Giuseppe Bergamini · Livio Presutti
Gabriele Molteni *Editors*

Injection Laryngoplasty

Springer

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The editors wish to acknowledge Dr Elisa Aggazzotti Cavazza for contributing with drawings to this volume.

ISBN 978-3-319-20142-9 ISBN 978-3-319-20143-6 (eBook)
DOI 10.1007/978-3-319-20143-6

Library of Congress Control Number: 2015952116

Springer Cham Heidelberg New York Dordrecht London

Printed on acid-free paper

Springer International Publishing AG Switzerland is part of Springer Science+Business Media (www.springer.com)

Preface

Oscar Wilde used to say that "education is an admirable thing, but it is well to remember from time to time that nothing that is worth knowing can be taught." Even if we are far away from Oscar Wilde's wisdom, we would like to transfer to our colleagues something that may be worth knowing in laryngology. Injection laryngoplasty is certainly a really small niche in ENT but nonetheless an interesting and valuable argument in which didactic material is lacking. This book aims to examine the most important features of injection laryngoplasty techniques, ranging from anatomo-physiological considerations and the principal causes of glottic and neoglottic insufficiency that may require a laryngoplasty to technique considerations. Injection laryngoplasty is considered in all its possibilities: by means of microlaryngoscopy, transcutaneous, and fibroscopic approach, both in adults and in pediatric population, and taking into considerations all material that can be used.

ENT surgeons who deal with phonosurgery and laryngeal cancer surgery should keep in mind that injection laryngoplasty is an important weapon at their disposal that may have a relevant impact on patients' quality of life.

This book is meant to be a simple and "ready to use" manual for surgeons who want to approach injection laryngoplasty techniques for the treatment of glottic and neoglottic insufficiency.

Modena, Italy
October 2015

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1 Introduction to Injection Laryngoplasty

Giuseppe Bergamini, Livio Presutti, and Gabriele Molteni

Injection laryngoplasty was introduced by Bruning in 1911, more than a century ago, but it does not look so old. Indeed, despite having had phases of varying fortune mainly linked to the characteristics of the materials available, since injection laryngoplasty (IL) was introduced it was never abandoned and still has many supporters despite the competition of the structural surgery and laryngeal reinnervation. In fact, IL is a technique that does not require an external approach and is able to solve important voice problems, often related to accidental or inevitable consequence of cervical and thoracic surgical procedures.

IL is a procedure that in some situations and with certain materials can be carried out under local anesthesia and mild sedation by fiberendoscopy or by transcutaneous access under endoscopic vision. This procedure is minimally invasive and very fast even when it is performed by means of microlaryngoscopy under general anesthesia. For these reasons IL is generally accepted even by those patients who have suffered iatrogenic damage and are reluctant to face further surgical procedures.

The main indication for injection laryngoplasty is unilateral vocal fold paralysis, but it also finds application in atrophy and cordal scars. Beyond the purpose of vocal fold filling that justifies the definition of "laryngoplasty," IL is also used for infiltration of drugs into the vocal cords (botulinum toxin, corticosteroids, growth factors, etc.).

This technique, after the initial experience of Bruning, was revisited many years later (1945) by Arnold, [1] but a new and decisive impulse came when Ford [2] in 1986 proposed clinical use of bovine collagen after a preliminary study on dogs. He designed also an injection kit that is still available and it can be used also with other materials. Since then many materials have been introduced with characteristics of high biocompatibility and persistency of variable duration at the injection site.

For this reason early surgical rehabilitation in case of laryngeal monoplegia is possible alongside definitive interventions after the certainty of the irreversibility of the paralysis and to treat other diseases of the superficial layer of the lamina propria with biological therapeutic purposes.

Milestones are those determined by the introduction of new materials that were subjects of numerous clinical experience: Mikaelian [3] in 1991 introduced autologous fat, Sittel [4] in 2000

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G. Bergamini et al. (eds.), *Injection Laryngoplasty*, DOI 10.1007/978-3-319-20143-6_1

polydimethylsiloxane, hyaluronic acid was introduced by Hallen [5] in 2001, and hydroxylapatite by Rosen [6] and Belafsky [7] in 2004.

