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Voice Rest versus Exercise: A Review of the Literature

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Abstract

Voice rest is commonly prescribed after vocal fold surgery to promote wound healing of the vocal fold. Currently, there is no standard protocol that is established based on biological evidence. In orthopedic rehabilitation, long-term rest is found to be less effective for connective tissue healing than exercise. Connective tissue healing is also an important factor for successful voice rehabilitation; however, whether this concept can be extrapolated to voice rehabilitation is unknown. The purpose of this article is to review current clinical and basic science literature to examine the effect of voice rest in post-surgical rehabilitation. First, we present a summary of clinical literature that pertains to voice rest. Second, description of connective tissue that are involved in orthopedic and voice rehabilitation, specifically, ligament and lamina propria, respectively, and their wound healing process are offered. Third, a summary of the literature from orthopedic research on the effect of rest versus exercise is presented. Lastly, it summarizes *in vitro* and *in vivo* studies that examined the effect of mechanical stress on vocal fold tissue. Current literature suggests that there is a lack of clinical evidence that supports a specific type and duration of voice rest, and extrapolation of the findings from orthopedic research may be unreasonable due to the morphological and biochemical difference between the tissues. In order to determine the effect of voice rest, further elucidation of vocal fold wound healing process and the effect of mechanical stress on vocal fold tissue remodeling are needed.

It has been hypothesized that uncontrolled voice use after vocal fold surgery may result in scarring of vocal fold mucosal tissue.¹ Scarred tissue significantly limits vocal recovery due to increased tissue stiffness and viscosity.² To prevent scarring and to promote mucosal healing, clinicians often prescribe voice rest.³⁻⁷ Although this may be common practice, there is no established standard protocol for voice rest and current literature reports that type and duration of voice rest varies among clinicians.^{3, 7} This variation is more than likely due to a paucity of data to support its efficacy.

The effect of rest versus exercise has been a controversial topic in orthopedic rehabilitation research for more than a century.⁸ Rest, in other words, immobilization of a surgical site may intuitively appear beneficial for wound healing of any tissue. However, literature from orthopedic rehabilitation may indicate otherwise. Recent investigations support that

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uncontrolled, excessive mobilization of a joint does result in unfavorable functional recovery; however, controlled remobilization in the early stage of healing leads to favorable functional recovery.⁹⁻¹¹ Long-term immobilization is also considered detrimental to the recovery, therefore it is not generally recommended.^{9, 11-15} Such outcomes rely largely on the degree of connective tissue healing.¹² It has been shown that early remobilization results in restitution of connective tissue architecture that is closer to normal.^{9, 16, 17} Connective tissue also constitutes the lamina propria of the vocal fold,¹⁸ whose structure is crucial for the biomechanical characteristics of the vocal fold tissue.^{19, 20} Whether immobilization or mobilization of vocal fold tissue would help healing of the lamina propria is unknown.

Clinicians in voice and orthopedic rehabilitation share challenges. A patient has a control over his/her movement, and excessive mechanical stress negatively affects tissue healing.^{1, 9} Therefore, they must consider the following questions when developing their post-surgical treatment plans – 1) Does rest facilitate better healing than exercise? 2) If exercise is more effective, what kind of, and how much exercise is appropriate? and 3) When should the patient resume use of tissue after surgery? Currently, voice rehabilitation clinicians do not have enough evidence that provides answers to these questions. Whether research findings in orthopedic research would generalize to rest versus exercise in voice rehabilitation is an intriguing question. The purpose of this review paper is to explore this question by examining current findings in clinical literature that pertains to voice rest and basic science literature in orthopedic and voice rehabilitation from a translational perspective.

Clinical Studies on Voice Rest

There is a paucity of clinical studies investigating the efficacy of voice rest. Koufman and Blalock attributed this lack of data to three factors which make systematic research on this topic challenging.³ The first factor being that variability in patient compliance for voice rest due to its impracticality. The second is inconsistencies in the type and duration of voice rest recommended. Third is lack of standards in the diagnostic approach of voice disorders. These authors conducted a retrospective study to define contributing factors for the outcome of vocal fold surgery. Along with several factors such as patient compliance with reduction of vocally abusive behaviors and type of lesion, they examined whether absolute voice rest versus voice conservation influenced their outcome -- duration of post-operative dysphonia. Type of voice rest did not significantly influence the outcome measure, yet patient compliance did. Based on these findings, the authors concluded that voice conservation may be more practical approach, and as effective as absolute voice rest.

