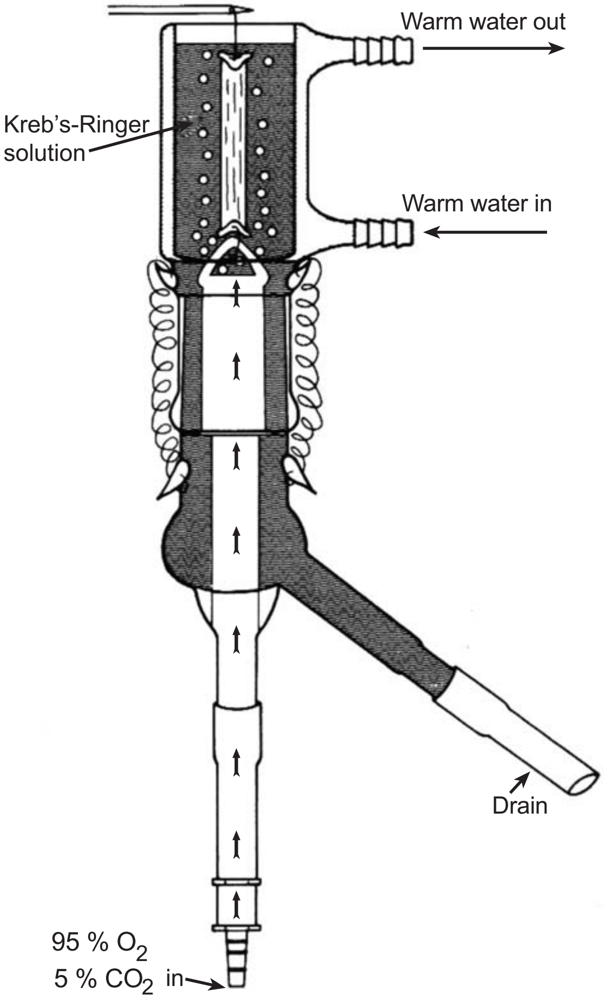

Fig. (3). Perlman/Titze ergometer device [43].

First let us review how these devices operate. A sample of the material to be tested, of known width and diameter, is sandwiched between two plates. This material can be just lamina propria, ligament or muscle; or a combination. Thus these devices are able to discriminate between the biomechanical properties of the different layers of the vocal fold. One of the plates can rotate and thus apply a rotational shear stress. By measuring the torque and the angular displacement it is possible derive the underlying rheometric properties of the material. One great advantage of using a rotating mechanism is that it can be driven using a sinusoidal driver, thus it applies at sinusoidal stress that mimics the oscillatory nature of the mucosal wave. We are then able to derive both the pure elastic and viscous properties of the tissue under test. Early devices could only operate at low frequencies, typically 15Hz [22], but more recent advances in torsional mechanical design has enabled studies to be taken at higher frequencies, typically 80Hz. A new publication in press states that data was obtained at 250Hz [46]. The significance of this advance will not be missed by laryngeal researchers, as 250Hz brings the test equipments operating range into frequencies that are within the range of

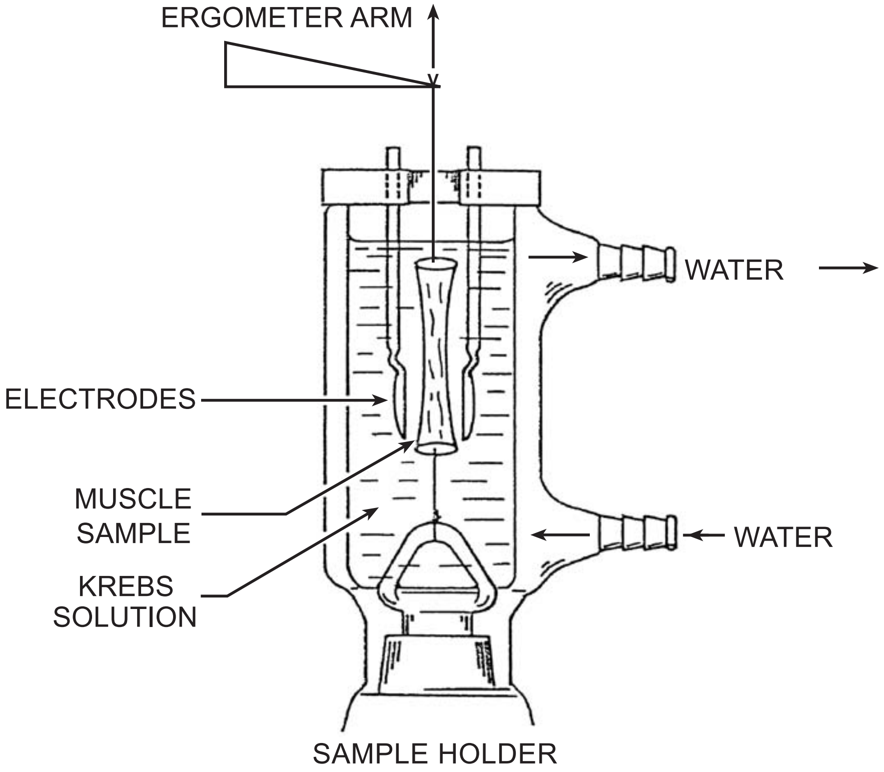

Fig. (4). More recent ergometer apparatus devised by the Iowa team [44].