Since open partial laryngectomies are widely used especially in Europe (supraglottic, sopracricoid, and supratracheal laryngectomies), indications of IL have expanded from a strictly phonological indication to the surgical rehabilitation of dysphagia after open partial horizontal laryngectomies [8].

"IL is an easy technique but results are not always predictable" might be the main objection!

In order to obtain optimal results it a good knowledge of the available materials and the capability of selection of the same in relation to the objective and to the clinical situation is fundamental. Furthermore, a correct technique of infiltration regarding the points of insertion, the headquarters of infiltration and the quantity is extremely important. Nonetheless, what stays before the surgery (i.e. diagnosis) and what comes after (speech therapy rehabilitation) are essential components that lead to the desired result.

Authors think that a book that points out and clarifies this technique which, if applied properly, is able to solve problems arising from a paralyzed vocal cord with similar results compared to the framework surgery [9, 10] might be useful. IL also finds application in other diseases and is able to solve swallowing disorders after open partial laryngectomies, alone or in combination with other interventions.

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Anatomo-Physiology of the Glottis and Neoglottic Region

2

Elio Maria Cunsolo, Elisabetta Genovese,
Maria C. Guarnaccia, and Marco Giuseppe Greco

2.1 Introduction

The larynx, commonly called the "voice box" or "organ of voice," is placed at the upper part of the air passage and seems to be a tube comprising a complex system of mucosa, muscle, cartilage, connective tissue, and nerves. It is situated between the trachea and the root of the tongue, at the upper and forepart of the neck, and is suspended from the hyoid bone. The "evolutionary" origins of the larynx lie in the need to protect the lungs of amphibious organisms from water, and airway protection remains its most important biological task [1].

Until puberty, the larynx of the male differs little in size from that of the female. In the female, its increase after puberty is only slight; in the male, it undergoes a considerable increase. All the cartilages are enlarged and the thyroid cartilage becomes prominent in the middle line of the neck, while the length of the "rima glottidis" is nearly doubled [2].

There are nine cartilages of the larynx (Fig. 2.1): three single (thyroid, cricoid, and epiglottis) and three paired (two corniculate, two cuneiform, and two arytenoid) (Fig. 2.2).

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2.1.1 Cricoid Cartilage

The cricoid cartilage is a ring of hyaline cartilage, which in youth is situated at the inferior part of the larynx and is the only complete ring of cartilage of the airways. It may be considered the structural unit of the larynx. It is smaller, but thicker and stronger, than the thyroid, and it forms the lower and posterior parts of the wall of the larynx. It consists of two parts: a posterior quadrate lamina, and a narrow anterior arch, one-fourth or one-fifth of the depth of the lamina [2].

2.1.2 Thyroid Cartilage

In youth, the thyroid cartilage consists of hyaline cartilage, is located above the cricoid, and is the largest cartilage of the larynx. It consists of two halves fused anteriorly in the middle line of the neck and creating an acute angle. The angle is more acute in men than in women.

2.1.3 Epiglottis

The epiglottis is a thin sheet of elastic cartilage, at every age, covered by mucous membrane; it has the shape of a leaf and its lower part represents the "petiole," also called the "epiglottis petiole." The petiole continues with the thyro-epiglottic ligament, which represents the epiglottis anchor to the dihedral angle of the thyroid cartilage.

