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Impact of Medialization Laryngoplasty on Dynamic Nanomechanical Vocal Fold Structure Properties

Gregory R. Dion, MD; Peter A. Benedict, BA; Paulo G. Coelho, DDS, PhD; Milan R. Amin, MD; Ryan C. Branski, PhD

Objectives/Hypothesis: Although the primary goal of medialization laryngoplasty is to improve glottic closure, implant placement is also likely to alter the biomechanical properties of the vocal fold (VF). We sought to employ novel, nanoscale technology to quantify these properties following medialization based on the hypothesis that different medialization materials will likely yield differential biomechanical effects.

Study Design: Ex vivo.

Methods: Nine pig larynges were divided into three groups: control, Silastic (Dow Corning, Midland, Michigan, U.S.A.) block medialization, or Gore-Tex (W.L. Gore & Associates, Newark, Delaware) medialization. Laryngoplasty was performed on excised, intact larynges. The larynges were then bisected in the sagittal plane and each subjected to dynamic nanomechanical analysis (nano-DMA) at nine locations using a 250-μm flat-tip punch and frequency sweep-load profile across the free edge of the VF and inferiorly along the conus elasticus.

Results: Silastic block and Gore-Tex implant introduced increased storage and loss moduli. Overall, storage moduli mean (maximum) increased from 38 kilopascals (kPa) (119) to 72 kPa (422) and 129 kPa (978) in control, Gore-Tex, and Silastic implants, respectively. Similarly, loss moduli increased from 13 kPa (43) to 22 kPa (201) and 31 kPa (165), respectively. Moduli values varied widely by location in the Silastic block and Gore-Tex groups. At the free VF edge, mean (maximum) storage moduli were lowest in the Gore-Tex group, 20 kPa (44); compared to control, 34.5 kPa (86); and Silastic, 157.9 kPa (978), with similar loss and complex moduli trends.

Conclusion: Medialization laryngoplasty altered VF structure biomechanical properties; Silastic and Gore-Tex implants differentially impact these properties.

Key Words: Larynx, vocal fold, mechanical testing, storage moduli, loss moduli, complex moduli, voice.

Level of Evidence: NA.

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INTRODUCTION

A range of therapeutic options is available to address glottic insufficiency associated with vocal fold (VF) immobility. A recent review identified over 500 studies regarding four primary procedures for VF immobility; however, no differences in outcomes were observed between these rehabilitative techniques.1 First described by Isshiki, medialization laryngoplasty involves the insertion of an implant into the paraglottic space through a lateral window in the thyroid cartilage at the level of the VF.2 Numerous materials have been employed for medialization. No reports exist on how these differing materials affect the dynamic biomechanical properties of the VF.

To date, the majority of laboratory research regarding medialization laryngoplasty has focused on the immunogenic/histologic effects of implants, and the gross static changes in VF length, position, and tension following surgery.3–6 Because phonation is a dynamic process dependent on the appropriate production of a mucosal wave propagating from the conus elasticus through the free edge of the true VF, we hypothesize that investigation regarding the viscoelastic VF properties have substantive clinical relevance. Despite intense interest in the varying viscosities of compounds used in injection medialization laryngoplasty, only one review and a single study investigated the effects of implant stiffness on dynamic outcomes following medialization.7,8 In that study, Zhang et al. investigated the effect of three silicone implants of varying rigidity on acoustic function in excised human VFs.8

A number of recent dynamic models have shown promise for the assessment of VF properties, including force-elongation measurement, linear skin rheometry, simple-shear parallel plate rheometry, torsional parallel plate rheometry, and indentation.9–11 Recently, our group...
described the utility of a nanoindentation-based method using dynamic nanomechanical analysis (nano-DMA) to precisely explore VF biomechanics on a micrometer scale. Although other indentation techniques have been used to analyze the static, elastic properties of the VFs (specifically Young’s modulus), our laboratory described dynamic, viscoelastic biomechanical properties of the excised VFs. Nano-DMA involves the application of a nondestructive force to tissue via a small probe, followed by the recording of a displacement of the tissue in response to this force. Specifically, nano-DMA allows for quantification of the dynamic elastic characteristics of a material through the storage modulus and the viscous properties of a material through the loss modulus. Nano-DMA has been used previously to analyze a wide range of materials and tissue types. Most recently, our laboratory investigated the properties of the VFs via nano-DMA in the context of an intact, healthy hemilarynx ex vivo. In the current study, we sought to assess the sensitivity of nano-DMA to manipulation of the VFs (e.g., medialization) and to quantify the effects of medialization on the biomechanical properties of the VFs as a function of the material employed (e.g., Silastic [Dow Corning, Midland, Michigan, U.S.A.] block or Gore-Tex [W.L. Gore & Associates, Newark, Delaware] strip) for medialization.