The inconsistencies in the treatment approach among clinicians were observed by a survey study by Behrman and Sulica. They examined current opinions on voice rest among 1,208 otolaryngologists in the United States.⁷ Specifically, they evaluated these otolaryngologists' preference for absolute voice rest versus relative voice rest as well as the duration of voice rest following vocal fold surgery for vocal fold nodules, polyps, and cysts. It was found that 51.4% of the clinicians preferred absolute voice rest and 62.3% preferred relative voice rest. Surprisingly, 15% responded that they never recommend either absolute or relative voice rest. The most common duration was seven days with the range of zero to 14 days for absolute voice rest and zero to more than 21 days for relative voice rest. There was no significant difference with the type of voice rest recommended based on the lesion type.

The clinical studies noted above indicate that patient compliance may be necessary for a successful outcome and there exists a wide variety of clinician's preference for voice rest. The rationale for the type and duration of the recommendation does not appear to be based upon wound healing physiology. While there is a great need of prospective clinical

evaluation of voice rest, one way to ameliorate this problem may be to evaluate the current practice based on the current understanding in vocal fold biology.

Biological Aspects of Voice Rest

The effect of immobilization versus remobilization on connective tissue healing has been extensively studied utilizing a knee ligament model. In order to examine whether it is reasonable to extrapolate findings using this model to the lamina propria of the vocal fold, we must first understand characteristics of these tissues.

Animal tissue is categorized into four types: epithelial, connective, muscle, and neural. Epithelial tissue is represented by the outermost layer of organs, such as the skin, respiratory and intestinal tract. Its primary function is to cover surfaces that are exposed to an external environment. Connective tissue provides a support for the surrounding tissue. It has many forms including dermis, ligaments, tendons, cartilage, bone, and adipose tissue. Density, type and orientation of fibrous tissue, and cellularity influences the mechanical characteristics of these tissues.²¹ Muscle tissue is a specialized tissue that has ability to contract. Broadly, it is categorized into three types: skeletal striated, cardiac striated, and smooth. Skeletal striated is the only type of muscle that allows voluntary control. Cardiac striated muscles are found in the heart and some major blood vessels such as aorta. Smooth muscles are found in vascular tissues and the walls of visceral organs. Nervous tissue allows communication between body parts by conducting electrical impulses. It consists of two main cell types: neurons and neuroglia. Neurons are specialized for rapid communication and they are composed of three parts: cell body, axon, and dendrites. Neuroglia form a scaffolding of nervous tissue and support, insulate and nourish the neurons.²²

Connective tissue is composed of cells and extracellular matrix (ECM). The cells in the connective tissue are categorized into two major types: stationary and migrating cells.²¹ Stationary cells are represented by fibroblasts, which secrete substances for maintenance of the ECM. Migrating cells include mast cells and leukocytes. Mast cells secrete substances that are involved in inflammatory process when activated by chemical and/or mechanical stimulation. Leukocytes remove foreign substances such as bacteria and cellular debris from the site of injury.

ECM constitutes the major portion of connective tissue, and is composed of fibers and ground substances. ECM fibers include collagen, elastic, and reticular fibers.²¹ Collagen fibers provide a scaffold to amorphous materials in the ECM and contribute to mechanical characteristics of the tissue.^{21, 23} The fundamental unit of collagen fiber is α -chain, which is produced by fibroblasts. Alpha chain is a polypeptide chain with three types of amino acids.²¹ It is the unique characteristic of these chains that it has a sequence of glycine-X-Y.^{24, 25} X and Y can be any amino acids, but proline and hydroxyproline occur frequently in these chains. Three α -chains fold to make a rope-like triple helix called procollagen. Hydroxyproline plays an important role for the maintenance of the triple helical form.²⁵ When fibroblasts secrete procollagen to extracellular space, it becomes converted to tropocollagen. Multiple tropocollagen bind to each other to form collagen fibrils, and multiple fibrils aggregate and form collagen fibers.²³ There are at least sixteen types of collagen.²⁶ Variations in the type and sequence of amino acids, as well as the combination of the polypeptide chains distinguish one type from another. Multiple type of collagen are present in connective tissues; however, collagen type I is found most commonly. Elastic fibers are composed of the protein called elastin. Elastin provides elasticity to the tissue. Reticular fibers are collagen fibers with small diameters and they are found around cells in the connective tissue.²¹

Ground substance is a viscous material that is composed of large carbohydrate molecules called glycosaminoglycans (GAGs) and proteoglycans. These molecules play a number of roles for maintaining the tissue's characteristics. One of these functions is to attract water molecules to provide hydration to the tissue, which allows the tissue to withstand compressional forces.²⁷ They also regulate physicochemical environment for cellular activities.