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Fig. 2.1 Cartilages of the larynx and ligaments

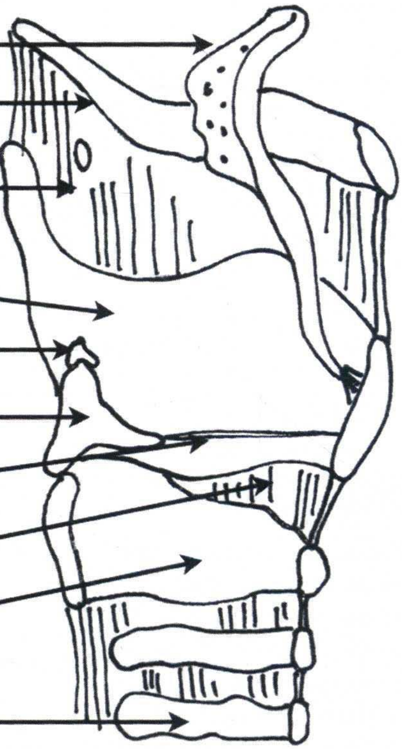
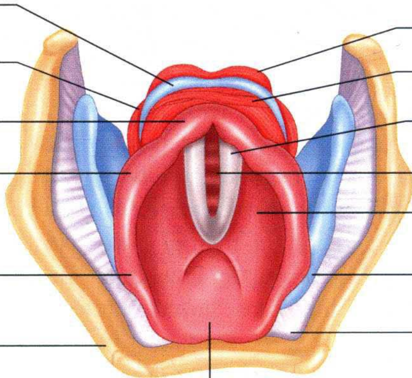


Fig. 2.2 Larynx viewed from above



2.1.4 Arytenoid Cartilages

The arytenoid cartilages form the part of the larynx to which the vocal ligaments and vocal folds are attached. Their "body" consists of hyaline cartilage and their "vocal process," elastic cartilage. The arytenoids are situated at the upper border of the lamina of the cricoid cartilage, at the back of the larynx. They have a pyramidal form, three surfaces, a base, and an apex.

The posterior surface is triangular and concave, and is attached to the arytenoid muscle and transverse muscle [2]. The anterolateral surface is convex and presents two depressions for attachment to the false vocal cord (vestibular ligament) and the vocal muscle. The medial surface is narrow and smooth, and it presents a mucosal lining that forms the lateral aspect of the respiratory part of the glottis.

The base of each cartilage is broad with a concave smooth surface, for articulation with

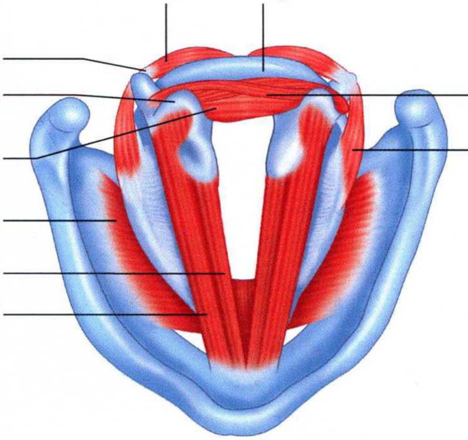


Fig. 2.3 Intrinsic muscles of the larynx

the cricoid cartilage. Its lateral angle is short, rounded, and prominent, it projects backward and laterally, and is termed the muscular process. It gives insertion to the posterior cricoarytenoid muscle behind and the lateral cricoarytenoid muscle in front. Its anterior angle, also prominent, but more pointed, projects horizontally forward; it gives attachment to the vocal ligament, and is called the vocal process. The apex of the cartilages is pointed, curved backward, and surmounted by a small conical, cartilaginous nodule, the corniculate cartilage [2].

2.1.5 Corniculate Cartilages

The corniculate cartilages are two small conical nodules situated in the posterior parts of the aryepiglottic folds of the mucous membrane; they are sometimes fused with the arytenoid cartilages. They articulate with the apices of the arytenoid cartilages, serving to extend them posteriorly and medially.

2.1.6 Cuneiform Cartilages

The two small cuneiform cartilages rest one on either side, in the aryepiglottic fold. They form small, whitish elevations on the surface of the mucous membrane just anterior of the arytenoid cartilages [3].