MATERIALS AND METHODS

Tissue Preparation and Setup

Nine flash-frozen (−80°C) female pig larynges thawed at −20°C for at least 24 hours were either bisected with a tissue band saw to maintain an intact hemilarynx for testing or thawed at 4°C for 2 to 4 hours for medialization laryngoplasty. Three specimens underwent Gore-Tex medialization, and three specimens underwent Silastic block medialization. To accurately simulate medialization laryngoplasty, both types of medialization (Gore-Tex and Silastic block) were performed on intact larynges (Fig. 1). Specimens undergoing medialization laryngoplasty were bisected immediately after the medialization and tested. Nanomechanical testing was completed using a previously developed testing apparatus to hold the hemilarynx in place, with testing for each larynx completed during a single sitting. Specimens were marked at three locations across the free edge of the VF: anterior commissure region, midmembranous VF, and just anterior to the vocal process. Similar markings were made in the same anterior–posterior position 5 mm and 1 cm inferiorly.

Gore-Tex Medialization Laryngoplasty

Using three intact, thawed, pig larynges, a 4 mm by 8 mm window was made in the thyroid cartilage 3 mm above the lower border of the thyroid cartilage and 5 mm posterior to the anterior border of the thyroid cartilage (Fig. 1A). While visualizing the larynx from above, a 0.6 mm Gore-Tex Cardiovascular Patch (W.L. Gore & Associates) was carefully folded horizontally into the window until the ipsilateral true VF (TVF) was at midline (Fig. 1B). After medialization, the larynx was sagittally bisected, maintaining an intact anterior commissure on the medialized side and immediately subjected to mechanical testing.

Silastic Block Medialization

Using three intact, thawed, pig larynges, a 4 mm by 13 mm window was made through the thyroid cartilage 3 mm above the lower border of the thyroid cartilage. A Silastic block wedge was sculpted to fit within the window, carefully adjusted in each larynx to medialize the VF to midline (Fig. 1C). The larynx was then sagittally bisected and subjected to mechanical testing.

Dynamic Nanomechanical Testing

A Hysitron TI950 TribolIndenter (Hysitron, Inc., Minneapolis, Minnesota, U.S.A.) was used for nanomechanical testing equipped with a 250 μm, round, flat-tip punch. The tip was calibrated in both dry and wet environments using Hysitron TriboScan software (Hysitron, Inc.) calibration procedures. A variety of load function values and profiles were assessed to ensure adequate tip-tissue contact and the collection of data throughout a variety of frequencies. A 1,000 μN force was applied over a frequency sweep from 10 to 105 hertz (Hz) with a 220 μN set-point value. All data were recorded using the Hysitron TriboScan software. An automated pattern collection of four indentations was programmed for each of the nine indentation locations (Fig. 2A), designed far enough apart to avoid local tissue changes from indentation.

Data Analysis

Similar to prior nanoindentation work on the intact hemilarynx, nine locations were tested on each specimen at eight different frequencies between 10 and 200 Hz (10, 23.6, 37.1, 50.7, 64.3, 77.9, 91.4, and 105 Hz) for comparative data. Average indentation depth at each location was 2,763 nanometer for each cycle. Room temperature saline was applied to the hemilarynx every 20 minutes throughout implantation surgery as well as during Nano-DMA testing to maintain tissue hydration. Intra- and inter-intervention group data were compared at the nine separate indentation locations. Loss and storage moduli are described as a function of intervention type; specimen; frequency; and inferior, superior, anterior, and posterior positions using scatter plots constructed using R and RStudio (v0.99.879).
Data Presentation

A nine-segment grid following the contours of the nine regions tested by in each specimen was designed. Storage/loss moduli data were then input using the data visualization software Tableau 10.0 (Tableau Software, Seattle, Washington, U.S.A.) to produce heat maps of laryngeal nanoindentations. These heat maps are visual representations of data in which values were hierarchically color-coded by anatomic subsite to allow for intuitive recognition of areas with high or low storage/loss moduli values.