Connective tissue is categorized into two major types: specialized and proper. Tissues such as cartilage, bone and blood are considered specialized connective tissues. Connective tissue proper is further categorized into three types based on the density and arrangement of the collagen fibers: dense regular, dense irregular, and loose irregular. Dense regular connective tissue is characterized by parallel orientation of densely packed collagen fibers. It contains relatively less amount of ground substance than loose connective tissue. Tendons and ligaments are considered dense regular connective tissue. Dense irregular connective tissue is characterized by randomly oriented or interwoven collagen fibers. Some examples of dense irregular connective tissue are dermis of the skin and joint capsules. Lastly, loose irregular connective tissues are characterized by sparsely distributed collagen fibers that are in random orientation. It contains greater amount of elastin, which provides elasticity to the tissue.¹² A summary of structure and characteristics of these tissue types is provided in Table 1. The vocal fold has not been classified based on its connective tissue type. The following section will present anatomical descriptions of the vocal fold connective tissue in attempt to make a comparison with other tissues mentioned above.

The Vocal Fold

The vocal fold has been described both anatomically and functionally. Anatomically, there are five layers that include three tissue types. The most luminal layer is the epithelium. The basal side of the epithelium is attached to relatively amorphous connective tissue called lamina propria via the basement membrane zone. The lamina propria consists of three layers; superior, intermediate, and deep layer of lamina propria. The innermost layer of the vocal fold is the thyroarytenoid muscle.¹⁸ Functionally, the epithelium, and superficial and intermediate layers of lamina propria construct the “cover,” and the deep layer of lamina propria and the thyroarytenoid muscle construct the “body” part of the vocal fold.²⁸

Lamina propria: Connective tissue of the vocal fold

The “cover” is more pliable than the “body,” therefore, pliability of the lamina propria affects voice quality to the greatest degree. Mechanical property of connective tissue is determined by the proportion of its constituents and their orientations. The lamina propria of the vocal fold contains glycoaminoglycans and proteoglycans such as hyaluronic acid, decorin, fibromodulin, versican, and heparin sulfate. These interstitial molecules affect viscosity of the tissue.¹⁸ In terms of extracellular fibers, collagen constitutes approximately 43% of total protein in the lamina propria of the human vocal fold. This is roughly 30% less than that of the dermis.²⁹ Collagen types I,^{18, 29, 30} III,^{18, 29, 30} IV,^{18, 29, 30} VI³¹ and VII^{18, 29} have been observed with types I and III being the predominant types.^{18, 29, 32-35} The proportion of these fiber types have been reported as type III greater than type I.^{32, 36} In terms of morphology, both collagenous and reticular forms have been observed in types I and III.³² A Picrosirius-polarization study have reported that thick fibers that appear as collagen type I is found immediately below the epithelium and above the thyroarytenoid muscle while thin fibers that appear as type III is found between those layers of type I.³⁴ These collagen fibers have “wicker-basket” orientation, which allows the tissue to withstand mechanical stress from multiple directions.³⁴ Reticular fibers have been observed in the superficial and intermediate layers of the lamina propria, surrounding glycoprotein and glycosaminoglycan in the tissue.^{37, 38} Elastin is also found in the lamina propria of the

human vocal fold. The amount of elastin in the lamina propria has been reported as roughly 8.5% of total protein, which is approximately twice as much as found in the dermis.³⁹

Ligament

Characterization of ligament is somewhat difficult because of its wide structural diversity. Even between two ligaments in a knee joint, the anterior cruciate ligament and the medial collateral ligament, there are differences in their cell shape and blood supply.⁴⁰ In general, ligament is a dense fibrous connective tissue which connects bones or bones to internal organs. Fibroblasts constitute the cellular part of this tissue type. Collagen constitutes 70-80% of ligament tissue in dry weight.⁴¹ Type I collagen is the predominant collagen type in ligament, and it constitutes 90% of all collagen fibers,⁴⁰ with type III, V, X, XII, and XIV also being described. Collagen fibers in the ligament are longitudinally arranged. Ground substance is composed of proteoglycans and GAGs, and these molecules constitute approximately 1% in dry weight. Elastin fibers constitute 3-5% of dry weight.⁴¹