2.1.7 Ligaments

In the larynx, there are ligaments that connect the thyroid cartilage and epiglottis with the hyoid bone and the cricoid cartilage with the trachea (extrinsic ligaments) and others that connect the numerous cartilages of the larynx to each other (intrinsic ligaments). Extrinsic ligaments are the thyrohyoid membrane, the lateral hyothyroid ligament, the hyo-epiglottic ligament, and the cricotracheal ligament, and intrinsic ligaments are the conus elasticus and the quadrangular membrane.

2.2 Muscles of the Larynx

The muscles of the larynx are extrinsic, passing between the larynx and parts around it, and intrinsic, confined entirely to the larynx.

The infrahyoid strap muscles (the sternothyroid, the sternohyoid, and the thyrohyoid), the mylohyoid, digastric, geniohyoid, and stylopharyngeus muscles all act in concert to provide laryngeal stabilization and allow the vertical displacement of the larynx, by changing the length of the vocal tract.

The intrinsic muscles (Fig. 2.3) of the larynx are responsible for altering the length, tension, shape, and spatial position of the vocal folds, by changing the orientation of the muscular and vocal processes of the arytenoids with the fixed anterior commissure [4]. The muscles are usually categorized as follows: three major vocal fold adductors, one abductor, and one tensor muscle.

2.2.1 Tensor Muscle

The cricothyroid muscles produce tension and elongation of the vocal folds by drawing up the arch of the cricoid cartilage and depressing the posterior portion of the thyroid cartilage lamina; the distance between the vocal processes and the angle of the thyroid is thus increased and the folds are, consequently, elongated [2].

2.2.2 Abductor Muscle

The posterior cricoarytenoid muscles act to rotate the arytenoid cartilages laterally, thereby abducting the vocal cords. Their action opposes that of the lateral cricoarytenoid muscles [2].

2.2.3 Adductor Muscles

The lateral cricoarytenoid muscles function to rotate the arytenoid cartilages medially, thereby adducting the vocal cords. They receive innervation from the recurrent laryngeal branch of the vagus nerve (CN X).

The inter-arytenoid muscle, or transverse arytenoid muscle, consists of oblique and transverse parts. Its main function is adduction of the vocal cords and it is innervated by both recurrent laryngeal branches of the vagus nerves (CN X) [2].

The thyro-arytenoid muscles function to draw the arytenoid cartilages forward, thereby relaxing and shortening the vocal cords, while also rotating the arytenoid cartilages inward, thus adducting the vocal folds and narrowing the rima glottis. The thyro-arytenoid muscles receive innervation from the recurrent laryngeal branch of the vagus nerve (CN X).

2.3 Innervation of the Larynx

The vagus nerve descends into the carotid sheath, giving off three major branches: the pharyngeal branch, the superior laryngeal nerve (SLN), and the recurrent laryngeal nerve (RLN) [4]. The SLN arises from the inferior ganglia of the vagus nerve and divides into internal and external branches. The internal laryngeal branch is almost entirely sensory, but some motor filaments are said to be carried by it to the arytenoideus. The external laryngeal branch supplies the cricothyroid muscle. The RLN supplies all the muscles of the larynx except the cricothyroid muscle and perhaps a part of the arytenoideus muscle. It also supplies the glottic and subglottic mucosa and the myotatic receptors of the laryngeal musculature [4].

2.4 Vessels of the Larynx

2.4.1 Arteries

The superior and inferior laryngeal arteries supply most of the blood to the larynx. The superior laryngeal artery is a branch of the superior thyroid artery, which arises from the external carotid artery. The inferior laryngeal artery originates from the inferior thyroid branch of the thyrocervical trunk, which is a branch of the subclavian artery.

2.4.2 Veins

The superior and inferior laryngeal veins drain the larynx and follow the same course as the arteries.

2.4.3 Lymphatics

The lymphatic vessels, which drain above the vocal folds, travel along the superior laryngeal artery and drain to the deep cervical lymph nodes at the bifurcation of the common carotid artery [5].