RESULTS

Sample storage modulus values from repeated indentations at a single point of interest (the midpoint along the true VF margin in Fig. 2B) are shown in Figure 3. These data are from one of the control larynges and demonstrated that, although the local indentations (as shown in Fig. 2A) were separated by at least 125 μm, progressive “stiffening” of the local area was observed on repeated measures. As a result, only initial successful indentation data were used in the remaining analyses. In rare cases in which the initial location did not make contact with the tissue specimen under the automated program (resulting in all 0 values), data were only analyzed from the first spot where contact was made. Eight locations out of 81 failed to yield meaningful data related to this confound. In these cases, the second programmed spot was employed. In one case, data from the fourth programmed spot was collected.

During initial review of the data, it became apparent that, for one of the control samples, the tip never fully contacted the specimen in a single region (the furthest posterior horizontal location along the TVF margin) and all resulting values were 0; this location was omitted from the data presentation and analysis.

Each of the nine indentation locations across all nine larynges had corresponding curved storage and loss modulus relationships across the sampled frequencies, similar to those in Figure 3. The curve shapes and values differed between locations within a sample as well as locations between samples of the same kind of intervention (or control) and across intervention types. As such, the range, mean, and median kilopascal (kPa) values were determined at each point for both the storage and loss modulus to use for comparison and analysis. During each indentation, the nanoindenter tip maintained contact with the surface of the specimen as it cycled through rotations at the eight specified frequency bins. The standard deviations (SD) within these individual measurements during indentation varied between 0.01 kPa and 4.42 kPa for storage moduli and between 0.01 kPa and 5.6 kPa for loss moduli.

Fig. 2. The sequence (numerals 1–4) of four indentations made by the 250 μm diameter, round punch of the Hysitron Ti950 Tribolindenter (Hysitron, Inc., Minneapolis, Minnesota, U.S.A.) at each of the nine indentation locations (A). Image of a sectioned hemilarynx with the testing locations marked with black ink between the anterior commissure (thin arrow) and vocal process (arrowhead; B). [Color figure can be viewed at www.laryngoscope.com.]

Fig. 3. Storage modulus data from repeated indentations at a single point (specifically the midpoint along the true vocal fold margin) plotted as storage modulus (kPa) versus frequency (Hz). Note increased storage modulus values at every frequency with each subsequent trial, indicating progressive stiffening of the local area.
Figures 4, 5, and 6 display heat maps for each of the larynges tested as well as aggregate data for each intervention (control, Silastic block, or Gore-Tex implant). These maps are divided into nine regions based on each of the nine indentation locations and provide a visual representation of the experimental data. Table I and Table II include the aggregate range, mean, and median kPa storage and loss modulus data, respectively, calculated among the three specimens as a function of intervention type at each of the nine points sampled.

Globally, storage moduli mean (maximum) increased from 38 kPa (119) to 72 kPa (422) and 129 kPa (978) in control, Gore-Tex-, and Silastic-implanted larynges. Similarly, loss moduli increased from 5 kPa (43) to 22 kPa (201) and 31 kPa (165), respectively. Aggregate storage moduli values (Fig. 4B) (Table I) varied widely in the Silastic and Gore-Tex groups compared to controls. This finding was particularly pronounced along the row of measurements 5 mm below the TVF margin where mean (median) values were 25 kPa to 39 kPa (29 kPa –38 kPa) compared to 54 kPa to 269 kPa (39 kPa–67 kPa) in the Silastic group and 44 kPa to 143 kPa (40 kPa–90 kPa) in the Gore-Tex group. In addition, variability was observed in the anterior-most and posterior-most position along the free VF margin in the Silastic group, with the aggregate storage modulus values for all specimens within the group ranging from 19 kPa to 978 kPa anteriorly and 5 kPa to 92 kPa posteriorly, compared to 28 kPa to 86 kPa anteriorly and 0 kPa to 77 kPa posteriorly in the aggregate of the control specimens. At these locations, the data from the Gore-Tex group were closer, although lower in value, comparatively at a range of 9 kPa to 42 kPa anteriorly and 9 kPa to 42 kPa posteriorly.