There is a clear difference between the lamina propria of the vocal fold and ligament in terms of their structure and constituents. Considering how they differ in their functional role and morphology, this is not surprising. Based on the arrangement of the collagen fibers, the vocal fold may be closer to the skin, thus categorized as “dense irregular connective tissue.” On the other hand, ligament is categorized as “dense regular connective tissue.” Their collagen orientation follows the direction of mechanical stress applied to these tissues. Proportion of their ECM constituents also significantly differs from each other. Table 2 summarizes these differences in the ECM constituents. It should be noted that direct comparison is difficult due to an inconsistency in their quantification methods. However, the lower proportion of collagen and the higher proportion of elastin in the vocal fold lamina propria appear to explain greater pliability of the tissue.

Characteristics of scar: general, ligament, vocal fold

Pathology of the vocal fold includes both benign and malignant. Malignant lesions typically progress from epithelial tissue to deeper tissue, potentially affecting all of the tissues that make up the vocal fold.⁴² On the other hand, benign lesions typically involve only the epithelium and lamina propria. For a malignant lesion, the primary goal of vocal fold surgery is resection of diseased tissue to prevent its recurrence. A secondary goal is to restore voice quality, as is the primary aim of vocal fold surgery for a benign lesion.⁴² For best results in both cases, restitution of the tissue without a scar is ideal.

Scar is the result of incomplete remodeling of the wounded tissue. Disorganized arrangement of collagen fibers is a hallmark of histological characteristic for scarred tissue.^{43, 44} Scar is biochemically different from normal tissue. For example, the level of hyaluronic acid⁴⁴ and collagen type III⁴⁵ is lower in dermal scar tissue. The level of glycosylation of hydroxylysine residues is also lower in the dermal scar,⁴⁵ which indicates alteration in the process of procollagen formation.⁴⁶ Scar of ligament tissue has also been shown to be characterized by disorganized collagen, and its collagen fibril size is smaller than that of normal ligament. Other characteristics of ligament scars that have been reported in the literature are defects between collagen fibers, immature cross-links of collagen, and greater amounts of collagen type III. Taken together, these factors are thought to be the reason for mechanical inferiority of the scarred ligament.⁹

Vocal fold scar shares some of these characteristics of dermal and ligament scar. Animal studies with canine and rabbit model report disorganized elastin and collagen fibers. Stiffness and viscosity of the tissue were greater than normal.^{2, 47, 48} A decrease in elastin and collagen fibers and increase in procollagen have been observed with rabbits at two

months after injury.^{2, 47} After six months of injury, collagen fibers increased, formed thicker bundles, and their arrangement was more organized.⁴⁷

Wound healing

For the details of wound healing process, the reader is referred to more comprehensive reviews.^{43, 49-51} Briefly, the general wound healing process is grossly divided into three phases; inflammation, proliferation, and maturation.⁴³ These phases overlap, and are not independent of each other. The wound healing process of the vocal fold described by Thibeault and Gray⁴⁹ are divided into seven phases; hemostasis/coagulation, inflammation, mesenchymal cell migration and proliferation, angiogenesis, epithelialization, protein and proteoglycans synthesis, and wound contraction and remodeling.⁴⁹

Ligament wound healing

Ligament wound healing follows the same general wound healing sequence of hemostasis, inflammation, proliferation and remodeling.⁵² The specific timeline of each phase may be difficult to define due to its structural diversity among subtypes and the extent of injury created in various studies. In general, the first event is exudation of blood and associated blood products which trigger the inflammatory response between cells and mediators. In the rat MCL, inflammation (measured by changes in neutrophils and macrophages continues from day 3-5 post injury. Vascular endothelial growth factor a potent angiogenic factor, also peaks during this time period. Fibroblast proliferation and collagen synthesis follow. Collagen type III is laid down first and provides mechanical strength to the newly formed ECM. While collagen type III is increasing, type I collagen is decreasing. For the partially or completely transected medial collateral ligament, fibroblasts become the predominant cell type and immature collagen fibers appear in two weeks after the injury. Collagen type III is the substantially higher for at least 4 weeks post injury when compared to type I levels. Cellularity gradually decreases and collagen fibers mature over time.⁴⁰ Collagen fibers also become more organized along the axis of ligament. The remodeling process may continue for several months or even years.