2.5 Anatomy of the Glottic Region

The laryngeal cavity extends from the laryngeal entrance to the lower border of the cricoid cartilage, where it is continuous with the lumen of the trachea (Fig. 2.4).

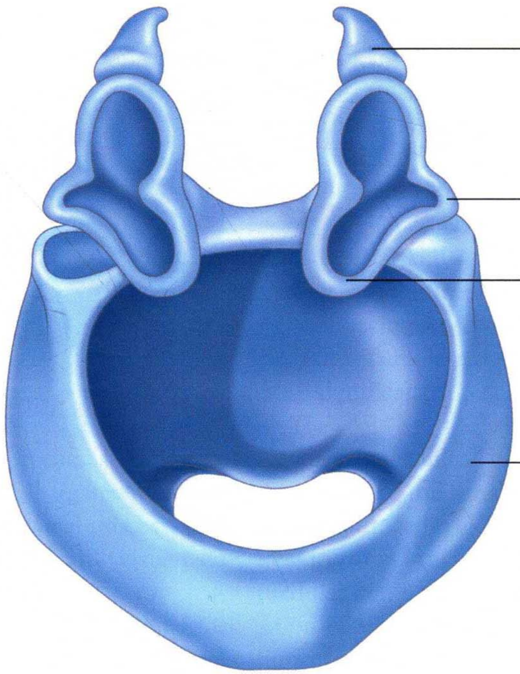


Fig. 2.4 Cricoid cartilage and arytenoid cartilages

It is divided into two parts by the projection of the vocal folds, between which there is the "rima glottidis." The portion of the cavity of the larynx above the vocal folds is called the vestibule; it contains the ventricular folds and, between these and the vocal folds, there are the ventricles of the larynx. The portion below the vocal folds is, at first, of an elliptical form, but lower down it widens out, assumes a circular form, and is continuous with the tracheal lumen.

The entrance of the larynx is a triangular opening, bounded in front by the epiglottis; behind by the apices of the arytenoid cartilages, the corniculate cartilages, and the inter-arytenoid notch; and on either side, by a fold of mucous membrane enclosing ligamentous and muscular fibers, stretched between the side of the epiglottis and the apex of the arytenoid cartilage. This is the aryepiglottic fold. On the posterior part of the margin where the cuneiform cartilage forms a more or less distinct whitish prominence lies the cuneiform tubercle.

The ventricular folds ("plicae ventriculares") are two thick folds of mucous membrane, each enclosing a narrow band of fibrous tissue, the ventricular ligament, which is attached in front to the angle of the thyroid cartilage, immediately below the attachment of the epiglottis, and behind to the antero-lateral surface of the arytenoid cartilage. The lower border of this ligament constitutes the upper boundary of the ventricle of the larynx [2].

The vocal folds (true vocal cords) are concerned with the production of sound, and enclose two strong bands, named the vocal ligaments ("ligamenta vocales"; inferior thyro-arytenoid).

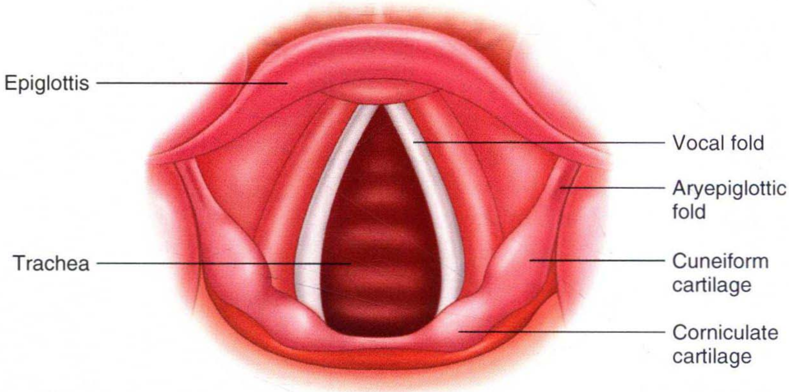
Each ligament consists of a band of yellow elastic tissue, attached in front to the angle of the thyroid cartilage and behind to the vocal process of the arytenoid. Its lower border is continuous with the thin lateral part of the conus elasticus. Its upper border forms the lower boundary of the ventricle of the larynx ((Fig. 2.5).

