

Pre-clinical evaluation of a minimally invasive laryngeal pacemaker system in mini-pig

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Abstract Microlaryngoscopic enlargement techniques have been the standard treatment for bilateral vocal fold paralysis (BVFP) for decades. Laryngeal pacing is a promising alternative treatment based on the electrostimulation of the posterior cricoarytenoid (PCA) muscle. This paper reports on the results of a pre-clinical study aiming to evaluate this method. Eight Göttingen mini-pigs were implanted with a laryngeal pacemaker (LP) implant prototype and with two LP electrodes, one in each PCA muscle. The 6-week follow-up included endoscopic stimulation controls in general anaesthesia and radiographic controls of electrode integrity and position stability. Stimulation parameters for optimal glottal opening were evaluated via videolaryngoscopy. Histopathology was performed upon conclusion of the study. 7/8 (87.5 %) animals were successfully implanted with the LP implant prototype and two LP electrodes. In general, stimulation was effectively delivered and correlated with the expected PCA muscle activation. 2/14 (14.3 %) electrodes dislocated and 1/14 (7.1 %) electrode tip broke. The LP system used in this experiment to induce vocal fold abduction by

means of selective functional electrical stimulation of the PCA showed promising results. It may be a valid alternative to the current golden standard for BVFP treatment. Clinical studies are needed to confirm the medical relevance of the LP.

Keywords Mini-pig · Larynx · Posterior cricoarytenoid (PCA) muscle · Neurostimulation · Laryngeal pacemaker · Middle term

Introduction

Bilateral vocal fold paralysis (BVFP) is a serious medical condition, which often has a significantly detrimental impact on quality of life and can, in extreme cases, become life threatening. The most frequent cause of BVFP is iatrogenic injury to both recurrent laryngeal nerves (RLNs). Functional recovery is unpredictable and often poor despite neuromuscular regeneration [1–5]. Post-injury RLN reinnervation is usually non-selective to abductor and adductor muscles and is often delayed in the posterior cricoarytenoid (PCA) muscle. Thus, RLN reinnervation is commonly associated with vocal cord paralysis in the paramedian position and, in the case of a bilateral paralysis, with the consequent severe breathing impairment symptoms. Standard therapy comprises tracheotomy for immediate relief and microlaryngoscopic glottal enlargement techniques commonly carried out with a laser or as a laterofixation of one vocal fold or with combined techniques. Glottal enlargement, however, often correlates with impaired voice quality and fails to restore good exercise tolerance [6, 7].

Functional electrical stimulation of the PCA muscle seems to be a promising approach to restoring vocal fold

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mobility. Based on the analysis of the results obtained by Zealear and other groups [5, 8, 9], we developed a minimally invasive device, the laryngeal pacemaker (LP), capable of selectively stimulating the PCA muscle via an electrode placed into the PCA via a transcricoidal insertion method. The procedure used to insert the LP electrode was previously tested in mini-pig larynx [10].

In this paper, we described the results of the bilateral implantation of the LP system in these mini-pig over a 6-week follow-up period. Electrode–tissue interaction, mechanical stability of the LP system, and stimulation effectivity over time were evaluated.

Materials and methods

Subjects

The subjects were eight Göttingen mini-pigs (designated MP 1 IMP to MP 8 IMP), Ellegaard, Dalmose, Denmark, aged 6 months to 1 year, weighing 23–5 kg.

Material

The LP system used in this study was manufactured by MED-EL Medical Electronics, Innsbruck, Austria. The animals were implanted with either the LP implant prototype I or the LP implant prototype II (Fig. 1c). Both prototypes consisted of an implantable stimulator connected to two bipolar inline connectors (ground at the tip of one electrode and three active channels). Prototype II had the same design as the LP implant that would be used in subsequent clinical investigations. The only difference between the prototypes was the external housing.

