

# Chapter 10

## Laryngeal Framework Regeneration

Masaru Yamashita, Yoshiharu Kitani, and Shin-ichi Kanemaru

**Abstract** The laryngeal framework consists of complex tissues including the thyroid cartilage, cricoid cartilage, and arytenoid cartilage. This framework contributes to protecting the inner aerodynamic tract from external impact and aids in maximizing vocal fold motion through intra-laryngeal muscular contraction. This structure is affected by malignant tumors, laryngeal trauma, congenital anomalies, stenosis, or intractable inflammatory diseases. Once this rigid structure is damaged, restoration of mechanical power sufficient to compensate for normal laryngeal function is quite difficult. Conventionally, laryngeal defects *have been* reconstructed with autologous tissues or flaps. These reconstructive surgeries, however, required donor tissue, skilled technique and multiple surgeries. Locoregional blood supply also had to be carefully considered to maintain living donor tissue. Tissue regeneration strategies for the laryngeal framework, therefore, have been sought to alleviate these problems. Recently, tissue engineering has attracted great attention as a means of recreating organs. There are three fundamental components in tissue engineering: cells, scaffolds, and growth factors. Among these, scaffolds play a central role in laryngeal framework regeneration because great mechanical power is required immediately after surgery to maintain airway structure. In situ tissue engineering techniques, which allow in vivo regeneration of organs through the application of scaffolds, have shown recent advancement due to biomaterial innovations. In this chapter, current progress and limitations of laryngeal framework regeneration will be discussed. To date, intraluminal epithelialization and subepithelial tissue regeneration have achieved some success after laryngeal resection. Research into the next steps, including functional tissue regeneration and development of suitable scaffolds for children, is now warranted.

**Keywords** Larynx • Framework • Cartilage • Regeneration • Tissue engineering • Scaffold

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## 10.1 Background

The laryngeal framework protects the intricate inner structure, the vocal folds, which are finely controlled during phonation, breathing, and swallowing. This framework is composed of a cartilaginous complex including the thyroid cartilage, cricoid cartilage, and arytenoid cartilage. This framework not only contributes to protecting the inner aerodynamic tract from external impact, but also aids in maximizing the effects of the underlying vocal fold motions through intra-laryngeal muscular contraction.

Treatment for various disease conditions such as malignant tumors, laryngeal trauma, congenital anomalies, stenosis, or intractable inflammatory diseases may require resection of the laryngeal tissue. This results in persistent postoperative problems in voice production and/or swallowing. Thus, a conflict exists between effectively treating tumors and functional restoration when handling laryngeal malignancies. Once this rigid structure is damaged, it is very difficult to restore the mechanical power needed to compensate for normal laryngeal function as well as to restore the barrier between the inner lumen and subcutaneous tissue. Furthermore, factors including airway pressure changes between inspiratory negative pressure and expiratory positive pressure, saliva from the pharynx, laryngeal secretions, and nonsterile conditions may all contribute to a decreasing rate of success during laryngeal framework reconstruction.

Laryngeal defects have conventionally been reconstructed with various autologous tissues or flaps, including muscle flaps [1–3], myocutaneous flaps [4, 5], thyroid gland flaps [6, 7], and cartilage grafts [8–10], among others. These reconstructive surgeries, however, often require donor tissues, highly skilled techniques, and multi-staged surgeries. Locoregional blood supply must also be carefully considered in order to maintain living donor tissue. The scope of these surgeries was generally limited to complete “tissue obliteration” of the extirpated area.

Laryngeal transplantation has also been studied in cases of hemilaryngectomy [11] and total laryngectomy [12–15] in various animal models. In 1998, a successful case of human laryngeal transplantation was reported [16]; however, many barriers remain before laryngeal transplantation becomes a standard treatment. Some of these challenges include managing donor larynges, potential tissue-borne transmitted diseases, ethical issues, and the inevitable administration of immunosuppressive reagents, which could lead to malignant tumor progression.

