Real-time light-guided vocal fold injection: An in vivo feasibility study in a canine model

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Conceptualization: WC. Data curation: SK, RTM and GH. Formal analysis: RTM and GH. Methodology: HK and WC. Project administration: WC. Visualization: HYS and WC. Writing - original draft: HYS and WC. Writing - review & editing: HYS, WJJ and WC.
Highlights

✓ Real-time light-guided vocal fold injection (RL-VFI) provides precise localization and visual-motor feedback in vivo animal model.

✓ RL-VFI would be helpful in various trans-cutaneous approaches including the cricothyroid approach, the trans-thyroid approach and the thyro-hyoid approach.

✓ The clinical application of RL-VFI is expected to improve safety and precision in VFI.

✓ This study establishes the rationale for an upcoming clinical trial.
ABSTRACT

**Purpose:** The trans-cutaneous approach is a good option for office-based vocal fold injection (VFI). However, precise localization requires a high level of experience because the needle tip is invisible in small and complex laryngeal spaces. Recently, a new technique, real-time light-guided VFI (RL-VFI), was proposed; it allows simultaneous injection under precise needle localization by light guidance. Herein, we aimed to verify the feasibility of RL-VFI in an *in vivo* canine model and tried to explore its clinical usefulness.

**Methods:** The device for RL-VFI comprised the light source (light-emitting diode modules [10W] of red color [650nm]) and the injectors (1.5 inches, 23 gauge). An adult male beagle was used for the experiment. After tracheostomy, a rigid laryngoscope was inserted and suspended to expose the larynx. A flexible naso-laryngoscopy system was used to visualize the vocal folds.

**Results:** RL-VFI was performed using various trans-cutaneous approaches, including cricothyroid (CT), trans-thyroid (TT), and trans-hyoid (TH) approaches. Light guidance helped identify the path of the needle and prevent inadvertent penetration. The location of the needle tip was accurately indicated by the light. The lighted needle could be easily placed at the intended points in the vocal fold with real-time visual-motor feedback. Hyaluronic acid could be simultaneously injected lateral to the vocal process under light guidance without manipulation of the device.

**Conclusion:** RL-VFI is safe and feasible in an *in vivo* canine model, providing precise localization and visual-motor feedback. The clinical application of RL-VFI is expected to improve safety and precision in VFI.

**Level of Evidence:** N/A

**Keywords:** vocal fold injection; real-time light-guided vocal fold injection; *in vivo* animal study; trans-cutaneous approach; vocal fold paralysis;
Introduction

Vocal fold injection (VFI) is a laryngological procedure that delivers drugs or materials to the vocal folds through a needle and has a history of over a hundred years.\textsuperscript{1,2} It can be used to treat various vocal fold pathologies, including vocal fold atrophy, vocal fold scarring, vocal fold papillomatosis, vocal fold nodules, vocal fold polyps, vocal fold granuloma, Reinke’s edema, and vocal fold paralysis (VFP).\textsuperscript{1,2} Recently, this minimally invasive technique has been re-highlighted in laryngology with the advancement in material science and endoscopic technology.\textsuperscript{2,3}

Currently, the approaches of VFI were classified according to the route of injection: the trans-cutaneous (the trans-cricothyroid [CT] membrane, the trans-thyroid cartilage [TT] and the trans-thyrohyoid [TH] membrane approaches), the trans-oral, and the trans-nasal approaches.\textsuperscript{2} The choice of approach usually depends on the anatomical characteristics of patients, the injection material, and the surgeon’s preference.\textsuperscript{4-6} Among various approaches, the trans-oral and the CT approaches are the most used in current practice. The trans-oral approach is very intuitive and easy to understand because of the good localization of the needle tip.\textsuperscript{2,4} However, it is difficult to handle the long-curved needle delicately during the trans-oral approach, and mucosal penetration may lead to spillage of the injectate, bleeding, and laryngeal spasm.\textsuperscript{2,7} In comparison with the trans-oral approach, the CT approach is advantageous due to good patient compliance, suitability as an office-based procedure, and a low complication rate.\textsuperscript{6-12}

However, the CT approach has a fundamental limitation despite its advantages; precise localization of the needle tip is very difficult because it moves inside the vocal fold.\textsuperscript{4,5} Most laryngologists usually estimate the location of the needle tip with the distortion of the vocal fold configuration indirectly. Therefore, a high level of experience is necessary to perform VFI with the CT approach proficiently, and mastery of this technique involves a steep learning curve.\textsuperscript{13,14} In our country, only a few laryngologists in referral hospitals perform the majority (90%) of VFI cases.\textsuperscript{15}