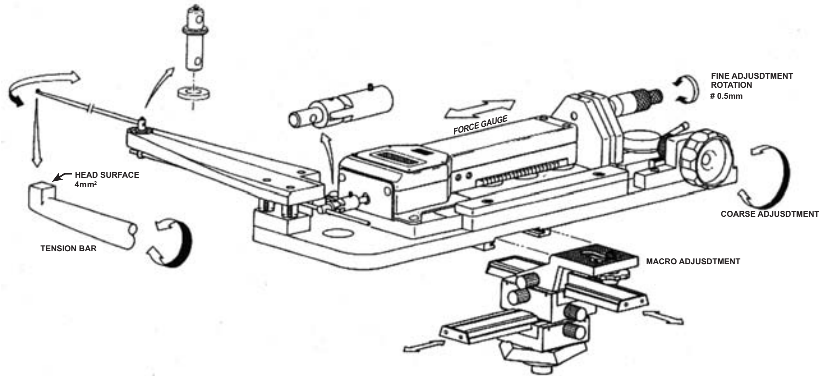

Fig. (5). The first *in vivo* laryngeal tensiometer [45].

normal phonation. The typical standard frequency for men is 100 Hz, and for women is 200 Hz [47].

3.3. A Return to Linear Devices

The great advantage of torsional rheometers is that they are capable of providing repeatable data, at frequencies that are within audible range, and can present both pure elastic and viscous data. There disadvantage is two-fold; they require the tissue to be dissected out of anatomical context, and they cannot resolve out orthogonal data.

There are now appearing in research papers results obtained from linear rheometers. These have been successfully deployed using excised tissue, and to measure vocal folds that are still in their anatomical context. Linear devices also have the mechanical advantage that the stress is applied to the soft vocal fold cover, rather then the complete structure that would include ligament and underlying muscle; thus they are more likely to be able to measure the biomechanical properties of the lamina propria only without dissecting it out of its anatomical context. There therefore now follows a brief review of the history of linear rheometers.

3.3.1. The Gas Bearing Electrodynamometer (GBE)

The GBE was developed in the 1960s by Dr Hargens specifically to measure the elastic properties of the stratum corneum. A detailed description can be found in Bioengineering of the Skin [48,49]. The device uses a moving coil that is arranged such that it moves linearly when a current is applied. The air bearing is achieved by applying a continuous airflow around the moving coil such that it is floating in free space, and thus has frictionless linear motion. An LVDT is used to measure displacement. Maes [50] established the methodology for the use of GBE for biomechanical studies. The coil is calibrated to determine the electrical current required to deliver 3g force. A long probe is attached to the floating coil, with the far end attached to the skin such that a shear force is applied. A sinusoidal current is applied to the coil, thus causing a sinusoidal shear

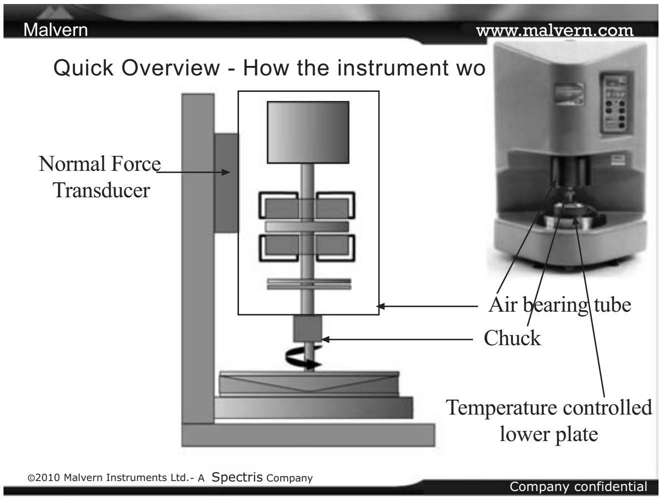

Fig. (6). The bohlin parallel plate rheometer.

force to be applied to the skin. The resultant strain is logged. The early device used a storage scope to capture the trace, which was then analyzed using a ruler. A team led by Eric Goodyer modified the GBE apparatus in 1980's for the Procter & Gamble corporation to use a PC to capture and analyze the data in place of the storage scope. In 1992 Goodyer was commissioned by P&G to re-engineer the GBE using modern components and this led to the development of Linear Skin Rheometer see Fig. (**7**).

One feature of the GBE method is that the force is not measured, but inferred by the current delivered to the moving coil. This overcomes a major difficulty when devising instrumentation to measure vocal fold biomechanics, which is the availability of small and easy to use load cells. Perhaps there is scope for future advances in mechanical systems that also employ this concept, which could be achieved with piezo-electric drivers to deliver a known force as opposed to trying to measure force?

3.3.2. The Linear Skin Rheometer

The LSR design was commissioned by P&G in 1992 to be a modern replacement for the GBE that was widely used by researchers examining skin conditions and treatments, as well as the efficacy of cosmetic formulations. It is still mainly used in that field, the most recent study being the effectiveness of a medicated bandage for use on geriatric skin [51].

A full description can be found in the first published paper that described the device, and provided results [52].

A collaboration between DeMontfort University, Harvard medical School and Universitätsklinikum Hamburg-Eppendorf (UKE) led to various modifications to the device, which enabled it be used in a series of *in vitro* studies [53-55]. The final solution is a device that uses the LSR electromechanics, operating such that a cyclical force of 1g is applied to the vocal fold, using a suction attachment. The key value of this arrangement is that it has enabled data to be obtained from vocal fold tissue that remains within its anatomical context, as shown in Fig. (**8**). This device has now been used to map the variations of vocal elasticity with respect to anatomical position, and to quantify the anisotropic nature of the vocal fold cover in situ. Results for shear modulus range from a few 100 Pa to a few 1000 Pa dependant upon anatomical context and the direction of applied stress.