Regenerative medicine has made great strides in sparing clinicians from the above-mentioned problems, with the goal of repairing affected organs using live cells or tissues without the need for donor tissues. There are two major approaches in regenerative medicine, one is cell therapy and the other is tissue engineering. Beginning in the early 1970s, cell culture for therapeutic purposes was studied using chondrocytes [17]. While cell therapy is appropriate for hematopoietic diseases, it is very difficult to mechanically sustain even moderately sized defects using cell pellets or cell clusters. Tissue engineering has thus emerged to solve this issue and is becoming widespread in the medical field. The basic concept of tissue

engineering was proposed by Langer and Vacanti in 1993 [18]. Their approach was to create tissue *ex vivo* with the use of three fundamental components: cells, scaffolds, and regulatory growth factors, under the appropriate environmental conditions. The intent was to subsequently implant the created tissue into a live recipient at a later time. When considering laryngeal framework regeneration, scaffolds are considered to play the most important role of *these* three fundamental components, because great mechanical power is necessary immediately after resection surgery in order to maintain airway structure.

In contrast to the original concept of *ex vivo* tissue engineering, an “*in situ*” tissue engineering technique has been developed, which allows for the regeneration of organs *in vivo* by introducing an appropriate scaffold for migrating cells from regions surrounding the affected site. This technique was first proposed and investigated in canine models beginning in 1995 in studies involving the trachea [19, 20], stomach [21, 22], and small intestine [23]. Omori et al. reported the first human case of tracheal regeneration using this technique in 2005 [24]. Polypropylene, a commonly used plastic material, was used in the study. The achievements in this field were mainly attributed to innovations in the available biomaterials. This technique eliminates the time required for harvesting organs that are created *ex vivo* as well as the need for repeated highly skilled surgeries. With *in situ* tissue engineering, patients can undergo repeated replacement surgeries if needed in cases where the initial treatment results in failure. Although infection control is a major consideration when using scaffolds, a wide variety of biomaterials, together with improvements in tissue biocompatibility, have greatly aided this problem.

The key to success in regeneration of the laryngeal framework is based on designing ideal artificial scaffolds. Once the ideal scaffolds have been identified, modifications *intended* to enhance regeneration by the addition of cells and/or regulatory growth factors can be considered, assuming strict quality control and patient safety as prerequisites.

## 10.2 Scaffolds: The Innovation of Bioartificial Materials

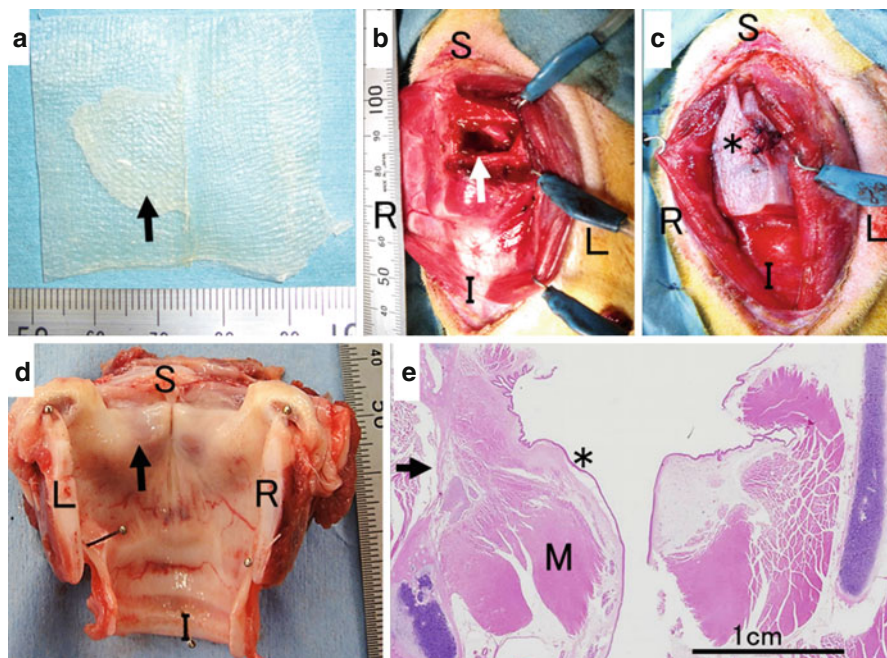
*In situ* tissue engineering studies on the laryngeal framework have mainly been conducted using canine models [25–31]; this is because the structure and size of the canine laryngeal framework is similar to that in humans. The scaffolding materials utilized in these studies to date can be divided into two types: (1) chemically or mechanically decellularized materials used as allogeneic or xenogeneic grafts and (2) synthetic materials. The incorporation of cells into scaffolds is necessary to create living tissues. Appropriate inflammatory responses at the surgical site and the integration of the scaffolds with stem cells or other differentiated cells are presumably enhanced by regulatory growth factors as part of the regenerative cascade. During the normal repair process in injured tissues and organs, endogenous factors are secreted at the damaged sites and serve as regulatory factors [32].