2.5.1 Microanatomy of the Vocal Fold

Anatomically, the vocal folds have three layers: the epithelium and its basement membrane, the lamina propria (a connective structure that is divided into layers), and the vocal muscle (Fig. 2.6).

Functionally, according to Hirano [6–8], we must consider two layers: the cover (an elastic structure) and the body (a rigid structure).

Fig. 2.5 Laryngoscopic view of the interior of the larynx



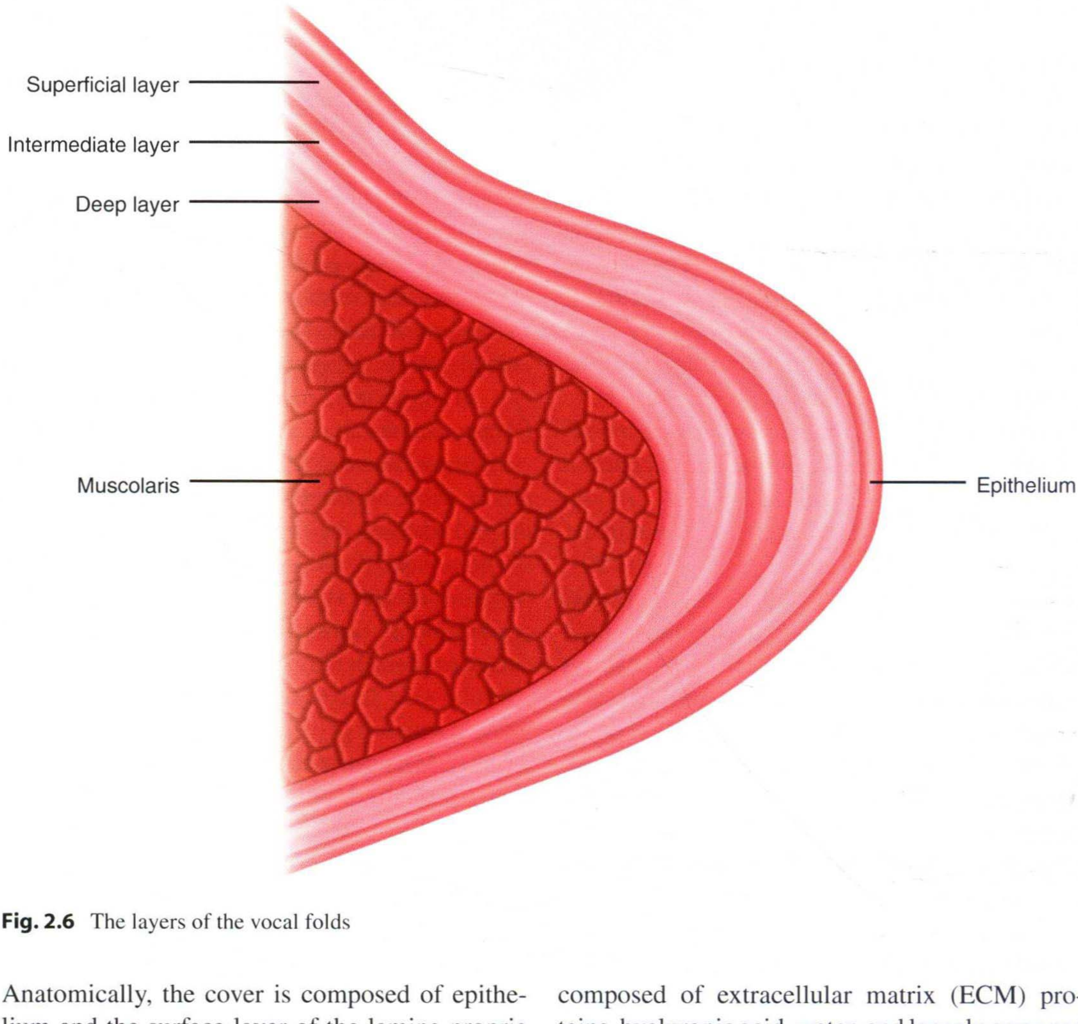


Fig. 2.6 The layers of the vocal folds

Anatomically, the cover is composed of epithelium and the surface layer of the lamina propria (Reinke's space), which is mostly acellular and composed of extracellular matrix (ECM) proteins, hyaluronic acid, water, and loosely arranged fibers of collagen and elastin.

The body is made up of the intermediate layer and the deep layer of the lamina propria (vocal ligament) and the underlying vocal muscle.

The intermediate and deep layers of the lamina propria (ILP and DLP) are composed mostly of elastin and collagen; in the deepest layer (DLP) the fibrillar component is greater and is made mainly of tightly arranged collagen fibers.

The cover moves freely over the underlying vocal ligament and muscle. This is the mechanism for the production of the vocal sound.

The vocal fold mucosa and vocal ligament cover the vocal muscle and extend from the anterior commissure to the vocal processes of the arytenoids. The mucosa and vocal ligament extend posteriorly to cover the entire vocal process. The posterior third of the endoscopically visualized true vocal fold, then, is the "aphonatory" (respiratory), or cartilaginous, portion, while the anterior two thirds of the endoscopically visualized vocal fold is the "phonatory," or membranous, portion.

The epithelium is stratified, squamous, and non-keratinized, has a thickness of 0.05–0.1 mm, and adheres to the underlying lamina propria owing to the "basement membrane zone."

Recent research has shown the different functions of the vocal epithelium:

1. Physical barrier, maintaining a high degree of differentiation that is expressed in the realization of a "malpighian epithelium" and, at the same time, inhibiting the evolution toward keratinization