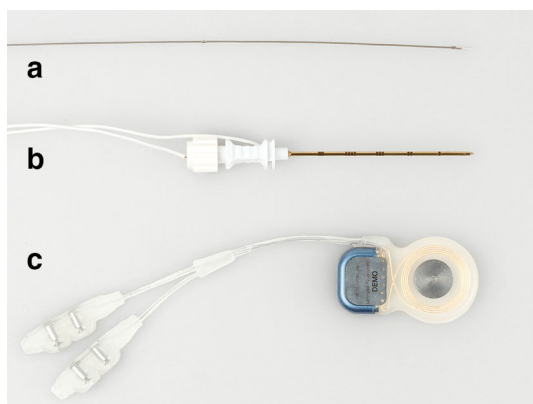


Fig. 1 LP electrode (a), LP insertion tool (b), and LP implant prototype II consisting of a hermetic implant housing containing the electronics, the receiver coil of the inductive link to the external processor or external digital interface box, and two electrode connectors (c)

The interface between the LP implant prototype I or II and the external stimulator was provided by MED-EL Digital Interface Box (DIB), connected via an external conductive coil. The output circuitry allowed a unilateral bipolar stimulation (one electrode with ground and one active channel) or a bilateral stimulation (ground on one side and one of the active channels of a second electrode on the other side), (Fig. 2). Biphasic stimulation with 400 μ s pulse length was used. The amplitude of the stimulation ranged between 0 and 4.8 mA. The stimulation frequency ranged between 35 and 45 Hz.

All the animals were implanted bilaterally with the LP electrodes (Fig. 1a). A bipolarly stimulatable LP insertion tool (Fig. 1b) was used to place the LP electrode in the PCA muscles. Both the LP electrode and the LP insertion tool share the design with the same components that will be used in subsequent clinical investigations. A NEUROSIGN N100 external stimulator (Magstim Inc., Whitland, UK) was used to guide the placement of the LP electrode. A PAL video camera system (Tele Pack, Storz, Tuttlingen, Germany) and rigid rod lens optics (Storz, Tuttlingen, Germany) were used to monitor surgery and the postoperative outcomes. Mini-DV recordings were made for comparison and later analysis.

Surgery

Surgery and follow-up were performed in accordance with the “Guide for the Care and Use of Laboratory Animals” [11] and local standards. The local ethics committee approval was obtained.

The animals were anaesthetized and intubated. Under sterile conditions, a prelaryngeal longitudinal skin incision was made, exposing the cricoid cartilage and the upper part

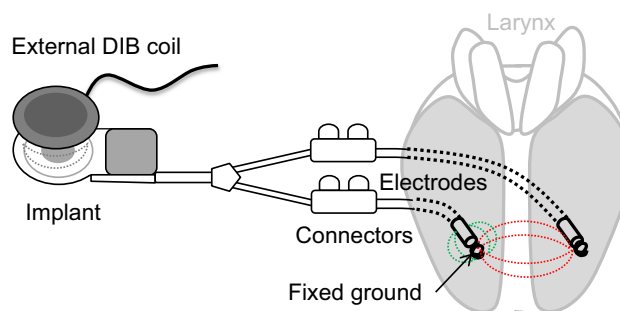


Fig. 2 Line draft implant system: the implant is controlled via an external conductive coil of the digital interface box (DIB), two bipolar LP electrodes are connected to the LP implant prototype, electrode tips within the PCA stimulate intramuscular nerve endings unilaterally (electrical field with green dotted line) or bilaterally (electrical field with red dotted line)

of the trachea. A temporary tracheostomy, closed at the end of surgery, freed the glottis from the ventilation tube. One LP electrode was placed into the PCA muscle of each vocal fold by means of the LP insertion tool via the cricothyroid membrane, the subglottic soft tissue space, and the cricoid lamina [10], and fixed with sutures to the cricoid cartilage. Electrical stimulation under visual monitoring via laryngoscopy was used to confirm the placement of the LP electrode into a PCA “hot spot”, i.e. a stimulation position with a low threshold and selective vocal fold abduction [12]. Threshold, i.e. stimulation parameters eliciting the smallest detectable vocal fold abduction, and optimal parameters, i.e. stimulation parameters eliciting full abduction, for unilateral and bilateral stimulation were obtained under laryngoscopy monitoring. Thresholds for adductor co-activation were also evaluated. The LP implant prototype was placed in the upper chest region, in a small, subcutaneous pocket and connected to the LP electrodes. The correct connection between the LP implant and the LP electrodes was tested before the wound was closed and dressed.