### 10.2.1 Decellularized Materials

Huber et al. [25] reported the utility of porcine-derived xenogeneic extracellular matrix (ECM) for canine laryngeal framework regeneration in 2003. This scaffold, made from porcine urinary bladder submucosa ECM (UBS-ECM), is decellularized bladder tissue and contains not only extracellular components but growth factors as well, namely, vascular endothelial cell growth factor, basic fibroblast growth factor, and transforming growth factor  $\beta$  [33, 34]. Since this material is acellular and degradable, it was stated to have low antigenicity. Huber et al. made a partial hemilaryngectomy in 30 dogs and applied multiple layers of UBS-ECM for the framework and a single layer of UBS-ECM for the vocal fold prominence. Histological data demonstrated that rapid ingrowth of mononuclear and polymorphonuclear cells from surrounding tissue occurred within 1 week, followed by scaffold degradation and replacement by recipient tissue within 2 months postoperation. Epithelialization was completed by a simple squamous cell layer by the first postoperative month. Cartilage, skeletal muscle, and glandular tissues without chronic inflammation were later identified and persisted through the 12-month follow-up period. Although the components were not well-structured and the length of the regenerated vocal fold was shortened compared to the naïve fold, this study clearly demonstrated that porcine-derived ECM scaffolds can be utilized in laryngeal framework regeneration.

Ringel et al. [35] have reported the detailed characteristics of this urinary bladder ECM. The surface of the original ECM was designed to be implanted toward the luminal side to enhance epithelial growth, while the opposite side, the coarsely textured tunica propria surface, was designed to provide enhanced conditions for vascularization and host cell migration. Anatomical and histological examinations were performed in two dogs 24 weeks after hemilaryngectomy; one was reconstructed using ECM and the other with a strap muscle flap. Although the sample size was small, the hemilarynx reconstructed with ECM showed better results than the hemilarynx reconstructed with a strap muscle flap.

Kitamura et al. [31] also used this ECM in a canine hemilaryngectomy model (Fig. 10.1a–c) and evaluated the animals 6 months postoperatively; five dogs were utilized for this study. Functional analyses including vibratory and phonation threshold pressure were performed along with endoscopic and histological examinations. Normal to near-normal phonation threshold pressure and normalized mucosal wave amplitude were observed in approximately half of the dogs. Endoscopic results showed the formation of granulation tissue within 1–2 weeks; one case of infection was reported and resolved within 2 weeks. Re-epithelialization was complete in every dog by 1 month, which was consistent with the previous report [25]. A macroscopic intraluminal view showed a good prominence at the surgically resected site 6 months postoperatively (Fig. 10.1d). Histological data also revealed the partial appearance of newly generated cartilage and muscle tissue within the original portion of the larynx (Fig. 10.1e). The vocal fold was also regenerated; however, fibrotic contraction and the absence of a layered structure ~~was~~*were* noted in the lamina propria, and was defined as a “scarred vocal fold”. The data thus



**Fig. 10.1** (a) A designed porcine urinary bladder-derived ECM. An arrow indicates a flap for a vocal fold ridge. (b) A left partial hemilaryngectomy was made as indicated by an arrow. *S* superior, *I* inferior, *R* right, *L* left. (c) An asterisk indicates the implanted scaffold. (d) An intraluminal image 6 months after the operation. An arrow indicates the surgically affected site (left vocal fold). (e) A hematoxylin- and eosin-stained image indicates regenerated epithelium (asterisk), muscle tissue (*M*), and cartilage (arrow) at 6 months postoperatively

support the use of ECM scaffold as a promising tool for both physical and functional regenerative surgeries after laryngeal resection, although further studies are warranted.

Recently, a decellularized laryngeal scaffold has been explored as a new material for laryngeal regeneration [36, 37]. Hou et al. [37] showed the potential for laryngeal regeneration using a chemically decellularized whole rabbit larynx. They found that the scaffold contained few intact cells and, when implanted into the omentum after recellularization with mesenchymal stem cells, vascularization was clearly seen within 4 weeks after implantation and integrated cartilage frameworks remained after 8 weeks.