Vocal fold wound healing

Inflammation, hemostasis, and epithelialization start immediately after vocal fold injury. Normally, completion of hemostasis takes 24 hours, inflammation continues four to seven days, and epithelialization takes about six days in vocal fold.⁴⁹ Inflammatory response has been observed in rat vocal fold as measured by changes in the expression level of various genes^{53, 54}. A study utilizing rabbit vocal fold observed fibrinous clots 24 hours after injury.⁵⁵ Re-epithelialization has been observed to start one⁵⁶ to three days⁵⁷ after the injury with rats, with completion of re-epithelialization occurring five days after injury in rabbits,⁵⁵ seven days after injury in dogs,¹ and 14 days after injury in rats.⁵⁷ Proliferation phase starts two to four days after the injury.^{43, 49} Proliferation of mesenchymal cells, multipotent stem cells that can differentiate into different type of cells, and angiogenesis start two days after the surgery.⁴⁹ A peak of proliferation of the fibroblasts has been observed three days after injury in both rabbits⁵⁵ and rats.⁵⁶ Protein and proteoglycan synthesis, wound contraction and ECM remodeling start around three days after injury. Protein and proteoglycan synthesis can take up to two months, and wound contraction and remodeling can take up to a year. Animal studies have observed changes in the level of ECM constituents such as collagen,^{2, 57} elastin,^{2, 48} procollagen,⁴⁸ decorin,⁵⁸ fibronectin,⁵⁸⁻⁶⁰ fibromodulin,⁵⁸ and hyaluronan⁶⁰⁻⁶² during this period. The process of ECM remodeling varies among studies, however. Some indicate complete remodeling by two weeks¹ while others indicate continuation of healing process. The variation may be attributed to design of these studies (e.g. different species,⁶³ degree of injury,⁶⁴ surgical techniques and characterization methods).

Rest versus Exercise

It is well-recognized that cells are responsive to the mechanical stress which is important for maintaining tissue homeostasis.⁶⁵ For example, astronauts experience a decrease in bone density and muscle mass from long-term spaceflight,⁶⁶ and weight lifting helps increase bone density.⁶⁷ These tissue changes occur due to the metabolic change in the cells that are the source of bone. A number of studies with other types of tissue have demonstrated that application of mechanical stress influences wound healing. Repetitive loading of mechanical stress affects cell shape, proliferation and differentiation.⁶⁸⁻⁷⁰ Specific outcomes depend on the amount and type of stress.⁷¹ For example, tension applied on wounds may stimulate collagen synthesis and help align collagen fibers in the direction of force applied, but prolonged application of tension may also result in hypertrophic scarring.²³ Inflammatory responses in the vascular wall of artery depends on the type of stress such that laminar-shear stress elicits anti-inflammatory effect while oscillatory or disturbed flow elicits inflammatory effect.⁷² An *in vitro* study using airway epithelial cells, has shown that application of cyclic elongation and compression significantly slowed airway epithelial repair.⁷³

In orthopedic rehabilitation, it is generally considered that long-term immobilization is detrimental for functional recovery. This is not only because of atrophy of the muscles and bones but also because of alterations that occur in connective tissue. Immobilization slows down turnover of ECM constituents,⁷⁴ and leads to more disorganized ECM arrangement.¹⁷ It has been demonstrated that immobilization leads to deposition of collagen in a random, “haystack” arrangement.¹² Twelve weeks of knee joint immobilization in rabbits caused loss of collagen mass, which lead to significantly decreased tensile strength and elastic modulus of the ligament.¹⁵ Long-term immobilization not only affects collagen synthesis and deposition, but also production of ground substance. A decrease in ground substance results in a loss of water in the ECM¹⁷ decreasing the ability of the joint to be a shock-absorber. A decrease in the ground substance also reduces distance between the collagen fiber, causing these fibers to more than likely to adhere to each other and lose their normal configuration.