Post-surgery treatment

The wound was dressed with a clean dry bandage for the first 4–7 days postoperatively. Antibiotics were administered, as appropriate, to treat potential local infections until the wound completely healed. Generally, the post-operative medication treatment included antibiotics with ampicillin (0.5 g) twice per day for 4 days and Baytril (0.2 g) once per day for 4 days.

Follow-up

The position of the LP electrodes was radiographically verified 1, 7, 14, 28, and 42 days postoperatively (Fig. 3). A videoendoscopic examination was performed at the same intervals in the anaesthetized animals. After ketamine–midazolam induction, anaesthesia was maintained with propofol in a dosage low enough to maintain spontaneous ventilation. Stimulation parameters corresponding to threshold and optimal abduction were determined via laryngoscopy (Fig. 4). Maximal glottal opening angles for unilateral and bilateral stimulation were calculated as the angle difference between the resting position and maximal abduction. The angles were measured among the following points: the anterior border of one vocal process, the anterior commissure, and the anterior border of the other vocal process in static image frames from all DV recordings using the angle-measuring tool of an open source Dicom-viewer software OsiriX^R 3.8.1 GNU. Data were processed with Excel^R, Microsoft, Redmond, USA.

Animal disposal

After 6 weeks postoperatively, the animals were euthanized with an overdose of pentobarbital and magnesium sulphate.

Computer tomography (CT) scans of larynx specimens

To visualize the exact position of the LP electrodes, the whole larynx specimens were scanned in 1 mm slices in a CT before being further processed for histopathology. The data were 3D reconstructed using the surface rendering mode of OsiriX^R 3.8.1. GNU. The PCA muscles were manually marked as “regions of interest”.

Histopathological examination

Whole larynx specimens were formalin fixed. Cross-sectional blocks were decalcified and paraffin embedded. The thin slices of electrode beds and the subglottal regions were stained with haematoxylin–eosin and Elastica van Gieson. Inflammatory cell infiltration was classified either as a normal foreign body reaction (i.e. some plasma cell and lymphocyte infiltrations within the connective tissue without granulocytes) or as an ongoing local infection (i.e. additional presence of granulocytes). The thickness of the connective and/or fibrotic tissue surrounding the LP electrodes was measured (in mm) and referenced with the LP electrode diameter. LP electrodes were removed after fixation and before cutting.

Results

The LP implant prototype I was implanted in 5/5 (100 %) animals and the LP implant prototype II in 3/3 (100 %) animals.

One LP electrode was successfully implanted into the PCA of each vocal fold in 7/8 (87.5 %) animals. One animal (MP 2 IMP) was excluded from the study because we could not observe any abduction on either vocal fold during the surgery upon implantation. Postmortem dissection and histopathological analysis showed that both LP electrodes had been placed caudally to the PCA muscle. To prevent the re-occurrence of such a problem, intraoperative radiographic controls were implemented when laryngoscopy alone could not provide sufficient confirmation of the LP electrode position.

No perioperative adverse events occurred, regardless of the type of LP implant used. Regular monitoring of the animals did not detect any signs correlating with pain, respiration impairment, and/or swallowing problems. This