Decellularized (acellular) materials are useful tools for laryngeal framework regeneration. Decreased antigenicity contributes to reducing the immune-allergic reaction, excessive inflammation and rejection of scaffolds. Biodegradable scaffolds are also ideal when considering the long-term side effects caused by implanted materials. In the future, regenerative surgeries using biodegradable scaffolds may be preferred for use in children and adolescents, once the mechanical strength of these materials has been fully established.

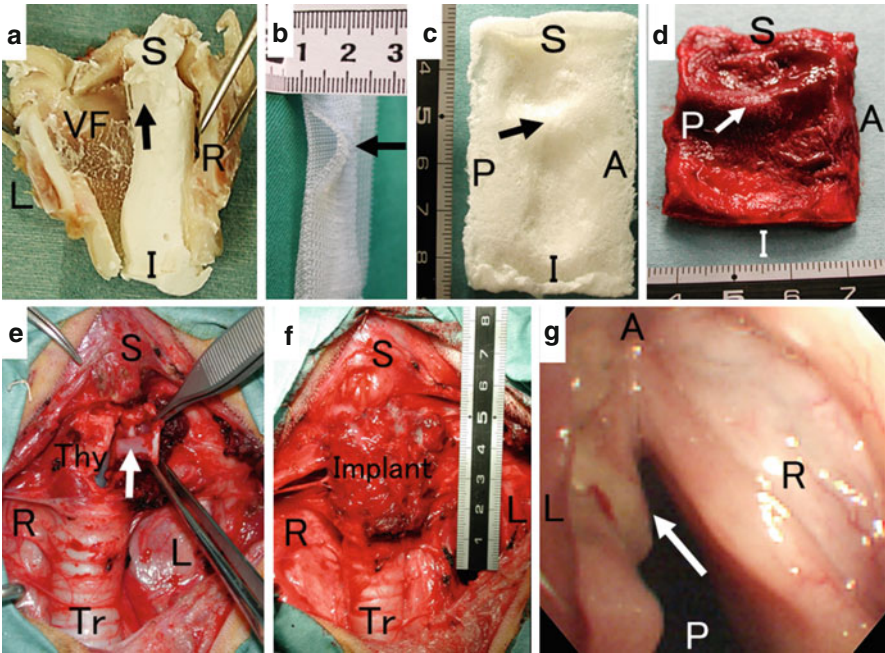
### 10.2.2 Synthetic Materials

Various studies on tracheal prostheses have been conducted since the 1960s; however, the materials used have not been ideal. Nakamura et al. [19, 20] reported successful tracheal regeneration in canine models using polypropylene-based artificial scaffolds, which were designed to be infiltrated with native recipient tissue based on the concept of in situ tissue engineering. This scaffold was composed of a cylindrically shaped polypropylene mesh framework with spongy porcine dermal collagen. Polypropylene is a commonly used plastic material. This type of polypropylene mesh has already been utilized widely in the clinical setting as a permanent and nondegradable implant for the surgical repair of abdominal herniation cases. This polypropylene mesh has benefits in terms of its high biocompatibility and morphological flexibility. The polypropylene mesh framework is covered in freeze-dried spongy collagen derived from porcine skin. This collagen is composed of 70–80 % collagen type I and the remaining 20–30 % of collagen type III. This collagen treatment is the key to better cellular attachment and sealing off from the airway after the preclotting procedure with autologous peripheral blood during surgery. This preclotting procedure can enhance the processes of tissue regeneration when bone marrow aspirates or bone marrow-derived stem cells [38] are applied to the scaffolds. Nakamura et al. [38] observed faster epithelialization and fewer complications in these types of regenerative experiments in canine trachea.

Following the successful use in tracheal regeneration, Omori et al. [26] applied this material to cricoid regeneration in a canine model. They utilized a total of nine dogs for cricoid cartilage resections ( $n=5$ ) and cricoid with cervical tracheal resections ( $n=4$ ). Two animals showed minor formations of granulation tissue and one showed exposure of the mesh framework; however, these lesions were asymptomatic. Mechanical tests of the regenerated cricoid showed equivalent mechanical strength *comparable* to native cricoid cartilage. Favorable luminal epithelialization of this material was observed 6 weeks after implantation. Regeneration of ciliated epithelium was confirmed by scanning electron microscopic examination.