Recently, Cha et al. suggested that the invisibility of the needle tip is the primary reason for the technical limitation of the CT approach. And they proposed a new technique to overcome the invisibility of the needle and termed it the real-time light-guided VFI (RL-VFI); it allows simultaneous injection under precise needle localization by light guidance.\textsuperscript{4,5} In previous studies, the concept models for RL-VFI were developed, and its technical feasibility was validated in an ex-vivo canine larynx model.\textsuperscript{4,5} The RL-VFI device could intuitively provide visual information for the precise location of the needle tip by light guidance during VFI.
However, in a real office-based VFI, the vocal folds should be visualized by a flexible laryngoscope through a dark naso-oropharyngeal tract, and the needle passes through the more complex anatomical layers from the skin to the vocal fold. The clinical setting is somewhat different from that of the previous studies using the extracted larynx. Notwithstanding the promising previous results, the experience of RL-VFI in an ex vivo model would not be sufficient to conduct a clinical trial. In this study, we aimed to verify the feasibility of RL-VFI in an in vivo canine model with the trans-cutaneous approaches and to establish the rationale for conducting the clinical trial in humans.

Materials and Methods

Ethical consideration

The animal care and use protocol of this study was reviewed and approved by the Institutional Animal Care and Use Committee at Pusan National University Yangsan Hospital (PNUYH-2018-126), and all methods were carried out in accordance with approved guidelines.

The Device for RL-VFI

The device for RL-VFI was developed for this experiment. The device comprised two components of the light source and the injectors. The light source had light-emitting diode modules (10W) of red color (650nm) and emitted light via a single optic fiber. The injectors consisted of a needle (1.5-inch, 23-gauge) and a connector with an optic fiber cable (Figure 1A and B).

Animal Model and Experimental Setting

A 10-month-old adult male beagle (Hanabio Technology Co.Ltd., Pyeongtaek, Korea) weighing 13 kg was used for the experiment. The dog was humanely anesthetized by the veterinarian. After tracheostomy, a rigid laryngoscope was inserted and suspended to expose the larynx.

A full high-definition video-laryngoscopy system consisted of a video processor (DEFINA, EPK-3000; PENTAX Medical, Tokyo, Japan) and a flexible video naso-pharyngo-laryngoscope (VNL11-J10; PENTAX Medical). To mimic office-based trans-cutaneous VFI, the flexible laryngoscope was inserted via the rigid laryngoscope to visualize the vocal folds. The injector of the RL-VFI device was inserted through the cervical
skin (Figure 1C and D). Hyaluronic acid (HA; Neuramis Light Lidocaine, Medytox Inc., Cheongju-si, Korea) was used as the filler.

After the experiment, the larynx was excised from the fourth tracheal ring to the hyoid bone to examine histologic change.

**Histologic Analysis**

The canine larynx was fixed in formaldehyde and cut axially at the level of the true vocal folds. The specimen was processed for 8 hours with a rapid hydrochloric acid decalcifier (Shandon TBD-I, Thermo Fisher Scientific Co., Waltham, USA) to remove the calcium. The specimen was embedded in paraffin blocks, sectioned, and stained (hematoxylin and eosin) for histologic analysis. Slides were scanned with Pannoramic®, 250 Flash III (3DHISTECH Ltd, Budapest, Hungary).

**Results**

In this study, we validated various approaches of trans-cutaneous RL-VFI in an *in vivo* canine model (Figure 2). In the CT approach, the needle was inserted between the inferior border of the thyroid cartilage and the superior border of the cricoid cartilage. In the TT approach, the needle was placed above the inferior border of the thyroid cartilage and introduced perpendicular to the cartilage. In the TH approach, the needle was placed just above the thyrohyoid notch and inserted into the subcutaneous tissues at a downward angle.