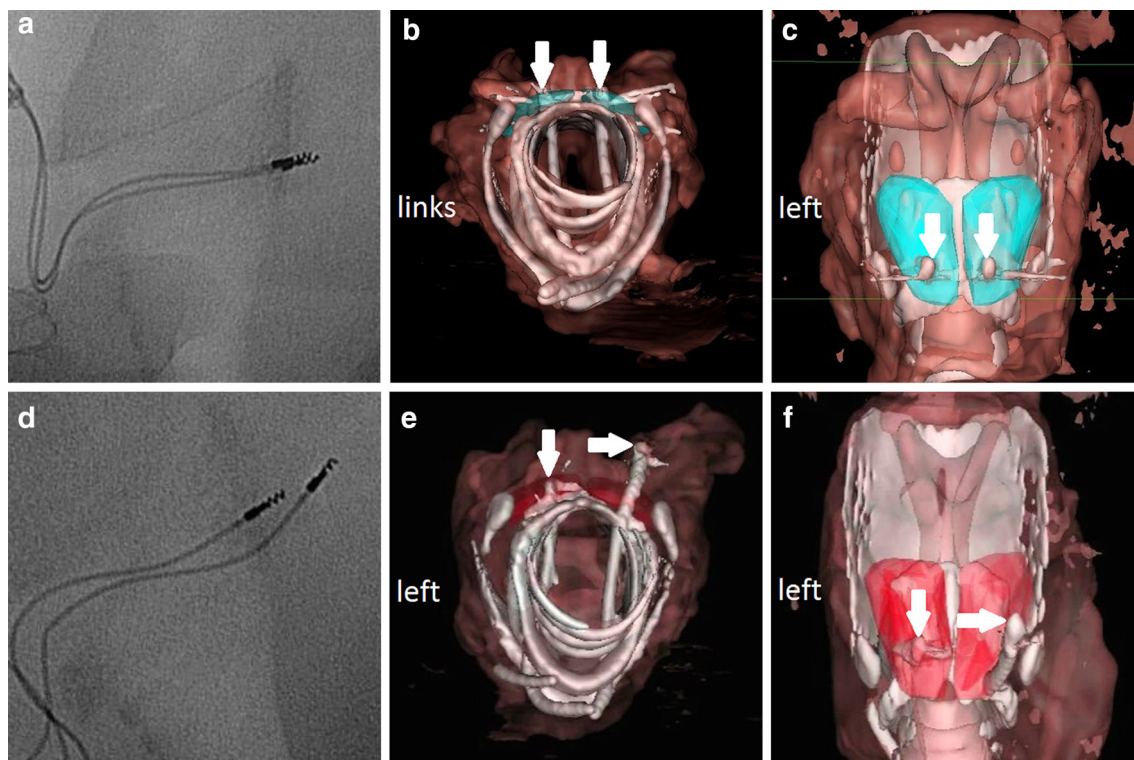
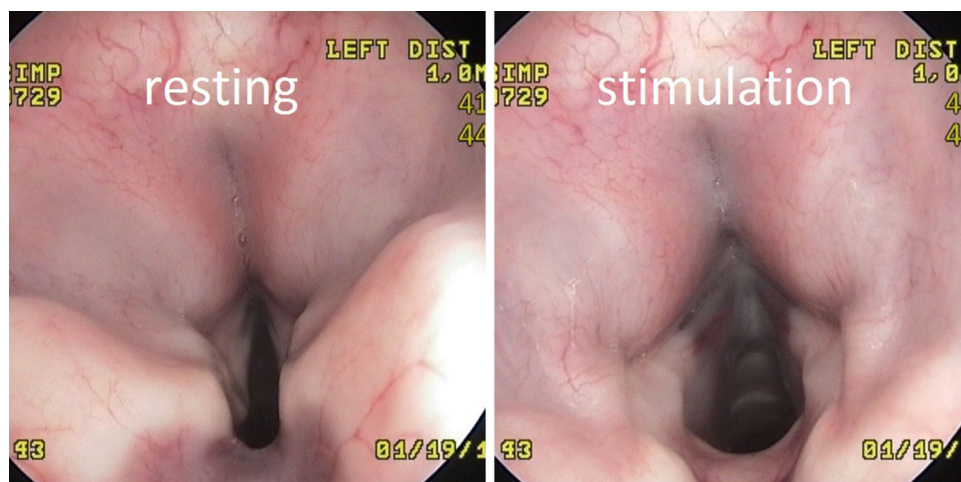


Fig. 3 Electrode positions on lateral-view radiographs in situ (a and d), and 3D CT reconstructions of whole larynx specimen (b, c, e, f). PCA colour marked as surface rendered regions of interest, mini-pig MP 3 IMP (upper row, a–c, PCA in blue), mini-pig MP 1 IMP (lower row, d–f, PCA in red), electrode tips marked with arrow, example of

a dislocated electrode in MP 1 IMP marked with *right arrow* (e and f *right side*); diameter of electrodes magnified due to metal artefact in CT scans and subsequent 3D reconstructions (2 mm in the images, 1 mm in reality)

Fig. 4 Videolaryngoscopy example during final control at 42 days in mini-pig MP 3 IMP. Left resting position, right maximal abduction during bilateral electrical stimulation



was further confirmed by perioperative and postoperative videolaryngoscopic sessions. Minimal swelling of the laryngeal region was observed, but was generally limited to the region proximal to the electrode implantation for a postoperative period not longer than 1 week. In the mini-pig MP 5 IMP, the videolaryngoscopy revealed the presence of a supraglottal oedema, likely caused by mechanical

stress produced by the prolonged use of a supraglottal suction catheter used to optimize the endoscopic view during the surgery. The supraglottal oedema disappeared completely 1 week postoperatively.