Recent orthopedic studies indicate mobilization is more favorable to ligament restitution.^{10, 14, 75} Remobilization prevents the adhesion of collagen fibers and stimulates the production of ground substance.¹² It increases collagen synthesis⁹ and helps deposition of collagen fibers in the direction of the movement.¹² Animal studies report that biomechanical and morphological property of healed ligament was closer to normal with early initiation of remobilization.^{11, 14} The mobilization produces better outcome possibly due to increase in blood flow which results in the faster resolution of inflammation.⁹

It should be noted that the time from injury to initiate exercise is not the only consideration in orthopedic rehabilitation. The amount of mechanical stress applied in post-surgical orthopedic rehabilitation must be also carefully controlled so that it will not exceed a stress level that the tissue will not be able to tolerate. The outcomes depending on the timing and amount of stress are shown in Figure 1. For better control, stress passively applied by others, during the early stage of healing has been advocated. Although this effect is controversial, a technique called “continuous passive motion” (CPM) has been shown effective in healing of the cartilage, ligaments and tendons.^{76, 77} It has been proposed that that continuous movement minimizes scar formation by stimulating cellular differentiation, nutrition, and metabolic activity at the injury site.^{8, 78}

The biological effect of mechanical stress on the vocal fold

The effect of mechanical stress on wound healing process has not been studied extensively in vocal fold tissue. Current literature presents two approaches that have been utilized to

study the effect of phonation on the vocal fold tissue. One way is with the use of animal models and the other is with the use of bioreactors. Both approaches have their advantages and disadvantages. Animal models provide *in vivo* environments that are biochemically closer to the system of interest (i.e. human vocal fold) than engineered *in vitro* bioreactors would provide. Animal models also provide morphological information. However, the differences between animal and human vocal fold such as structure, metabolic rate,⁶³ and innate ability to withstand mechanical stress from phonation may limit the animal model's generalizability. Bioreactors detour this issue by allowing one to study cells of interest thus providing information that is unique to the species and organ of interest.

Detrimental effects of excessive phonation on the vocal fold tissue have been shown by a histological study using a canine model.⁷⁹ After two hours of artificially induced phonation, scanning electron microscopy revealed no damage to the basement membrane, basal cell layer, or lamina propria. However, four to six hours of phonation resulted in significant destruction of the area. Injury was characterized by loss of the surface microridges, separation of hemidesmosomes and presence of interstitial fluid beneath the basal cells and the basement membrane.

An animal study by Cho and his colleagues evaluated the effect of voice rest after vocal fold surgery. Bilateral excision of vocal fold mucosa was performed followed by simulated "voice rest" induced with resection of the left recurrent laryngeal nerve.¹ The healing process of this group was compared to "phonation" group, which did not receive the recurrent laryngeal nerve resection. Both groups showed complete re-epithelialization one week after the surgery. However, while the basement membrane of the "voice rest" group showed complete rearrangement two weeks after the surgery, the basement membrane of the "phonation" group showed various alterations. At four weeks, the "phonation" group showed a basement membrane that was separated from the basal cell layer and formed a multiple-layer structure. This multiple-layer structure was unique to the "phonation" group, and observed even after twelve weeks post surgery. Furthermore, restitution of the basement membrane zone anchoring fibers and collagen type III fibers was observed at the fourth week with the "voice rest" group; however, not with "phonation" group until the eighth week. Based on these findings, the authors concluded that voice rest promotes restoration of the tissue and recommended two weeks of voice rest and eight weeks of vocal hygiene after phonosurgery.

More recently, the effect of phonation on gene expression was studied using a rabbit model. Rousseau and his colleagues induced phonation by passing air through adducted vocal folds with electrical stimulation of the recurrent nerve. Vocal folds were collected one hour after three hours of phonation. Gene expression of matrix metalloproteinase-1 (MMP-1), an enzyme that breaks down collagen I and III, significantly increased while gene expression of MMP-9, an enzyme that degrades basement membrane collagen, and IL-1 β , a cytokine involved in inflammation, did not change. Because measurements were taken at one time point only, the study did not provide information regarding how different durations of the non-phonatory period may have influenced the outcome. However, changes in gene regulation found in this study suggest that mechanical stress from phonation influences the biochemical pathway for the maintenance of vocal fold connective tissue.