3.3.3. Endurtec Linear Rheometer

A major failing with the LSR is that it is unable to operate at speeds anywhere near those that occur during phonation. Roger Chan has solved this problem by devising a high speed linear rheometer, that can deliver shear stress and monitor the resultant strain at frequencies up to 250 Hz [1]. The team constructed an apparatus consisting of two parallel plates which held the tissue sample in place. An EnduraTEC ElectroForce linear actuator was modified by the Bose Corporation to provide a high frequency sinusoidal linear action. A piezoelectric force sensor (PCB Model 209C12) measured the resultant linear force, and a Schaevitz LVDT measured displacement. Excellent results have been obtained from this device, which is capable of providing both elastic and viscous data. The elastic shear moduli reported range from just over 20 to just under 900 Pa and the viscous shear moduli reported range from around 70 Pascal to about 700 Pa; both these data sets are for a frequency range of 1 to 250Hz. The main advantage of linear shear

Fig. (7). The linear skin rheometer [52].

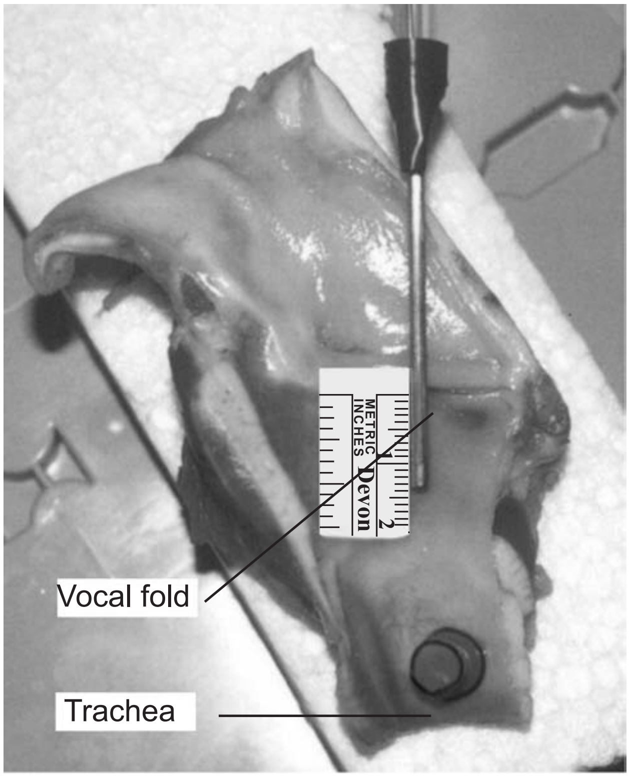

Fig. (8). A typical Linear Skin Rheometer (LSR) measurement arrangement, showing a suction probe attached to a porcine hemi-larynx.

measuring devices over the torsional motion provided by a Bohlin Parallel Plate Rheometer is that it can determine the variation in biomechanical properties with respect to the direction of the applied force.

3.4. Optical Devices

The future for both *in vitro* & *in vivo* vocal fold studies lies with optical techniques. The key reason is that optical measurements are non-contact and therefore the act of taking a measurement has no impact on the way that the tissue deforms. Imaging of patterns or dots on moving vocal folds for *in vivo* studies is well known, but as yet does not seem to have been taken up for *in vitro* use. However there are some highly innovative optical measuring techniques now being developed. The most interesting new arrival is optical coherence tomography. A particular advantage of an optical technique is that it is well suited to the visualization of changes in the profile and height of the soft body cover of the vocal fold.

3.4.1. Optical Coherence Tomography

An experimental setup using Optical Coherence Tomography (OCT) see Fig. (**9**) has been successfully trialed by a team led by J Kobler at Center for Laryngeal Surgery and Voice Rehabilitation, Massachusetts General Hospital [56].

The potential use of OCT to image mucosal tissue was first presented in 1997 by Sergeev *et al.* [57], and there have been numerous references since then. Kobler's device appears to be a promising realization of the technique, and can provide high-resolution (~10-15µm/pixel) images of vocal fold microanatomy. The principle is based on interferometry. The fundamental optics is that a coherent light source is split along two paths, and then remixed. If the path lengths are the same the light sources are still in phase with each-other and the two light sources recombine back to their original amplitude levels. If however one beam travels a different length then the remixed light sources are now out of phase, and there will be a variation in the light intensity – this is known as interference and results in a change in intensity that is related to the extra distance travelled. By using a flapping mirror a line scan across the tissue surface is obtained, and by indexing the position of the sample these line scans can be combined to provide a surface image. The next operation is to trigger the line scan at different stages during the mucosal wave progression; thus the device can obtain a 3D image of the vocal fold surface with respect to time. One advance of this system over its predecessors is that by using Infra Red there is a degree of light penetration beneath the epithelium, estimated to be 1-2mm. By computational analysis of the data it has been possible to reconstruct 3D images with respect to time that also show underlying tissue boundaries.