The healing of the incision used to place the LP implant was closely monitored. Wound recovery was complete within 1 week postoperatively. In general, no infection

symptoms could be detected other than minimal localized infections around some skin staples. The animals were monitored for a postoperative period of 6 weeks.

In general, the LP electrodes did not dislocate and the stimulation parameters remained stable for the monitored 6 weeks postoperatively. The efficacy of the stimulation was confirmed by videolaryngoscopy, which showed a clear abduction of both vocal folds upon stimulation that correlated with an increased glottal gap.

The LP electrodes on the right side in two animals (MP 1 IMP and MP 5 IMP) dislocated between 4 and 6 weeks postoperatively. Plain radiography and 3D reconstructions of the larynx specimens showed that both had migrated inwardly towards the hypopharynx without penetrating the pharyngeal lumen.

In 1/14 (7.1 %) LP electrode (MP 8 IMP on the right side), the tip broke apart from the lead after 4 weeks.

In mini-pig MP 6 IMP, a protrusion of the parasternal implant through the skin occurred at the end of the study without affecting the stimulation outcome. In the zone where the protrusion occurred, scratch marks were visible and thus self-scraping by the animal was considered as the main cause for the skin defect. In one animal (MP 8 IMP), minimal subglottic mucosal damage was observed on the left side, close to the cricoid lamina where the electrode ran most superficially and thus had the highest risk of perioperative mucosal damage.

In general, perioperative abduction thresholds ranged between 0.1 and 1.0 mA. Thresholds for unilateral/bipolar stimulation (Fig. 5) increased slightly up to day 14 and then remained stable. Electrode dislocations (MP 1 IMP and MP 5 IMP) and breakage (MP 8 IMP) were excluded from this evaluation.

In all seven successfully implanted animals, stimulation via the LP system correlated with clear vocal fold abduction. Maximal glottal opening angles, calculated as the difference between resting angle and stimulated

maximal abduction, generally remained stable throughout the 6-week postoperative period (Fig. 6). Electrode dislocations (MP 1 IMP and MP 5 IMP) and breakage (MP 8 IMP) were excluded from this evaluation. For unilateral stimulation, the maximal glottal opening angle was 9.0°–14.5° (median 11.7°) perioperatively and 10.0°–16.7° (median 13.75°) at day 42. For bilateral stimulation, it was 10.0°–24.8° (median 18.25°) perioperatively and 18.0°–22.0° (median 20.25°) at day 42.

The amplitude thresholds inducing a detectable perioperative PCA muscle stimulation did not correlate with unselective co-stimulation of the adductor muscle in 14/14 (100 %) cases. Only in the case of MP 8 IMP did the unselective stimulation threshold almost overlap the selective PCA muscle stimulation threshold (0.6 vs. 0.5 mA). 3D CT reconstruction showed that the LP electrode was placed more laterally than usual.

Postmortem histopathological examinations (Fig. 7) revealed a normal foreign body reaction with thin connective tissue layers and mild to moderate round cell infiltrates around the LP electrode tip and lead in most cases. In three cases, MP 5 IMP (left PCA muscle), MP 6 IMP (subglottal, bilaterally), and MP 8 IMP (subglottal, bilaterally and on both PCA muscles), infiltrates indicative of an ongoing local infection, i.e. including granulocytes, were observed around the LP electrode (Table 1). In no cases could localized or total atrophy of the PCA muscle or fibre clustering be detected. In all cases, the observed endomysium was as thin as expected in healthy conditions. In some slices we also saw neural structures close to the LP electrode, which indicated the proximity of the said electrode to the nerve branches.

There was no correlation between the degree of connective tissue surrounding the electrode in the PCA muscle and changes in the stimulation threshold over time ($r = -0.18$) or between this connective tissue and changes in the maximal glottal opening angles over time ($r = 0.58$) (Table 2).