Aggregate loss modulus values (Fig. 4C) (Table II), which increased throughout the frequency bins opposing the behavior of the storage modulus in viscoelastic materials, varied in a similar profile to the storage modulus. Specifically, the aggregate range (median) of the control and Gore-Tex groups varied from 4 kPa to 23 kPa (13 SD = standard deviation.

Fig. 5. Individual heat maps of median storage modulus data for every specimen. The mean storage modulus of the sample medians was 80.61 with a SD of 140.68. The color-scale minimum and maximum were balanced to 1 SD from the mean to include 0 but avoid skew relating to outlying data points. [Color figure can be viewed at www.laryngoscope.com.]

Fig. 6. Individual heat maps of median loss modulus data for every specimen. The mean loss modulus value of the sample medians was 22.16 with a SD of 26.90. The color-scale minimum and maximum were balanced to 1 SD from the mean to include 0 but avoid skew relating to outlying data points. [Color figure can be viewed at www.laryngoscope.com.]

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kPa) anteriorly and 0 kPa to 27 kPa (5 kPa) posteriorly and 0 kPa to 18 kPa (8 kPa) anteriorly and 0 kPa to 26 kPa (8 kPa) posteriorly, respectively. The Silastic group aggregate range (median) varied from 0 kPa to 144 kPa (12 kPa) anteriorly and 2 kPa to 94 kPa (8 kPa) posteriorly.

The greatest variability occurred in the anterior and posterior regions in the Silastic and Gore-Tex samples based on analysis of individual specimens (Fig. 5 for storage modulus values; Fig. 6 for lost modulus values). Heat maps were generated using a color scheme from blue (lower values) to red (high values) corresponding to moduli values, with Figure 4B/4C serving as an illustration of the median data column in Table I and Table II. To create a scale, as well as lower and upper color limits between blue and red, the overall median storage modulus for the 81 locations (nine locations on nine specimens) was calculated at 81 kPa, with a large SD of 141 kPa. The scale for these data was set at ±1 SD from the average median value because 1 SD included 0. Therefore, any measurement of 222 kPa or above was represented as red, and 0 was set to blue. Of note, five values above 222 kPa were obtained, of which four occurred in one of the Silastic block samples and the other in one of the Gore-Tex samples. The colors on the heat map illustrate the numerical trend of increasing storage modulus extending inferiorly from the free VF edge in all specimens, with this increase highly variable and exaggerated in specimens that underwent Silastic block or Gore-Tex medialization. Similarly, heat map representations of the loss modulus in the nine specimens appear almost identical. The overall average of the 81 median loss modulus values was 22 kPa, with a SD of 27 kPa; thus, 0 was set as blue, and 1 SD above the aggregate median value 49 kPa (22 kPa + 27 kPa) was set to red. In the resulting heat map illustrating the numerical loss moduli values, values from Silastic block and Gore-Tex medialization were widely variable and increased more inferiorly than was seen on the heat map for control specimens.

DISCUSSION

Surgical interventions to address glottic insufficiency have largely failed to account for the effect of medialization on the inherent VF tissue properties. Consideration of these properties can likely further optimize

TABLE I.

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<td>Gore-Tex</td>
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This table is arranged spatially according to the anatomic position of each sampled point.

Gore-Tex: W.L. Gore & Associates, Newark, Delaware; Silastic: Dow Corning, Midland, Michigan, U.S.A.

TABLE II.

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<td>Gore-Tex</td>
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Values are organized spatially according to the anatomic position of each sampled point.

Gore-Tex: W.L. Gore & Associates, Newark, Delaware; Silastic: Dow Corning, Midland, Michigan, U.S.A.
these procedures, despite generally favorable outcomes. In that regard, we sought to employ novel technology to provide initial insight into the effects of medialization on the biomechanical properties of the affected VFs. Furthermore, we sought to better characterize nano-DMA as a meaningful tool to capture these data. Our preliminary work with nano-DMA was in normal, healthy excised larynges and we sought to query the sensitivity of this technique as a preliminary step for more wide-spread utility across VF tissue manipulation.