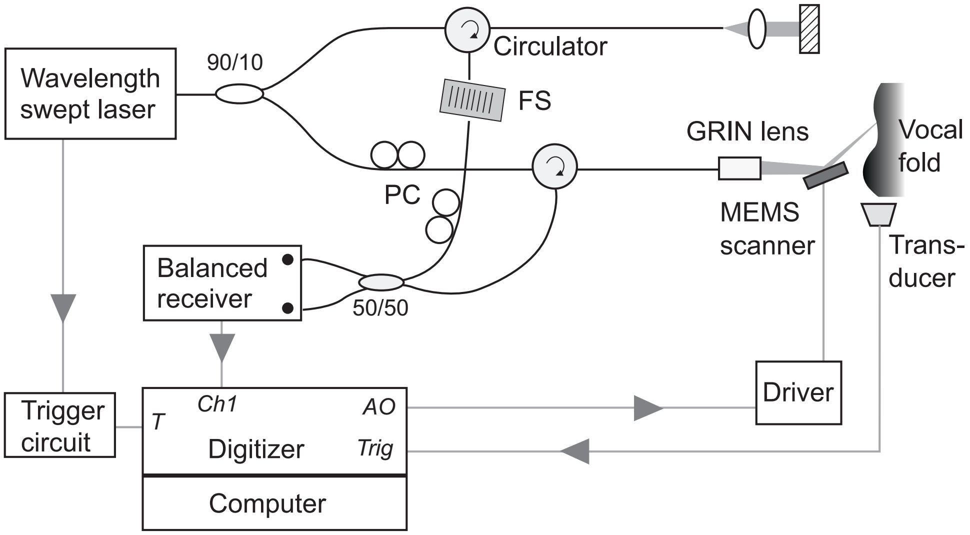

Fig. (9). Schematic of the optical frequency domain imaging, optical coherence tomography (OCT) [57].

3.4.2. Laser Triangulation

The use of lasers, and various imaging techniques, is a well established to determine distance. When coupled with some form of trigger that is related to the generation of the mucosal it is possible to visualize deformation with respect to time, and thus derive rheometric data. There are however few examples of this being done *in vitro*. One good example is an experimental set-up devised by Mannenberg, Hertegard & Liljencrantz [58]. In this device excised vocal fold tissue was mounted on a vibrating membrane to simulate vocal fold deformation during phonation. The laser beam is tracked using a CCD camera, and post-processing of the captured image is used to derive the change in height of the tissue with respect to time. A positional resolution of 30 microns is reported for this method. This *in vitro* device was derived from the extensive work carried out in Sweden, most notably at the Karolinska Institute, on *in vivo* optical measurement techniques of the vocal fold; which are excellently summarized in the doctoral thesis published by Hans Laarson [59].

4. SYNTHETIC PHYSICAL MODELS

Synthetic materials are of importance in voice production research mainly on two counts. First, viscoelastic materials of different stiffness serve as basis for the construction of vocal fold-shaped compliant constrictions in flow experiments. These experiments deal with the causes of flow-induced, self-sustained vocal fold oscillations. The complex fluid-structure-acoustic interaction is to be studied here [60]. Second, one is seeking for biomaterials which can potentially be used for vocal fold augmentation [61]. In both cases, precise biomechanical characterization of the synthetic materials is essential. In the following, several methods are discussed.

4.1. Rheometer Based Techniques

Basically, two different types of rheometers are utilized to identify the mechanical parameters of viscoelastic materials, the torsional rheometer and the linear rheometer. As the working principle and the use of those rheometers for the determination of mechanical properties of tissue has been discussed in Sec. 3, here, we focus on synthetic materials. In general, both types are based on sinusoidal shear deformations of small amplitudes which are applied to the samples. For the first type, the shear deformations result from rotary motion. With the prescribed torque, the measured angular velocity and angular displacement can be used to calculate the viscoelastic properties of the investigated material, such as the elastic shear modulus, the dynamic viscosity and the viscous shear modulus [62]. Due to the variation of the torque's oscillation frequency, the viscoelastic properties can be identified for rather small frequencies (< 0.1 Hz) up to 15 Hz. Chan and Titze [62] applied this technique for the characterization of hyaluronic acid. As the results clearly show, this material offers viscous shear properties similar to human vocal fold mucosa (see Table **1**).

On the other hand, the linear rheometer makes use of sinusoidal translation motion (see Fig. **10** and Sec. 3.3). In order to investigate the materials within the linear viscoelastic region, the strain amplitudes are usually limited to 2% [1]. By means of this rheometer type, the material parameters can be identified in the frequency range of 1 Hz to 250 Hz. Thus, the frequency band of the human phonation process is mostly covered. With the aid of this kind of rheometer, Chan and Rodriguez [1] determined the complex shear modulus and the dynamic viscosity of human vocal folds. The measurement results point out that human vocal folds exhibit extremely low values for the dynamic viscosity at low frequencies. Kimura *et al*. [61] utilized this rheometer type to compare human vocal folds to injectable biomaterials for vocal fold augmentation, like Juvederm, Cymetra, Atelocollagen and Radiesse (see Table **1**). The results for those biomaterials show higher values of the dynamic viscosity at low frequencies compared to human vocal folds. Hence, the investigated biomaterials are not quite appropriate for the reconstruction of the vocal fold lamina and there is still a lot of research necessary on this topic.