2. Transport of the mucous film, even in the absence of a mucociliary apparatus; this is because of the presence of microfolds in the apical pole of the outer layer of the chordal epithelium, with the realization of "tread epithelium."
3. Vibration of the vocal cords; in this case the role of the junctional complex is particularly important

The cells of the chordal epithelium are connected together by a system of molecular junctions composed of "tight junctions (TJs)" and "adherens junctions (AJs)," the expression of which is under genetic control.

The most important TJs are the occludin, claudin, and zonula occludens proteins (ZO), while the main AJs are the b-catenin and E-cadherin glycoproteins.

Rousseau et al. (2011) [9] have documented ultrastructural and molecular damage in the laryngeal epithelial barrier, after phonatory effort of about 30 min. In the study some "stretch marks" of interdigitation were noted in the membranes of the epithelial cells, while, for the bio-molecular aspects, a significant inhibition of gene expression for occludin and b-catenin were documented, with considerable reductions, though not statistically significant, for ZO-1 and e-cadherin.

The data of the study show a correlation between mechanical damage and a variation of the control of the gene expression.

The surface layer of the lamina propria is particularly important and its structural features (cellular component, characteristics, and composition of the ECM, metabolic processes) condition the rheological properties of large and regular undulation.

The cellular component is composed, in addition to fibroblasts, of "stellate cells" contained in two organelles (macula flava), which are located near the front commissure and at the back near the vocal apophyses.

The first mention of the macula flava (MF) was by Lanz and Wachsmuth [10], who in 1955 described only the anterior MF, calling it "nodulus elasticus."

In 1975, Hirano [7] described an anterior MF, placed immediately behind the Broyles ligament, and a posterior MF, in front of the vocal process of the arytenoid.

These elliptical masses contain a high density of stellate cells and extra-cellular proteins composed of fibrillar components (collagen, elastic, and reticular), glycoproteins, and glycosaminoglycans (hyaluronic acid).