**Identification of the Needle Tip in the CT Approach**

The needle tip could be identified using the RL-VFI device in the CT approach, and its anatomical location in the larynx could be intuitively determined by the red light. The vocal folds were visualized using a flexible laryngoscopy under a suspension rigid laryngoscopy (Figure 3A). When the injector of the RL-VFI device was gently introduced on the CT membrane, the red light was easily identified on the subglottic mucosa of the CT membrane and helped prevent the penetration into the airway (Figure 3B). By referring to the position of the needle on the CT membrane, the entry point of the needle on the skin was adjusted slightly outward. As the needle was advanced gradually, the light scattered around the vocal fold and indicated that the needle was properly introduced into the paraglottic space (Figure 3C). To treat unilateral vocal fold paralysis, the material
is usually injected on the point lateral to the vocal process in the thyroarytenoid muscle for deep vocal fold augmentation. Under light guidance, the needle tip could be easily positioned on the intended point lateral to the vocal process by real-time visual feedback (Figure 3D). The scattering and intensity of the light provided information about the depth of the needle in the vocal fold. When the needle tip approached the mucosa on the superior surface of the vocal fold, the intense light could prevent the needle from penetrating the mucosa and help it repositioned at the proper space of deep musculature (Figure 3E). When the needle approached the medial surface of the vocal fold, the visual information by the light prevented inadvertent penetration into the mucosa (Figure 3F). Depending on the degree of light scattering and transmittance, the depth of the needle could be identified and controlled delicately in the vocal fold.

Identification of the Needle Tip in TT Approach

In the TT approach, the needle tip could be easily localized in the vocal fold using the RL-VFI. The vocal folds were visualized using a flexible laryngoscopy (Figure 4A). The dispersed light could be identified around the vocal fold and the laryngeal ventricle after the needle was introduced through the thyroid cartilage (Figure 4B). Under light guidance, the needle was moved into the thyroarytenoid muscle and could be located in the target point lateral to the vocal process by visual feedback (Figure 4C). When the needle proceeded across the vocal process, the vocal process was shaded and easily identified (Figure 4D). Visual information by light emission and scattering in the vocal fold provided the direct orientation of the needle tip. Furthermore, the device could assist the needle tip to reach the posterior mucosa precisely and prevent unintentional penetration (Figure 4E). The anterior vocal fold mucosa was easily accessed using the light-guided needle (Figure 4F).

Identification of the Needle Tip in TH Approach

The route of needle tip could be identified by the red light of the device during the TH approach. The vocal folds and the epiglottis were visualized using a flexible laryngoscopy (Figure 5A). The needle should be inserted to the petiole near the vocal fold through the pre-epiglottic space and the epiglottic cartilage. The precise penetration of the epiglottic mucosa is important to perform successful VFI into the intended point. The needle was inserted through the TH membrane and identifiable around the petiole by the scattered light (Figure 5B). The light was observed in the sufficiently low-lying point around the petiole, allowing the operator to insert the needle on the vocal fold at a comfortable angle (Figure 5C). After the needle was positioned at the intended
point just lateral to the petiole, it penetrated the mucosa (Figure 5D and 5E). After the needle penetrated the appropriate point, it could be easily inserted into the vocal fold (Figure 5F).

**RL-VFI with HA via CT approach using the device for RL-VFI**

RL-VFI with HA was performed in the CT approach using the device. The lighted needle was inserted through the CT membrane and positioned in the deep thyroarytenoid muscle of the left vocal fold (Figure 6A and 6B). The injection of HA into the muscle was subsequently started under light guidance and medialized the left vocal fold slightly (Figure 6C). Because HA has higher transmittance of the light than muscle and mucosa, the lighted needle in the material showed stronger dispersion and could provide information on the extent of the injectate (Figure 6D and 6E). After removal of the needle, it could be confirmed that the vocal folds were sufficiently medialized (Figure 6F).

**Histologic Evaluation after HA injection using the RL-VFI**

In post-mortem analysis, the distance from the anterior commissure (AC) to the posterior commissure was 18 mm and the distance from AC to the vocal process was 8 mm in the canine larynx. The diameter of the trachea was 18 × 21 mm and the distance from the superior thyroid notch to the inferior thyroid notch was 17 mm. H&E stained section of the extracted vocal folds showed that HA was properly injected in the thyroarytenoid muscle of the left vocal fold (Figure 7). There were no histological findings related to thermal damage on the tissue around the materials in the left vocal fold.