4.2. Transmission of Mechanical Vibrations

For this kind of analysis, the mechanical properties of the investigated materials are determined by means of the transmission of mechanical vibrations. In one possible setup, the samples are excited at one side by mechanical vibrations propagating along the sample. For this experiment, samples of special shape are needed. On the other side, the mechanical displacements arising from the mechanical excitation are measured. Depending on both, the shape of the samples and the measuring points on the sample's surface for the displacement, different mechanical quantities can be identified. The mechanical excitation frequency can be varied allowing for the determination of the mechanical properties with respect to the frequency. Madigosky and Lee [63] presented a technique based on the propagation of mechanical vibration for the identification of the dynamic Young's modulus as well as the loss factor of viscoelastic materials. In particular, they used a thin strip of a rubber material which was excited at the bottom end by an electromagnetic shaker Fig. (**11**). The top end of the strip is loaded with a mass. A so-called phonograph cartridge is used to measure the mechanical oscillation of the strip. Since the whole setup was placed in refrigerator oven, the samples could be investigated at different temperatures. In their contribution, Madigosky and Lee estimated the mechanical properties of rubber materials in the frequency range 1 - 10 kHz and temperature range 4 – 47 °C. Additionally, they applied the time-temperature principle to calculate the so-called master curve, which can be used to determine the mechanical properties of viscoelastic material over a wider frequency range than they were acquired [64,65]. They found out, that both the Young's modulus and the loss factor of the investigated rubber materials strongly depend on the frequency. Extended techniques based on simple measurements of the transmitted mechanical vibrations in thin strip are explained in [66].

However, the presented methods can only be used for the determination of the dynamic Young's modulus and loss factor. Therefore, Willis *et al.* [67] developed a technique allowing for the identification of both, the dynamic bulk and dynamic shear moduli of viscoelastic materials. In contrast to the mentioned methods, the measurement setup consists of five independent laser interferometers. The dynamic material parameters are determined by means of a comparison of

Table I. Summary of Applied Devices, Methods, and Analysed Parameters

***In Vivo* Experiments**			
Study	**Method**	**Measured Parameters**	
Hsiao *et al*. [8]	CDI	Vocal fold stiffness: 30 to 120 kPa (men) 120 to 300kPa (women)	
Tran *et al*. [45]	Linear Ergometer	Shear Modulus: 2450Pa to 29400Pa	
***In Vitro* Experiments**			
	Method	**Measured Parameters**	
Alipour *et al*. [44]	Ergometer Measurements on Laryngeal Muscles	Contraction times: 32ms (PCA), 29ms (LCA), 32ms (IA)	
Goodyer [53]	Linear Skin Rheometer	Dynamic Spring Rate in dependence of direction relative to vocal fold axis: From 1.05 g/mm (90°) to 0.6 g/mm (0°) or 0.5 g/mm (180°)	
Hess *et al*. [54]	Linear Skin Rheometer	Dynamic Spring Rate along the vocal fold: at Vocal Process: 4 g/mm to 9g/mm at Anterior commissure: 0.5 g/mm to 1.0 g/mm	
Chan and Rodriguez [1]	Shear Rheometer	Elastic Shear Modulus: 22Pa (1Hz) to 900Pa (300Hz) Viscous Shear Modulus: 70Pa (1Hz) to 500Pa (300Hz) Dynamic Viscosity: 10Pa s (1Hz) to 0.13Pa s (250Hz) Damping Ratio: 3 (1Hz) to 0.5 (250Hz)	
Physical Models			
	Method/Material	**Parameters**	
Chan and Titze [22]	Shear Experiment	Elastic shear modulus @ 0.1Hz	@ 10Hz
	Hyaluronic Acid	~0.1Pa	~1Pa
	Gelatin	10kPa	10kPa
	Vocal Fold (f) Mucosa	10Pa	10Pa
Titze *et al*. [81]	Tecoflex SG-80A, Thermedics in a Special Bioreactor	Estimation of Young’s modulus in situ by probing for resonance frequencies E=7kPa	
Zhang *et al*. [60]	Two-Component Liquid Polymer Solution	One-layer artificial vocal fold, E=3-11kPa	
Pickup and Thomson [82]	Two Layer, Life-Size, Synthetic, Selfoszillating Vocal Fold Models with Asymmetric Stiffness	Silicone compound: DragonSkin^{TM} Young’s modulus: Body: 7.8-9.0kPa Cover: 2.9-8.7kPa PTP: 0.48-1.23kPa	
Kimura *et al*. [61]	Inject. Biomaterials:	Shear modulus @ 100Hz:	
	Juvederm	1980Pa	
	Cymetra	5390Pa	
	Atelocollagen	2030Pa	
	Radiesse	7150Pa	
	Human Vocal Fold	333Pa	
Numerical Models			
	Method	**Parameters Used**	
Hunter *et al*. [76]	Finite-Element Simulation	Poisson’s ratio = 0.47, Young’s modulus(cartilage) = 30MPa, Young’s modulus(tissue) = 20.7kPa, density = 1.043g/cm3, Rayleigh damping (alpha = 32.3, beta = 6.87e-4)	
Alipour *et al*. [83]	Finite-Element Simulation	Poisson’s ratio: longitudinal = 0.0, transverse = 0.9 Longitudinal shear modulus: body = 12kPka, cover=10kPa, ligament=40kPa, transverse shear modulus: body=1.05kPa, cover=0.53kPa, ligament=0.87kPa; Viscosity: body=6 poise, cover=3 poise, ligament=5 poise	

measurements and the results of a finite element (FE) simulation for harmonic excitation (simulation assisted measurement). With the aid of this method, different viscoelastic materials in the frequency range 500 Hz – 2.5 kHz were investigated. Besides the rather complicated calibration of the measurement setup (e.g., laser interferometers), the frequency range of the human phonation process is not covered. However, this method can be used to identify the whole mechanical parameter set of a homogeneous material with respect to the frequency.