As such, the dynamic storage and loss modulus values from the control group in this study correlated well with the ranges of 6 kPa to 55 kPa for storage moduli and 5 kPa to 20 kPa for loss moduli values at the free VF margin previously reported by our lab using nano-DMA. These values were, however, higher than some statically collected data from canines and humans, in which Young’s modulus varied from 2 kPa to 13 kPa in one study and from 30 kPa to 48 kPa in another study. With regard to medialization, increased storage and loss modulus were seen moving inferior to the free VF margin, particularly anteriorly and posteriorly with Silastic block, and to a lesser extent, Gore-Tex medialization. This finding can likely be explained by the mechanical alterations to the tissue from surgery, which resulted in a broad-based implant pushing medially and putting additional strain on the anterior and posterior portions of the VF complex tethered anteriorly to the anterior commissure and posteriorly to the vocal process. Similar to surgical procedures carried out in humans under flexible laryngoscopy visualization, these procedures were performed under direct visualization to optimize medialization. Because implant shape and size varied in the Silastic block group, and the amount of Gore-Tex folded into the larynx varied in the Gore-Tex group, variations in the resulting mechanical properties are not unexpected.

The wide variability of nano-DMA output was best illustrated in the differences between the aggregate mean and median data found in the Silastic and Gore-Tex groups (Table I). For example, the mean and median storage modulus values of the Silastic block group varied from 309 kPa and 43 kPa anteriorly and 141 kPa and 18 kPa posteriorly along the free VF margin, and 165 kPa and 67 kPa anteriorly and 269 kPa and 56 kPa posteriorly along the data collected 5 mm below the free VF margin. Comparatively, the mean and median values were close in all aggregate control group locations. In the Gore-Tex aggregate data, variations between the mean and median were most prominent in three regions: posteriorly 5 mm below the TVF margin with values of 143 kPa and 63 kPa, anteriorly 10 mm below the free VF edge with values of 116 kPa and 70 kPa, and in the midpoint with values of 111 kPa and 75 kPa. These storage modulus data variations can be visualized in Figure 5. Median values are represented for each of the nine sample locations across all specimens. Clear variability is evident within the Silastic and Gore-Tex groups, with both blues and reds in the same test location between specimens as compared to the control group in which colors are similar for the same location between specimens—illustrating the origin of the differences between aggregate mean and median values for the Silastic and Gore-Tex groups. With the wide range of data values in Table I and Table II and the small sample size, the integration of data into heat maps illustrating modulus values at tested locations allowed for visualization of differences between specimens, locations, and intervention types, as well as within each of the intervention groups.

Nanomechanical assessment of tissue properties with Silastic block and Gore-Tex implants had limitations. The generated heat maps generalized local tissue properties to a region outside the immediately indented point, which likely was not entirely accurate but provides visualization of measured differences. More clinically, no concomitant phonation was observed during medialization, as is the case during sedated surgery in humans; medializations were estimated based solely on adequate medialization of the VF prior to dissection. This limitation may have introduced variability in the size of the implant or quantity of Gore-Tex without confirmation of resulting phonation quality, altering both storage and loss modulus values in the medialization groups. Furthermore, permanent tissue deformations prevent testing on the same specimen before and after medialization.

Given the variability found in the implantation groups, larger sample sizes are necessary to make meaningful statistical comparisons between the data collected for the control and medialization groups. In addition, lack of viable data from the automatically programmed, locally spaced nanoindentations at each of the nine locations on each specimen resulted in the inability to collect repeated measures to assess reproducibility within a specimen. Additional determination of the extent of local tissue effects extending from the 250-μm indentation tip would be useful to determine how close indentations can be to allow for collection of multiple data sets in each region of interest. Despite these limitations, nano-DMA appears to hold value with regard to the acquisition of geographically precise biomechanical data from the VFs.

CONCLUSION

Nano-DMA of the swine hemilarynx after medialization laryngoplasty with both Silastic block and Gore-Tex implants demonstrated altered nanomechanical properties in different regions of the VF complex. These alterations warrant additional investigation to determine how implants may alter phonation not only through glottal shape but also through VF biomechanical properties.

BIBLIOGRAPHY