The presence of MF along the course of the vocal ligament allows a segmentation of the glottic region into five "glottic zones" [11]. The membranous glottis is composed of three zones: the first zone includes the Broyles' ligament and the anterior MF; the second zone includes the free "vibrant" chordal margin; and the third area includes the posterior MF.

The cartilaginous glottis comprises the fourth area, which corresponds to the insertion between the membranous glottis and vocal process of the arytenoid, and the fifth area, which corresponds to the vocal process of the arytenoid (intercartilaginous glottis). Functionally, this division indicates the presence of a zone of maximum chordal vibration (second zone) in addition to a front zone (first zone) and rear (third and fourth zones), where we see the gradual transition from elastic zones to rigid areas.

Currently, the first, third, and fourth zones are called "vibration dampers" [11]. This anatomical conformation, which represents differences age, reaches its highest expression in the young-adult. Stellate cells represent the cellular elements that characterize the MF and they undergo significant modifications with age.

The MFs are rich in stellate cells, with a cell density equal to two and a half times that of the lamina propria in which, however, there are only fibroblasts.

Stellate cells are so defined because of their morphology, characterized by cytoplasmic prolongations, through which the various cells are mutually connected. Fibroblasts and stellate cells are completely different types of mesenchymal cells.

Stellate cells are therefore cellular elements in the active and constant activity of the synthesis of structural elements that is essential to the maintenance of the metabolic and rheology of the lamina propria of the vocal folds. The intracellular organelles, rough endoplasmic reticulum (RER), and Golgi are well developed. These cells represent an abundant production of ECM.

A very interesting aspect is their propensity for vitamin A storage. This significantly affects the activity of the enzyme adenosine triphosphate sulfurylase, which is related to the synthesis of glycosaminoglycans.

Fibroblasts are located in the lamina propria, present a fusiform morphology; intracellular organelles, RER, Golgi, and vesicles are rare. There is no accumulation of vitamin A and reticular, collagen, and elastic fibers are also rare.

Numerous studies on mammals with vocal cords with MFs deprived of vitamin A showed alterations in the constitution of the ECM [12], epithelial metaplasia [13], and laryngeal malformations in the case of prenatal deprivation of retinol [14]. Another important aspect of the cellular physiology of vocal fold stellate cells (VFSCs) and given their strong sensitivity to radiotherapeutic treatment. Studies of irradiated human vocal folds showed broad damage to VFSCs, which display morphological aspects of cellular degeneration, to witness a reduced synthesis of the precursors of collagen and mainly elastic fibers. It is possible that this cell type represents the main target of post-actinic damage to the vocal fold [15].

At different ages there are different ratios among the membranous and the cartilaginous glottis. The ratio in length between the glottis from the front to the back is approximately 1:1 in the newborn and 3:2 in the adult (Fig. 2.7).

The structure of membranous glottis changes over time; according to its characteristics, we can distinguish five age ranges: newborn, childhood, adolescence, young adult, and elderly.

In the newborn, the lamina propria shows as a uniform structure, with no vocal ligament and Reinke's space. The MFs of newborns have the same topography as those in adults, but are histologically immature and rich in stellate cells, with little ECM.

In the five age groups, the density of stellate cells of the MF declines gradually, while there is a substantially stationary density of fibroblasts in the "lamina propria". These data demonstrate the critical role of MFs, not only in maintaining chordal cell structure, but also in the process of chordal maturation and senile evolution [16].

The ECM contains collagen fibers (most represented at the level of the intermediate layer and the deep layer [ligament]) and elastic fibers, which are particularly rich in the surface layer. In addition, there are glycosaminoglycans (GAGs; hyaluronic acid, fibronectin, decorin, fibromodulin), which are particularly important for maintaining the rheological characteristics and preventing the excessive production of collagen fibers, and adhesion molecules (cadherin, syndecan-4, and syndecan-1), whose role is still unclear. Particularly interesting in view of possible interventions with injection techniques are the studies on the healing chordal process. From animal studies [17, 18] it appears that in the