Omori et al. [24] then applied this material to four patients in an attempt to repair the cricoid and trachea, including, in one case from 2002, a portion of the thyroid cartilage. One case of subglottic stenosis and three cases of thyroid cancer underwent in situ tissue engineering surgeries using this scaffold. During the 8–34-month observation period, every case showed a well-epithelialized airway lumen without any obstruction or major complication. Their study thus indicated that this technique could be a useful tool for laryngotracheal reconstruction, which *had* sometimes resulted in failure due to granulation, scar, and fibrosis. It took approximately 2 months to achieve sufficient luminal epithelialization in this study. Since the polypropylene-based scaffold is nonbiodegradable and free from growth factors, in situ tissue engineering of the cricoid and trachea may be a good indication for adult cases with stenosis and malignancies. Moreover, the adjustable mechanical power suits the need for sustaining inner airway structure, although this material cannot be utilized in children yet because of its rigid and nonbiodegradable structure.

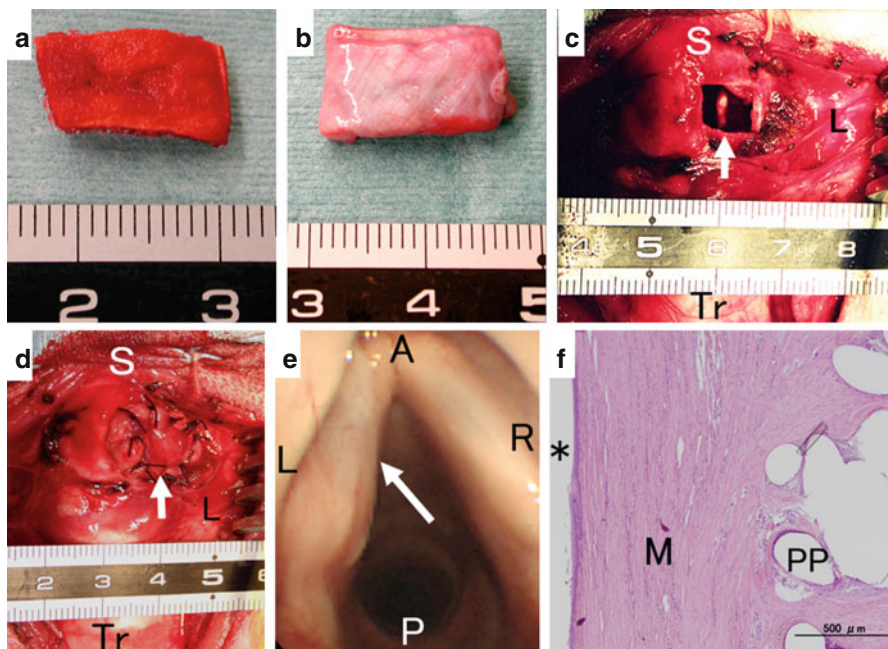




**Fig. 10.2** (a) Casting procedure. The intraluminal shape of left vocal fold (VF) was reflected on the cast (*arrow*). *S* superior, *I* inferior, *R* right, *L* left. (b) Contours of the vocal fold were replicated on a dual-layered polypropylene scaffold (*arrow*). (c) An image of the scaffold covered with freeze-dried spongy collagen. *P* posterior, *A* anterior. (d) An image after preclotting procedure with BSCs and peripheral blood. (e) A left hemilaryngectomy was performed. An *arrow* indicates an original left vocal fold. *Thy* thyroid cartilage, *Tr* trachea. (f) The scaffold implant was anastomosed to the surgical defect. (g) A fiberscopic image taken 3 weeks after the operation. A left vocal protrusion covered with epithelium was observed

Kanemaru et al. [39] reported the combination of this polypropylene-based scaffold *in conjunction* with a growth factor, basic fibroblast growth factor (b-FGF), in three clinical cases of cricotracheal stenosis after long term intratracheal intubation. Although the follow-up period only lasted up to 6 months, they showed preferable airway regeneration using the scaffold combined with *the* growth factor. The slow-release effect of b-FGF in combination with the surrounding spongy collagen was thought to enhance angiogenesis in the scaffold. Given the reduced possibility of tumor formation after local b-FGF application during the short postoperative period, this tissue engineering technique may be a viable approach for cases with cricotracheal stenosis.