An engineered bioreactor consists of mobile parts that allow an experimenter to control various parameters such as magnitude and duration of mechanical stress applied to cells of interest. Bioreactors have been used extensively in orthopedic⁸⁰⁻⁸² and cardiovascular⁸³⁻⁸⁶ studies. In both fields, it has been well recognized that different type, magnitude, and duration of mechanical stress alters the level of gene and protein expression, thus affecting the tissue regeneration process. It is believed that studying the cells in the

most biologically and physically realistic environment allows us to obtain an observation of cellular behavior that is closest to the phenomena *in vivo*.

Few studies have examined the effect of mechanical stress on fibroblasts from the vocal fold using such bioreactors. One study studied the anti-inflammatory effect of mechanical stress on fibroblasts from rabbit vocal fold. The cells were cultured under biaxial cyclic tensile strain (CTS) at various magnitude and frequency. The inflammation was induced by treating the fibroblasts with IL-1 β . It was found that CTS at low magnitude (6%) and frequency (0.5 Hz) decreased induction and synthesis of pro-inflammatory genes, which was interpreted as anti-inflammatory response. Another bioreactor study used a device that stimulated the cells at magnitudes and frequencies that are closer to phonation.⁸⁷ The level of mRNA expression of various matrix proteins and proteoglycan and hyaluronic acid associated gene products was studied with human vocal fold fibroblasts in three conditions: static, 20% strain, and 20% strain with vibration at 100 Hz. After six hours, an increase in the expression of these genes was greater with mechanical stress. Specifically, expression levels of matrix protein gene products and proteoglycans/hyaluronic acid gene products were significantly increased with the combination of strain and vibration. Additionally, a difference in the spatial distribution of cells and matrix was observed with 20% strain condition. The findings from these studies show that vocal fold fibroblasts are responsive to mechanical stress, thus it is an important factor to be considered in studying their behavior. Further investigation is necessary to understand specifically how cell respond to mechanical stress and if this changes depending upon type and frequency of mechanical stress.

Concluding Remarks

The review of current clinical literature suggests that voice rest is commonly recommended after phonosurgery, the primary factor that influences treatment outcome is patient compliance to voice rest, absolute voice rest may not be more effective than conservative voice use and vice versa, and typical recommended duration of voice rest is one week. The review of biological literature suggests that vocal fold wound healing closely follows the general wound healing sequence, vocal fold fibroblasts do respond to mechanical stress, the anatomy of connective tissue examined in the immobilization versus mobilization studies in orthopedic literature is significantly different from vocal fold lamina propria, and early, controlled mobilization is beneficial for connective tissue restoration for knee ligaments, however, its effect on vocal fold tissue is unknown.

The question of the effect of rest versus exercise in orthopedic rehabilitation arose from astute clinicians who observed the difference in functional recovery. It is this question that elicited a series of clinical and basic science investigations to assess these treatment approaches. The field of voice rehabilitation generally accepts that uncontrolled phonation immediately after the surgery is detrimental to wound healing; however, it has not found whether prolonged voice rest hinders the functional recovery. (Figure 1) Finding the answer to this question may be the first step. If it is found to be a negative approach, appropriate timing for exercise initiation, as well as the type and amount of exercise need to be defined. Prospective, randomized, double-blind clinical studies with objective outcome measures may provide more information on the functional outcome. Designing such clinical studies may be difficult. For example, it is challenging to control variables such as patient's susceptibility to scarring, lesion type, degree of post-surgical injury, surgical technique, type of voice rest, and compliance. These factors are under-investigated both clinically and biologically.

The studies of the vocal fold wound healing indicate that some findings in other fields may be generalizable to its healing process. One of these findings is that vocal fold cells are

responsive to mechanical stimulus. This finding supports clinical supposition that phonation affects the biological process of vocal fold wound healing. Changes in gene regulation in the vocal fold from phonatory stress have also been demonstrated both *in vitro* and *in vivo*. Although experimental conditions were not close to physical environment for phonation, the anti-inflammatory response to mechanical stimulus is an interesting finding.⁸⁸ These basic science studies will serve as a ground work for the further translational investigations. Another important finding is that vocal fold tissue undergoes the phases of wound healing similar to others. However, as suggested by the different wound healing pattern between the ligaments in the knee joint, healing process is tissue dependant. Therefore, it is reasonable to think that there may be a biochemical process and timing of wound healing that are unique to vocal fold tissue, and the extent of the difference is unknown. Currently there is only one study that compared healing process of vocal fold to dermal tissue.⁶⁰