Another resonant method is introduced by Titze *et al*. [68]. He measured the vibration pattern of periodically actuated elastic substrates in a bioreactor. In a frequency

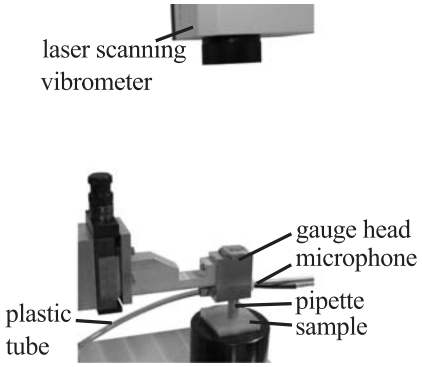

Fig. (10). Rheometer based on translation motion [22].

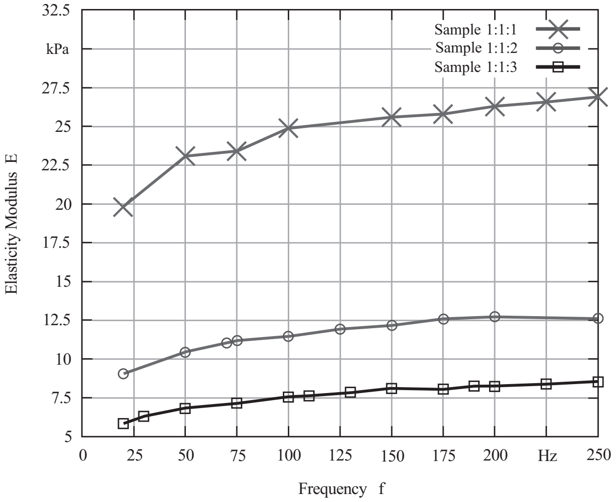

Fig. (11). Schematics of the measurement setup for the transmission of mechanical vibrations [63].

range of 20-200Hz, the vibration of stroboscopically measured patterns could be compared to calculated patterns. Also the viscoelastic properties could not be estimated in situ, the potential has been established. For the future, a combination of the above mentioned methods will offer great potential to develop resonant methods for the characterization of supplementary tissue material in the phonatory frequency range.

4.3. Pipette Aspiration Techniques

Contrary to the previous described approaches (rheometer and transmission of mechanical vibration), the pipette aspiration techniques can be used to identify the spatially resolved mechanical properties of viscoelastic materials. Originally, the pipette aspiration technique was introduced to measure the stiffness and intracellular pressure of red blood cell membranes [69-71]. Zörner *et al.* [3] extended the technique to determine the Young's modulus of soft tissues as well as viscoelastic materials. Their measurement setup is illustrated in Fig. (**12**). A pipette is placed onto the material sample to direct an alternating pressure on the sample inducing a vibration. The alternating pressure is generated by a so-called pistonphone which provides pressure frequencies covering the human phonation process. A laser scanning vibrometer is utilized to measure the mechanical displacement of the sample inside the pipette. The deviation between the measured displacements and the results of a finite element simulation is used to iteratively correct the Young's modulus of the investigated sample. Although, the Young's modulus can be determined spatially resolved due to variations of the pipette positions, the current measurement setup is not applicable for the identification of Poisson's ratio and damping coefficient. Hence, these quantities have to be estimated with alternative measurement principles.

Zörner *et al*. [3] utilized the pipette aspiration technique to investigate viscoelastic materials made of Ecoflex Platinum Cure Silicone Rubber. Three samples with different ratios of silicone thinner were fabricated, namely 1:1:1, 1:1:2 and 1:1:3. The numbers denote the ratio between the two-part silicone composite and the silicone thinner, respectively. Fig. (**12**) shows the resulting Young's modulus for the different samples with respect to the frequency. As one can see, the moduli strongly depend on frequency. For instance, the material 1:1:1 offers a Young's modulus of 5.8 kPa for 20 Hz but 8.5 kPa for 250 Hz. Therefore, the pipette aspiration technique is an important means of measurement for investigating biomaterials, as it works in the phonatory frequency range and on materials that meet the mechanical properties of laryngeal tissue.

5. NUMERICAL APPROACHES

5.1. Identification of Material Parameters

Opposed to most methods described in the previous chapters, the following methods are all based on the idea of

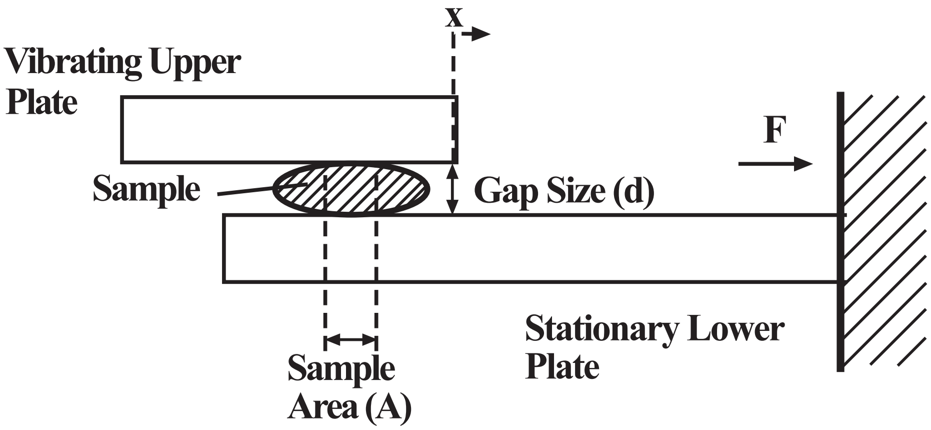

Fig. (12). Laser scanning vibrometer measuring the displacement of the silicone sample through the gauge head (left) and elastic modulus of the three investigated samples (1:1:1, 1:1:2, 1:1:3) plotted against the frequency (right). [3].

using a mathematical optimization approach to identify model parameters which yield agreement between experimental data and simulation results, which is as close as possible.