Yamashita et al. [27] revised the polypropylene scaffold framework for the subsequent regeneration of the thyroid cartilage. Replication of the intricate luminal shape of the canine larynx was performed using a dental cast (Fig. 10.2a). Two-ply polypropylene mesh sheets with similar concavity and convexity of the vocal folds were designed (Fig. 10.2b). In preliminary studies, the sheets were coated with spongy collagen (Fig. 10.2c) and were implanted after hemilaryngectomy in their

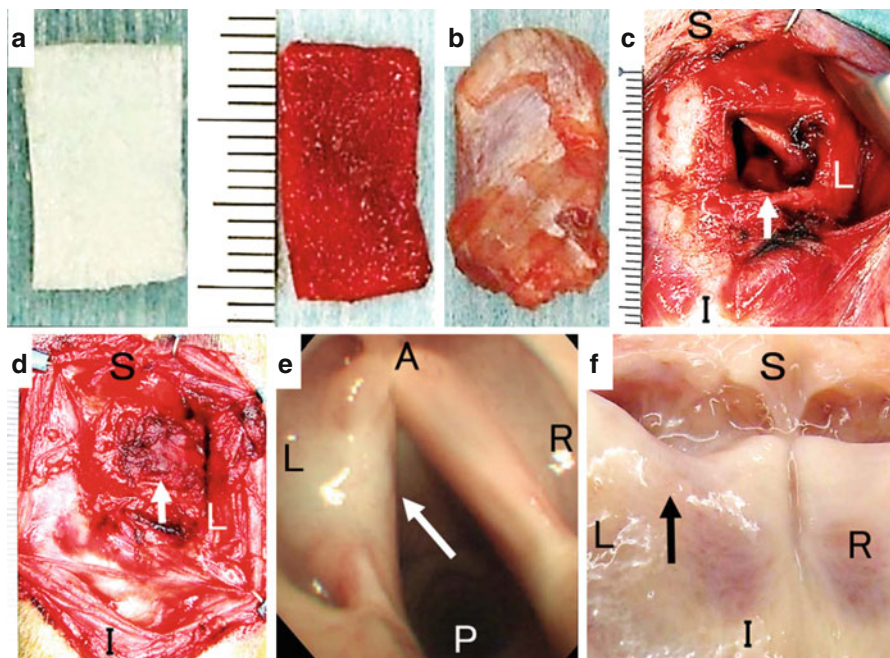


**Fig. 10.3** (a) A polypropylene-based scaffold preclotted with peripheral blood. (b) The scaffold was covered with autologous fascia lata. (c) Membranous portion of left vocal fold was resected through a window defect created in a left ala of thyroid cartilage. *S* superior, *Tr* trachea, *L* left. (d) The scaffold implant was anastomosed to the resected site. (e) A fiberscopic image taken 3 months after the surgery. A preferable luminal shape with epithelialization was obtained at the surgical site. *R* right, *A* anterior, *P* posterior. (f) A hematoxylin- and eosin-stained image 3 months postoperatively. Luminal surface is covered with squamous epithelium (*asterisk*). Sparse muscle tissue is observed (*M*) between epithelium and scaffold. No inflammatory reaction is seen. *PP* polypropylene framework

canine model; these initial attempts resulted in failure, even though the same methods were successfully used for cricoid and tracheal regeneration. This failure might be due to movements resulting from swallowing and barking as well as infection of the scaffold from secretions which are inevitable because of the anatomical location. Next, they utilized bone marrow-derived stromal cells (BSCs) as an enhancer of the regenerative process and peripheral blood for preclotting the scaffold (Fig. 10.2d) for their hemilaryngectomy model (Fig. 10.2e, f). One out of three dogs showed favorable epithelialization with preferable luminal contour (Fig. 10.2g), although no histological assessment was performed. While a beneficial contribution of BSCs was observed, it is still unknown how they behaved in the scaffold *in vivo*.

Yamashita et al. [29] reported *in situ* tissue engineering of the canine thyroid cartilage after a partial window defect (size of  $1.2 \times 0.7$  cm). In the experimental group ( $n=5$ ), they utilized a single polypropylene mesh sheet with spongy collagen covered with autologous fascia lata after preclotting with peripheral blood (Fig. 10.3a–c), and in the control group ( $n=3$ ) a strap muscle flap was used for their