Fig. 5 Threshold currents for unilateral stimulation, i.e. ground side (graph on the left) and bilateral stimulation, i.e. ground to tip (graph on the right). For the purposes of this experiment, the electrode with the lowest threshold was chosen as the ground side. Because of this, the thresholds related to unilateral stimulation were lower than those related to bilateral stimulation. Dislocated electrodes are not included in these graphs

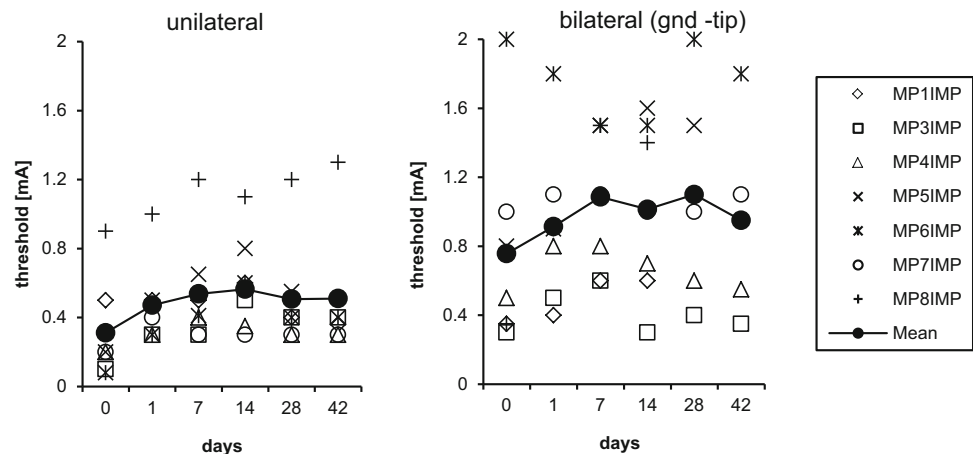


Fig. 6 Maximal stimulated increase of glottal angle over time during unilateral stimulation (graph on the left) and bilateral stimulation (graph on the right). Dislocated electrodes are not included in these graphs

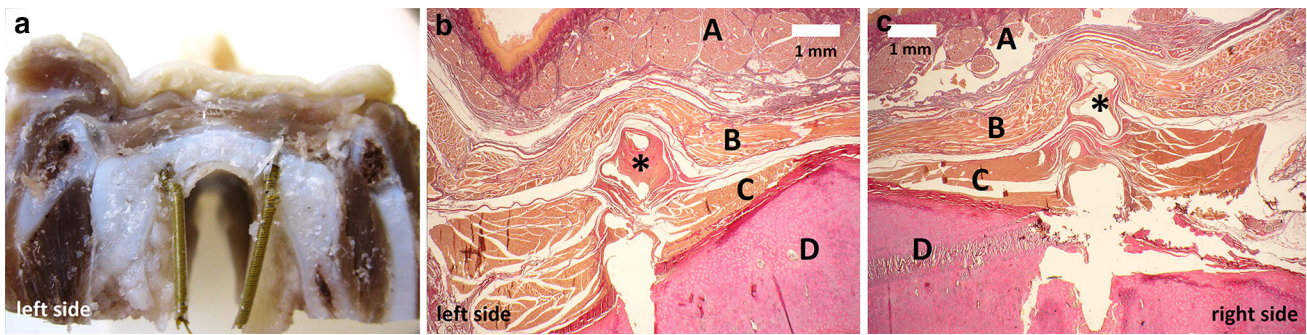
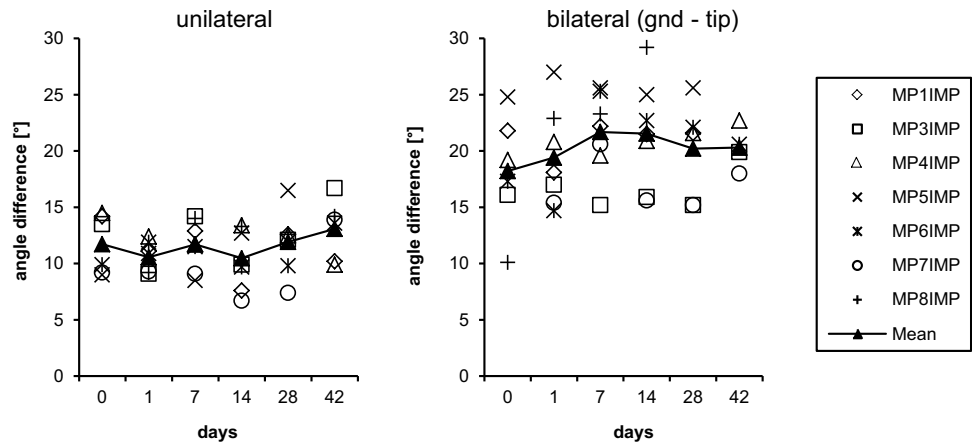