The morphological and biochemical differences between ligament and vocal fold lamina propria suggest that extrapolation of findings from orthopedic literature may be unreasonable. The rationale for mobilization of joint during the early stage of wound healing is to stimulate collagen synthesis and encourage the fibers to arrange themselves in the direction of the movement. Collagen fiber arrangement of the vocal fold tissue and the direction of mechanical stress applied during phonation are more complex than those of the ligament. Whether phonation can help formation of such complex architecture is questionable. Also, the rehabilitation goal for ligament healing is quite different from that for vocal fold healing. While orthopedic rehabilitation aims for increasing scar strength and stiffness of the tissue, voice rehabilitation aims for reducing stiffness and viscosity of the tissue. These differences indicate that assessing the effect of phonation on vocal fold tissue remodeling requires its own investigation.

Vocal fold wound healing literature reports that fibroblast proliferation, initiation of collagen production, and completion of re-epithelialization by a week after surgery.^{1, 55, 56} These observations imply that the typically recommended duration of voice rest may be biologically appropriate. However, it should be noted that these observations are with small animals; therefore, how the biological difference between the species may affect the generalization is unknown. These animal studies report that the wound healing process continues even after the recommended period of voice rest. This finding suggests that careful monitoring of recovery process and voice use after completion of voice rest is clinically important. Currently, voice therapy during this period aims to reduce excessive mechanical stress to the vocal fold tissue to prevent recurrence of phonotrauma. If application of mechanical stress is found to be effective in vocal fold tissue remodeling, voice therapy for “correct” voice production would gain another facilitative role.

In examining the effect of rest versus exercise in voice rehabilitation, it should be noted that absolute immobilization of vocal fold tissue is probably impossible. Although the amount of force may be negligible compared to the impact force from vocal fold collision during phonation, vocal fold tissue constantly experiences mechanical stress from non-phonatory laryngeal activities. In the early stage of ligament healing, passively applied force may be more effective for functional recovery than actively applied force; however, applying passive force to the vocal fold tissue *in vivo* to test this concept is neither possible nor meaningful.

In summary, the present review of current literature suggests that voice rest facilitates vocal fold wound healing better than uncontrolled phonation, especially in the early stages of healing. More clinical investigation is needed to examine how the treatment strategy, patient history and behavior interplay for successful outcome. More biological investigations are needed to assess the effect of voice rest versus exercise on tissue remodeling. Previous

investigations unveiled some of the biochemical events in the wound healing process. However, elucidation of this process is far from complete. Animal studies will provide further understanding of histological and biochemical changes occur during the wound healing process. *In vitro* studies will provide understanding of basic cellular response to injury and mechanical stress. Translational studies are needed to assess how much of the microscopic difference affects the function. Additional research in interrelationship between mechanical stress and vocal fold wound healing will aid evaluation of voice rest.

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Figure 1.
The effect of rest versus exercise in voice and orthopedic rehabilitation. (Adapted and modified from Frank, 1996.)

Table 1

Classification of connective tissue proper (adapted from Cantu & Steffe)

Dense regular	Ligaments, tendons	Dense, parallel arrangement of collagen fibers; proportionally less ground substance
Dense irregular	Aponeurosis, periosteum, joint capsules, dermis of skin, areas of high mechanical stress, <i>vocal fold?</i>	Dense, multidirectional arrangement of collagen fibers; able to resist multidirectional stress
Loose irregular	Superficial fascia sheaths, muscle and nerve sheaths, support sheaths of internal organs	Sparse, multidirectional arrangement of collagen fibers; greater amounts of elastin present

Table 2

Morphological and biochemical differences between lamina propria of the vocal fold and ligament

Collagen orientation	Interwoven, "wicker basket"	Parallel, longitudinal
Collagen types	I, III, IV, VI, VII	I, III, V, X, XII, XIV
Proportion of collagen	43% of total protein; Type III comprises ~40%	70% of dry weight
Proportion of elastin	8.5% of total protein	3-5% of dry weight
Ground substance	HA: 0.64% of total protein	1% of dry weight
	GAGs:	
	Chondroitin sulfate/dermatan sulfate – 23.9%	
	Keratin sulfate – 14.7 %	
	Heparin sulfate – 61.4%	