**Discussion**

The CT approach has the advantage that it has a low complication rate, suitability as an office-based procedure, and good patient compliance compared to other mucosa-penetrating procedures such as trans-oral or TH approaches. During the CT approach, inadvertent penetration of the mucosa can lead to cough, bleeding, failure, cessation or delay in the procedure, and low patient satisfaction. In our study, the RL-VFI device provided the precise location of the needle by light guidance and could intuitively prevent inadvertent penetration in the CT approach. Usually, the position of the needle can be indirectly identified by conventional
maneuvers, such as palpation of the CT membrane and alteration of the vocal fold configuration. Furthermore, the alteration of the vocal fold configuration can only be noticed when the needle enters into the thyroarytenoid muscle.\textsuperscript{2,7,12} Without anatomical or spatial orientation correlating external landmarks with internal laryngeal structures, it would be difficult to insert the needle into the muscle with a few attempts in a short time.\textsuperscript{4,5,12} The RL-VFI device can be useful in the identification of the needle during the blind pathway from the skin to the thyroarytenoid muscle.\textsuperscript{4,5} The injector of the device could radiate the scattered light and provide visual information about the direction and depth when it entered into the deep paraglottic space before reaching the thyroarytenoid muscle. The location and depth of the injection point can considerably affect the final configuration of the vocal fold after VFI.\textsuperscript{4,5,14} In the CT approach, precise injection is challenging due to the invisibility of the needle tip as widely known, and there has been nothing to provide the depth orientation of the needle. RL-VFI could provide information about the anterior-to-posterior or medial-to-lateral position as well as the depth orientation by alteration of intensity and scattering of the light.\textsuperscript{4,5}

Although the CT approach has advantages and is a preferred technique in our country, a previous history of operation or obesity would make it very difficult to perform.\textsuperscript{1,6} The TT or TH approaches are good techniques and could be alternative options when the CT approach is not possible.\textsuperscript{1,6,8} In the TT approach, the needle is inserted perpendicular to the thyroid cartilage and the TT approach is very useful in small larynges with less ossification.\textsuperscript{2,6,11} However, the ideal direction of the needle varies according to the insertion point. The possibility of penetration is higher than in the CT approach because the needle route is more perpendicular to the vocal fold.\textsuperscript{6,4,12} In this study, the lighted needle was very helpful to prevent the penetration of the vocal fold mucosa in the in vivo canine model. The RL-VFI device could lead the needle to the intended point without spatial orientation by real-time visual feedback.

The disadvantages of the TH approach include the inability to access the anterior vocal fold in small larynges, inability to access the posterior vocal fold in large larynges, and difficulty in passing the needle through a calcified TH membrane.\textsuperscript{1,2,7} These disadvantages result from the long pathway of this approach, which limits the range of movement of the needle. There is a potential need to pass the needle multiple times into the larynx, which can cause bleeding, gag, or cough reflex.\textsuperscript{2,4,8-10} The optimal penetration point can vary depending on the target location of the vocal fold: anterior or posterior. To overcome these current limitations, it is important to set an optimal point before penetrating the mucosa of the epiglottis.\textsuperscript{4,9,13} In this study, the lighted needle of the device could provide visual information on the position of the needle before penetrating the
mucosa. It would help to determine the optimal penetration point and to reduce the number of trial insertions in
the TH approach.

Since Seifert and Hirst introduced the CT approach a hundred years ago, the advancement of endoscopic
technology and the development of biocompatible materials have accelerated the refinement of this
 technique. However, there have been few signs of progress in the technical aspect, and most laryngologists
still depend on Hirano’s classical maneuvers to identify needle position: palpation of the CT membrane and the
examination of distortion and subtle movement of the vocal folds during the needle insertion. They have been
only precious lighthouses for successful VFI with the CT approach over the past decades. Nonetheless, these
maneuvers fundamentally require high clinical experience and learning curves. Several attempts were made
to improve the CT approach technically. Jin et al. suggested that the anatomical references regarding the CT
approach provide guidelines and the understanding of the procedure, eventually leading to precise access to the
vocal fold. However, it is difficult to solve these current problems using only the image-guided approach
because of the inter-individual variation in laryngeal anatomy. Hoffman et al. suggested a breakthrough
technique for the transillumination of the vocal folds, which utilized visual feedback during the procedures.
However, the optic fiber cable must be removed before injection and this removal-and-reinsertion technique is
hard to apply in real office-based practice.

RL-VFI was conceptualized as a technique that allows simultaneous injection under precise needle
localization by light guidance. The RL-VFI device was developed and the technical feasibility was validated in
the ex vivo canine model simulating the setting of office-based VFI. However, in actual patients, the needle
passes through more complex anatomical layers of the skin, the subcutaneous fat, the strap muscles, the CT
membrane, the paraglottic space, and the thyroarytenoid muscle. It is more challenging to determine the
optimal entry point because the external anatomical landmarks might be obscure. In this study, RL-VFI
was feasible in an in vivo canine model with the whole anatomical structures, using the CT approach in addition
to the TT and the TH approaches. The VFI of HA could be simultaneously performed with no additional
manipulation using the RL-VFI device. The dispersed light gives information about the extent of injected
materials due to its transparency and is helpful even in re-injection.