**Fig. 10.4** (a) A polypropylene-based scaffold covered with spongy collagen. (b) The scaffold after preclotting procedure. (c) Autologous fascia lata was used to cover the preclotted scaffold. (d) A left partial hemilaryngectomy was performed (*arrow*). *S* superior, *I* inferior, *L* left. (e) The scaffold implant was anastomosed to the defect site. An *arrow* indicates the scaffold implant. (f) A fiberscopic image taken 6 months after the operation in 3% collagen group. An *arrow* indicates regenerated left vocal prominence. *A* anterior, *P* posterior, *R* right. (g) A macroscopic intraluminal image from excised larynx revealed difference in vertical levels of vocal folds (*arrow*), which resulted in suboptimal vibratory data

window defect model (Fig. 10.3c, d). Four out of five dogs showed good vocal fold eminence under fiber-optic examination; typical cases showed residual tissue of the fascia covering until the 7th postoperative day and epithelialization was completed within 1 month (Fig. 10.3e). The histological data at 3 months postoperation revealed the regeneration of lined epithelium, subepithelial tissue, and muscle (Fig. 10.3f) in both groups without any new cartilage formation. Vibratory assessments from the experimental group showed suboptimal results. The protective effects from infection at early time points seemed very important for obtaining favorable regeneration.

Kitani et al. [30] reported on a canine partial hemilaryngectomy model (with a size of  $1.8 \times 1.0$  cm) and compared the 1% classical type ( $n=6$ ) and 3% new type ( $n=6$ ) of spongy collagen which covers the polypropylene mesh framework (Fig. 10.4a, b). Fascia lata was also used for their artificial scaffolds (Fig. 10.4c). While the 1% collagen model showed a half successful ratio without mesh implantation, the 3% collagen model showed successful results in all cases after

hemilaryngectomy (Fig. 10.4d–g). They stated that the 3 % collagen model contributed to tissue regeneration with harder mechanical stiffness and slower absorption than the 1 % collagen model. Histological data indicated the presence of a fine epithelial lining with subepithelial tissue without any cartilage formation in successful cases. Vibratory data were suboptimal mainly due to a difference in vertical levels of the regenerated vocal folds.

Metallic substances have also been investigated for cricoid regeneration. Tan et al. [40] examined the efficacy of a porous metastable  $\beta$ -type titanium alloy for regeneration of the cricoid and trachea (20 mm in length) in 10 mongrel dogs. The titanium alloy with tiny pores (diameters of about 70–90  $\mu\text{m}$ ) was designed to a final porosity of 30–35 %. The final prostheses showed a cylindrical C-shape and were 0.5 mm thick, 20–22 mm in inner diameter, and 20 mm in length. Two dogs died of an accidental complication with anesthesia and pneumonia. Granulation was observed in four cases out of eight, and one showed exposure of the metastable  $\beta$ -type titanium alloy plate, although these animals were asymptomatic. During the 3–8-month postoperative follow-up period, all of the prostheses had completely incorporated into the host tissue and histological data showed favorable epithelial lining with simple squamous cells at the midposition and ciliated columnar cells near the anastomoses of the prostheses. The mechanical power of this material was sufficient to prevent airway collapse; however, the scaffolds had to be designed and prepared prior to the regenerative surgeries. This means that the resection area must be precisely estimated before surgery. This study showed remarkable progress in manufacturing metallic scaffolds and their potential application as bioartificial materials. As they stated in the article, further studies related to the long-term outcomes and regenerative surgeries for larger defects should be performed in the future.

What is the ideal scaffold for laryngeal framework surgery? No one yet knows the answer. Extensive study into the various scaffolds should be performed. Major factors to take into consideration include materials, processing techniques to improve cellular attachment and/or ingrowth on the surface of the scaffolds, lower immune-rejection and antigenicity, better biocompatibility, and easier handling with sufficient mechanical power. Bioabsorbable scaffolds, which will be substituted with host cells over the long term, would be preferable if they could retain sufficient mechanical properties to prevent collapse of airway structures. These scaffolds could thus be applicable to stenotic lesions in children.

### 10.3 Cells and Regulatory Growth Factors

In 2000, Wambach et al. [41] reported potential cartilage regeneration in vitro using chondrocytes from canine thyroid cartilage and bovine-derived type I collagen matrix. The isolated and cultured chondrocytes grown on bovine type I collagen expressed type II collagen, which was produced by the chondrocytes. No gross cartilage formation was noted in this study. As of now, in order to generate

mechanically durable gross tissue using chondrocytes, other scaffold templates for chondrocytes or an in vivo prefabrication period is likely required.