Fig. 7 Mini-pig MP 3 IMP cross sections of electrode beds, macroscopic (a) and microscopic (b and c), Elastica van Gieson staining. A mucous membrane with submucosal glands, B muscular lining of pharynx, C PCA muscle, D Cricoid cartilage; electrode tips

slightly exceed the PCA, causing a connective tissue “head” (*) around the tip region and very little connective tissue within the PCA muscle. Collagen fibres stained red, and muscle in orange

Table 1 Inflammatory cell infiltration around the LP electrode

	Subgl. left	Subgl. right	PCA left	PCA right
MP 1 IMP	N	N	N	N
MP 2 IMP	N	N	- ^a	- ^a
MP 3 IMP	N	N	N	N
MP 4 IMP	N	N	N	N
MP 5 IMP	N	N	G	N
MP 6 IMP ^b	G	G	N	N
MP 7 IMP	N	N	N	N
MP 8 IMP ^c	G	G	G	G

N normal foreign body reaction with some lymphocyte and plasma cell infiltration. G infiltration including granulocytes, indicating the presence of an acute and localized infection

^a The LP electrode was placed outside the PCA muscle

^b Detection of a skin protrusion of the LP implant prototype towards the end of the experiment

^c Presence of a minimal subglottic mucosal damage where the LP electrode runs most superficially

Discussion

This middle-term pre-clinical study in mini-pigs aimed to evaluate the bilateral application of the LP system, developed for the treatment of BVFP via selective stimulation of the PCA muscle.

In particular, this study evaluated the functionality of the LP system and of the surgical procedure used to position and maintain in place its implantable components, i.e. the LP implant and the LP electrode for a period of 6 weeks. In general, the results showed that the LP implantation, its dedicated tools, and the respective surgical procedure were safe, as already described in a previous paper [10]. In addition, the study provided initial positive evidence regarding the middle-term functionality of the device.

In short, the surgical procedure adopted for LP implant placement was effective in 8/8 (100 %) of the cases; the surgical procedure used to identify the “hot spot” on the PCA muscle and to place the LP electrode was effective in

Table 2 Connective tissue thickness in correlation with threshold and angle changes

	Connective tissue (in mm)		Threshold change (mA)	Angle change
	PCA left	PCA right		
MP 1 IMP	* _{-a}	_{-a}	-0.14	-4.0°
MP 2 IMP	_{-b}	_{-b}		
MP 3 IMP	0.17	*0.15	0.30	3.2°
MP 4 IMP	*0.29	0.26	0.10	-4.6°
MP 5 IMP	0.77	*0.47	-0.20	
MP 6 IMP ^c	0.20	*0.12	0.32	3.7°
MP 7 IMP	_{-a}	*0.14	0.10	4.7°
MP 8 IMP ^d	*0.56	0.20	0.40	14.2°

Threshold change over time was calculated as difference between day 0 and day 42 with unilateral stimulation, and change of optimal abduction angle over time was calculated as difference between day 0 and day 42 also for unilateral stimulation. The side undergoing unilateral stimulation is marked with an asterisk. The correlation coefficient between the thickness of the connective tissue around the electrode tips and the threshold change over time was -0.18, while that for the connective tissue and the angle changes over time was 0.58

^a No reference; electrode for reference missed in histology

^b Electrodes were not within PCA

^c Mini-pig MP 6 IMP had a skin protrusion of the implant at the end of the trial

^d Mini-pig MP 8 IMP had a small subglottic defect of the mucous membrane covering the left electrode

7/8 (87.5 %) of the cases. Repeating the “hot spot” identification procedure did not correlate with any adverse event or with reduced functionality over time.

None of the implanted animals suffered from any adverse events related to either the surgical procedure or the middle-term implantation other than minimal and local infection that did not affect the animals’ well-being or the effectiveness of the LP system.