The canine model used in this study weighed 13 kg. And it had a relatively small larynx with an 8-mm-long
vocal fold considering that the average length of the vocal fold is 13.5 mm and 15.3 mm in female and male
adults. The successful injection in this small larynx indicated that delicate and precise manipulation is possible
with the aid of the device.
In modern laryngology, VFI has developed mainly into an awake in-office procedure. In our study, the animal model was inevitably sedated under general anesthesia and could not completely simulate an awake in-office practice. Thus, a clinical trial is mandatory to establish clinical safety and effectiveness of the RL-VFI device. We plan to carry out a pilot clinical study to apply the device in office-based VFI in the future.

After the introduction of RL-VFI in the clinical field, we anticipate the improvement of VFI in various aspects. RL-VFI will improve the precision and safety of VFI for the treatment of unilateral VFP. The RL-VFI device will facilitate customized VFI in the various shape of the paralyzed vocal fold with controlling the dose and location of injection. Also, it will help prevent inadvertent penetration and mis-injection into the superficial layer, which can lead to disastrous problems for the voice. From an educational perspective, RL-VFI will help trainees to understand the route of the needle during the transcutaneous VFI and learn it quickly. The training program using RL-VFI would be helpful for trainees to practice the transcutaneous VFI before trying it on actual patients. When counseling patients, it would be difficult to explain the concept of the transcutaneous VFI due to the invisibility of the needle. RL-VFI will help patients to understand the procedure intuitively and be reassured.

Conclusions

RL-VFI is feasible and safe in an *in vivo* canine model, providing precise localization and visual-motor feedback. This study demonstrates the feasibility of RL-VFI in an *in vivo* canine model and establishes the rationale for an upcoming clinical trial. The clinical application of RL-VFI is expected to improve safety and precision in VFI.
References


Figure 1. (A) The device for real-time light-guided vocal fold injection (RL-VFI); (B) The injector of the RL-VFI device; (C) The experimental setting. The flexible laryngoscope was inserted via the rigid laryngoscope to visualize the vocal folds to mimic office-based trans-cutaneous VFI; (D) The injector of the RL-VFI device was inserted through the cervical skin.

Figure 2. Various approaches of trans-cutaneous RL-VFI device in an in vivo canine model. (A) The CT approach; (B) The TT approach; (C) The TH membrane approach.

Figure 3. Identification of the needle tip in the CT approach using the RL-VFI device. The location of the needle tip can be identified by the red light: (A) before the needle insertion; (B) on the CT membrane; (C) in the paraglottic space; (D) lateral to the vocal process; (E) on the mucosa on the superior surface of the vocal fold; and (F) on the medial surface of the vocal fold. The scattering and intensity of the light can inform the depth of the needle in the vocal fold.

Figure 4. Identification of the needle tip in TT approach using the RL-VFI. The location of the needle tip can be identified by the red light: (A) before the needle insertion; (B) around the vocal fold and the laryngeal ventricle; (C) lateral to the vocal process; (D) around the vocal process; (E) on the posterior mucosa; and (F) on the anterior vocal fold mucosa.

Figure 5. Identification of the needle tip in TH approach using the RL-VFI. The location and the route of the needle tip can be identified by the red light: (A) before the needle insertion; (B) in the pre-epiglottic space; (C-D) on the mucosa of the petiole; (E) the penetration of the mucosa; (F) the insertion of the needle into the vocal fold.

Figure 6. RL-VFI with HA in CT approach. (A) before the needle insertion; (B) The placement of the lighted needle in the deep thyroarytenoid muscle; (C-E) The injection of HA into the muscle. HA has higher transmittance of light than muscle and mucosa, the lighted needle in the material shows stronger dispersion and
can provide information of the extent of the injectate; (F) After removal of the needle, it can be confirmed that the vocal folds is sufficiently medialized.

Figure 7. H&E stained section of the extracted vocal folds showed that HA was properly injected in the thyroarytenoid muscle of the left vocal fold. Histologically, there was no histological findings related to thermal damage on the tissue around the materials in the left vocal fold.
Figure 5