Katic et al. [42] examined the regenerative effects on thyroid cartilage defects (1.5 cm<sup>2</sup>) without opening the lumen in a canine model using thyroid cartilage allografts and human recombinant osteogenic protein-1 (OP-1; bone morphogenetic protein-7). The data revealed that OP-1 combined with thyroid cartilage allografts induced bone, cartilage, and ligament-like structures comprising up to 80 %. The borders of the defects were shown to have healed by formation of new bone in cases where bone resided within the old thyroid cartilage layers.

Tcencu et al. [43] published an article describing the regeneration of partial defects (2 mm) in the anterior portion of the rabbit cricoid cartilage utilizing recombinant human bone morphogenetic protein-2 (BMP-2) and collagen sponge as a carrier. They found new cartilage and bone formation 4 weeks after surgery when using BMP-2 with collagen sponge. No discontinuity at the boundaries of the implant was observed. Proteoglycans were also produced by the new cartilage.

Thus, regulatory growth factors are clearly able to modulate the tissue regeneration process, but may have unintended effects on cell behavior.

Nomoto et al. [44] reported on the effects of allogeneic dermal fibroblasts on polypropylene mesh with collagen sponge for cricoid regeneration in rats. In the group treated with heterotopic fibroblasts, epithelialization and changes in collagen fibers occurred more rapidly than in the scaffold group without cells. Although the fate of allogeneic dermal fibroblasts and the mechanism by which they accelerate tissue regeneration are unclear, this study indicated that the addition of cells could also directly modulate the tissue regeneration process.

When ex vivo cell culture is employed in clinical use, strict quality control is essential. Contamination by unintended cells or microorganisms and the risks of transmitted diseases from donors or culture serum may hinder its rapid progress in the field of regenerative medicine.

## 10.4 Future Perspectives

The recent advances in regeneration of the laryngeal framework are remarkable and very promising.

Clinical use of in situ tissue engineering has been applied in cricoid or cricotracheal regeneration since 2002 [45, 39]. Favorable epithelialization and tissue regeneration was achieved in canine studies with maximum resection size *during* hemilaryngectomy at the level of thyroid cartilage [30, 31].

Approaches utilizing tissue regeneration may be more preferable than the complicated and skilled repeated surgeries that involve damage to donor sites in order to reconstruct deficits in the laryngeal framework. To establish these approaches as standard treatment options, current techniques should be modified and refined.

Researchers should focus on the technological innovations pertaining to new scaffolds. Ideal scaffolds may eliminate the need for exogenous cells or growth

factors, making it easier to be applied in clinical practice. Rapid sealing of the defect site from the airway and subsequent epithelialization could also reduce the risk of infections, which is key for obtaining favorable results.

The use of growth factors could be accepted in limited situations. Thoughtful consideration should be taken to prevent tumorigenesis or relapse of malignant tumors. Release time, concentration, and duration could be controlled with the use of carrier compounds. If scaffolds could be successfully excluded from the immunorecognition system, immunomodulation with cytokines or other molecules combined with scaffolds could be beneficial after regenerative surgeries.

When considering the addition of exogenous cells to scaffolds, it becomes much more complicated to predict the types of interactions which could occur within host tissues and scaffolds. Progenitor or stem cells might differentiate into other cell types and might produce different factors over time. Embryonic stem cells and induced pluripotent stem cells might induce tumorigenesis, as they have the potential to become many different cell types. Bone marrow-derived stem cells and adipose-derived stem cells are *also* candidates for regeneration of mesenchymal tissues. Differentiated cells, including chondrocytes or fibroblasts, should not persist as long as stem cells; thus, they are generally considered to be safe due to their rapid disappearance. In general, cell culture media that includes serum enhances cellular activities, i.e., proliferation and differentiation; however, these sera are generally autologous or derived from other animals. Serum-free medium is one option to prevent donor-borne transmitted diseases; however, the proliferative ability of the cultured cells would be greatly reduced.

Once the long-term safety of these techniques is established, the cloning of other mammals for donor tissue harvest might surface for debate, although lifelong immunosuppressive treatments and ethical issues related to the lives of donor animals are inevitable.

The eventual goal of laryngeal framework regeneration is the fully functional restoration of the vocal folds. In order to achieve this goal, many challenging tasks lay ahead, namely, laryngeal re-innervation of both sensory and motor nerves, regeneration of the viscoelastic properties of the lamina propria, and vibratory function through muscle contraction, among others. Further ongoing studies will be required in order to restore laryngeal function in patients with intractable diseases.

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