In [3], a pipette aspiration method is used to determine the frequency dependent Young's modulus of a soft material (e.g. silicone) in a frequency range of 20-250Hz. For this purpose the Young's modulus is considered a variable in a finite-element model, which is adapted to reproduce the mechanical behavior measured in the physical experiment. This approach is local in the sense that it is suitable for determining spatial differences along the surface of the material. The authors apply their method to silicones with characteristics similar to human vocal folds.

A similar experimental setup for identification of viscoelastic material parameters is described in [72]. The authors perform *in vivo* and *in vitro* pipette aspiration experiments and optimize associated model parameters of a viscoelastic, non-linear, nearly incompressible, isotropic continuum to achieve a similar deformation profile in the simulation compared to the one measured in the experiment. Pressure is applied over a period of about 100 seconds, which is far from the frequencies occurring during human phonation. Although the experiments are performed on human uteri and frequency dependent effects in the simulation are not considered, the concept of this approach would also be suitable for identification of the viscoelastic parameters of the human vocal folds, when combined with experiments using higher frequencies (e.g. experiments as described in [3]). Another comparable approach is described in [73], where instead of the pipette aspiration experiment an indention test is used for the generation of the experimental data. A finite-element simulation and a Kalman filter algorithm are then used to fit isotropic elastic material parameters, i.e. Young's modulus and Poisson ratio, to this data.

Finally in [74] an approach using experiments with excised human hemilarynges is described. Different forces are applied and resulting deformation fields are measured. These deformation fields are then used as reference displacements in an optimization method to compute material parameters in finite element simulations see Fig. (**13**), so that the simulation exhibits deformations as close as possible compared to the measured ones. Isotropic, i.e. Young's modulus, Poisson's ratio, as well as transversal-isotropic parameters, i.e. Young's moduli, Poisson's ratios and shear moduli in different directions, are obtained with that approach. It is shown that in this optimization framework manufacturing constraints can be taken into account, which is particularly suitable for determining parameters of physical vocal fold models (e.g. multi-layer silicone).

5.2. Identification of Model Parameters

Not only elastic models, depending on traditional material parameters, e.g. Young's modulus, shear modulus, are considered to describe vocal fold dynamics. Other approaches for modeling the vocal fold tissue including Kelvin models for muscle tissue or chain and mass-spring models see Fig. (**14**) for simulating the dynamical behavior of the vocal folds are widely used. In these mass-spring models several masses connected with springs are subject to a simulation running over time. This is a comparably simple and computationally very fast way to approximate oscillations of a physical body, with however a lower approximation accuracy compared to finite element simulations of the complete elastic body. Though the parameters in such mass-spring models are not material properties in a classical sense, there is a strong relationship to material parameters in underlying physical models. The Kelvin models can be seen as a more realistic representation for muscles, by approximating muscle tissue with both an active and a passive component with different stiffness values and viscous damping. All these models can be used to gain more insight into the phonation process and allow observation of different causal relations; see also section 5.3.

In [75], a modified Kelvin model with parameters for vocal ligament, mucosa and all five laryngeal muscles is presented. These parameters were adjusted using a curve fitting method, a particular instance of inverse optimization techniques, and stress-strain measurements from an ergometer. The optimized parameters are then used to reveal functional differences in the load-bearing capacity of the muscles and stress-strain hysteresis effects. Previously a finite-element model, enhanced by such a Kelvin model, had

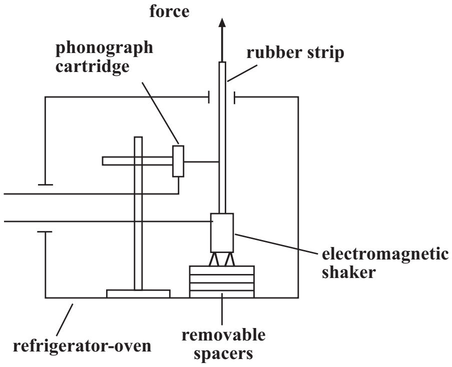

Fig. (13). Cut through a finite element mesh of a 3D three layered vocal fold model [74].