In total, 7/8 (87.5 %) of the implanted animals successfully completed the 6-week study. In 11/14 (78.6 %) cases, the successfully implanted electrode remained in place over 6 weeks and delivered selective PCA muscle stimulation as expected. It is important to note that both dislocations (the right electrode in MP 1 IMP and in MP 5 IMP) correlated with the use of resorbable sutures. When resorbable sutures were substituted with non-resorbable sutures, dislocation did not reoccur. No significant device deficiency was observed for the 6-week duration of the study. In particular, we could observe a damage of the LP electrode only in 1/14 (7.1 %) cases (MP 8 IMP on the right side), when the LP electrode tip broke apart at 4 weeks postoperatively.

The stimulation parameters for threshold and optimal abduction required some adjustments only for the first two postoperative weeks, in accordance with the well-known pattern for stimulating electrodes [13]. They remained stable for the subsequent follow-up period. The characteristics of the stimulation parameters observed in the seven mini-pig were comparable for the entire duration of the study follow-up. The stimulation was selective, i.e. it

did not induce co-adduction, and was thus extremely effective on the vocal fold opening. Indeed, although an unselective co-stimulation of the adductor muscle was observed in one case, it did not negatively affect the vocal fold opening by the selective PCA stimulation. The unselective co-stimulation correlated with a more lateral LP electrode placement than usual. This may indicate that if an adductor reaction is observed during the placement of the LP electrode, it would be beneficial to place the LP electrode into a more medial position and re-test the stimulation outcome.

In this study, the identification of the correct “hot spots” into the PCA muscle by means of the LP insertion tool was effective in 7/8 (87.5 %) animals, thus providing good evidence on the effectiveness of such procedure to place the LP electrode onto the respective PCA.

The encapsulation via connective tissue of the electrode tips was an expected and well-known mechanism of reaction to the introduction of any foreign body. Excessive connective tissue formation could have impeded the application of an electrical stimulus or the contractility of the PCA. This was not the case. The thickness of the connective tissue layer was found in the normal range (Table 2). Accordingly, it did not correlate with any increase of the stimulation thresholds or with decreased maximal glottal angles. This confirms that the encapsulation of the electrode tip does not constitute a potential issue for the use of the LP system.

In three cases, more pronounced infiltrates including granulocytes were observed. This indicates an ongoing

local infection. The presence of such infiltrates never changed the stimulation effectiveness, nor was there a correlation with changes in threshold. In one animal (MP 8 IMP), it was likely due to a mucosal lesion that most likely occurred during LP electrode placement. This observation strongly suggests that special care should be taken to prevent any mucosal perforations during LP electrode placement. In the mini-pig MP 6 IMP, the LP implant protruded through the skin. Visual inspection detected scratch signs around the protruded implant, pointing to manipulation of the animal as the most likely cause for the displacement accompanied by a local infection. No clear root cause could be determined for the local infection observed in mini-pig MP 5 IMP. Regular visual inspection had revealed a potential skin infection limited to the region where the wound clips were placed, 2 weeks postoperatively, suggesting this as the most likely cause for the observed granulocyte infiltration, or an infection in surgery via the nearby temporary tracheostomy could have occurred.

On the whole, it appeared likely that the occurrence of these local infections was connected with some of the techniques that, while commonly used in animal models, would not be suitable for clinical use. The outcomes of this experiment strongly suggested that in future animal testing, both self-scratching by the animal and the use of wound clips should be considered potential cause for infection and preventive actions shall be implemented to avoid it. The subglottic mucosal damage observed in the mini-pig MP 8 IMP points to the need of mastering the LP electrode placement in surgeons training in animal models before implementing the technique in clinic.

Conclusions

The LP system used in this experiment to induce vocal fold abduction by means of selective functional electrical stimulation of the PCA showed promising results in the middle-term period and may become a valid alternative to the current standard for BVFP treatment. Clinical studies are needed to confirm the medical relevance of the device.

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Compliance with ethical standards

Conflict of interest This work was financially supported by MED-EL (Medical Electronics) Innsbruck, Austria. The animal trials were sponsored by MED-EL Medical Electronics, Innsbruck. The authors declare no conflicts of interest.

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