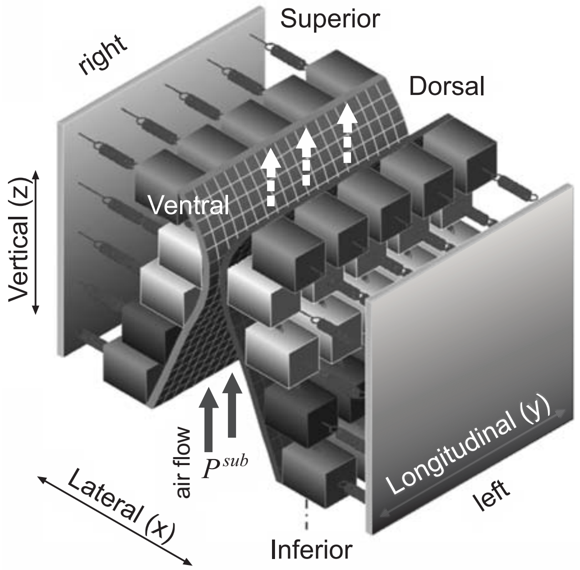

Fig. (14). Three-dimensional multi-mass-model with 25 coupled mass-spring oscillators for each vocal fold [78].

been used by the same authors [76] to show fine accordance in range and speed of motion compared to published vocal fold kinematic data. In all articles described above, the measured data were generated from mechanical experiments with excised larynges. As an alternative, high-speed videos can be used to capture the deformation behavior of the oscillating vocal folds. In [77] the masses, spring constants and damper constants of a symmetric two mass model of the vocal folds are optimized to have a similar dynamic behavior as observed in the video. It is stated that using this approach the mass spring model can accurately describe the vibration of the observed vocal folds. Moreover the authors claim that the increase of longitudinal tension due to the vocal fold elongation from the glottal area signal can be successfully detected. A similar approach is described by [78], where instead of a classic two mass model, a 3D mass-spring, cover-model using 50 masses is developed Fig. (**14**). The additional freedom in the model allows observation of the vertical motion and the entire medial surface of the vocal fold. Moreover symmetric as well as asymmetric vibration patterns can be considered.

Common to all the approaches described is the use of mathematical parameter identification methods, ranging from classical parameter fitting to involved inverse optimization methods based, for instance, on genetic algorithms.

5.3. Application of Constitutive Models

In the following two examples calibrated constitutive models are used to uncover causal relationships during phonation. In [79], the relationship between the phonation threshold flow (PTF) and different phonatory system properties is investigated using a numerical body-cover vocal fold model. It is stated that the PTF can be reduced by decreasing tissue viscosity, decreasing mucosal wave velocity, increasing vocal fold thickness, decreasing prephonatory glottal area, or decreasing vocal tract resistance. Furthermore it is claimed that a divergent glottis can also reduce the PTF. A later paper of the same group [80] uses a chain model with 20 masses of the vocal fold and surrounding tissue to study the influence of mucosal loading, i.e. the loading caused by the interaction between the vocal folds and the surrounding tissue, on the phonation threshold pressure, phonation instability pressure and energy distribution. It is observed that mucosal loading directly influences voice production as it depends strongly on the stiffness constant as well as other constants used in the chain model. Both of these investigations work by just modifying different parameters of a constitutive model, running a simulation and comparing the simulation outcome for different parameters. Though usually these parameters are not material parameters of the vocal fold tissue themselves, many of the parameters are strongly correlated to the material properties, e.g. stiffness, viscosity.

6. SUMMARY

In vivo biomechanical is an important, yet difficult, aspect of current laryngeal research. Since biomechanical properties are so central to proper vocal fold function, their restoration after disease or injury is the goal of most treatment options. CDI, HDSI and OCT offer valuable *in vivo* information and have the potential to be valuable tools for treatment evaluation, but are limited by accuracy and reliability. In order to improve treatment design and evaluation methods, these *in vivo* methods of biomechanical characterization need to be further developed. Validation studies comparing these methods to more widely accepted *in vitro* methods of histological and rheological evaluation, as well as constitutive models, will lend credibility to their use in treatment evaluation.

In vivo and *in vitro* testing of biomechanical properties has seen great progress over the last decade. Imaging, histological and rheological characterization methods have made *in vivo* characterization of biomechanical properties more feasible and accurate.

Further research into the relationships between indirect measurement techniques and direct measurement techniques is required to make *in vivo* and noninvasive biomechanical analysis more reliable and widely applicable, and current progress on this front has created an avenue of research on which to continue. Current biomechanical methods have allowed for characterization of biomechanical properties of the vocal folds, biomechanical changes resulting from pathology, and evaluation of biomechanical changes as treatments attempt to restore function to disordered vocal folds. As these methods are further developed, more research into vocal fold treatment advances will be possible. The advance in optical techniques offers the possibility of future researchers and clinicians the ability to measure in real-time the phonation process *in vivo*, with simple handled devices. A summary of the discussed methods and results is given in Table **I**. The biomechanical characterization and quailfication of synthetic material both for the construction of vocal fold-shaped compliant constrictions in pipe flow experiments as well as for the potential use for vocal fold augmentation are ongoing tasks. The distinct advantages of rheometer based techniques, the mechanical vibrations transmission method and the pipette aspiration techniques have been highlighted. The rheometer based techniques offer the possibility to measure quite small samples at phonatory frequencies and, especially with rotatory principles, sinusoidal signals are easy to apply. The development of new rheometers that are able to operate at phonation frequencies opens up the opportunity for new studies to model the deformation of the vocal folds, and the resultant airflows, within normal pitch range. The mechanical vibration transmission experiments are especially useful to measure frequency dependent damping in combination with elasticity moduli. The pipette aspiration techniques offer the greatest advantages for spatially resolved measurements and possibly for inhomogeneous materials.

Only recently numerical simulation and optimization methods are applied in order to identify material parameters in physical models as well as to calibrate abstract parameters in constitutive models. Although the underlying numerical models are not yet capable of resembling the phonation process in its full complexity, already now they provide additional insight and a better understanding of the causalities in speech generation.

ACKNOWLEDGEMENTS

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