The injury of recurrent laryngeal nerve and thyroid regeneration after irreversible electroporation ablation of most part of thyroid gland—an experimental study on swine model

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Abstract. To study the thyroid regeneration and injury of recurrent laryngeal nerve after irreversible electroporation (IRE). 12 pigs were divided into three groups: six pigs underwent IRE, other pigs were used as controls. IRE was performed near tracheoesophageal groove, to ablate most part of thyroid gland. Parathyroid and thyroid function, recurrent laryngeal nerve injury and thyroid computed tomography (CT) imaging were regularly investigated. The histopathology results were analyzed to detect thyroid regeneration. Masson’s trichrome method for collagen and immunohistochemistry were performed for Soluble protein-100 (S100) and neurofilaments on nerve section. In IRE group, there were no symptoms of recurrent laryngeal nerve-related injury. No abnormalities of recurrent laryngeal nerve were shown on hematoxylin-eosin (HE) staining, Masson’s trichrome staining, Neurofilament (NF) staining and S100 staining. There were no significant changes for thyroid and parathyroid function in all pigs. Immediately after IRE, CT showed hypoattenuation in the ablated thyroid gland and it became swelling. 14 days after IRE, thyroid CT showed heterogenous attenuation in the electroporation zone, and the size and attenuation of thyroid gland were normal after two months. There was cell apoptosis in the thyroid gland after IRE. Seven and 14 days after IRE, there was fragmentation of nucleus within the follicle, and some follicles were empty. Two months later, complete regeneration of thyroid tissue was shown. IRE was shown to be both effective and safe with complete regeneration of thyroid tissue and preservation of the function and structure of the recurrent laryngeal nerve.

Key words: Irreversible electroporation, Thyroid regeneration, Recurrent laryngeal nerve, Computed tomography, Pathology

SUBTOTAL OR TOTAL THYROIDECTOMY was usually performed for patients with thyroid cancer or other thyroid nodule, and most thyroid tissue was lost postoperatively, along with the adjacent parathyroid glands. For these patients, they always need to take med-

icine orally in all their lives to keep their thyroid function normal, though part of thyroid gland regenerated in some patients. For those who have limited volume of thyroid gland or hypothyroidism, it is especially important to reserve the structure and function of thyroid gland. Thus, to improve the quality of life, it is necessary to preserve thyroid function and structure at the same time of curing thyroid disease.

In recent years, radiofrequency and microwave ablation have been used in the treatment of thyroid nodule, and part of thyroid can be reserved. Their major complications include bleeding and voice changes due to injury of recurrent laryngeal nerve, but life-threaten bleeding is rare [1]. There were reports about injury of recurrent laryngeal nerve in thermal ablation [2], which resulted in hoarseness, dyspnea and even brought life risk. To protect nerve, water isolation was often used; however, when water sometimes flows away, this protection is unreliable.
In clinical practice, when the ablated lesion is close to nerve, the risk of nerve damage or tumor residual would increase. Since nerve is sensitive to thermal injury, complete ablation may damage nearby nerve [3, 4]; or residual tumor may be left to avoid potential nerve damage. So, a selective ablation technique is needed and irreversible electroporation (IRE) may help to realize this goal. IRE can cause cell apoptosis, without destroying abundant collagen tissue and extracellular matrix, which is suitable for thyroid regeneration and tumor ablation while sparing nerve damage and preserving thyroid gland.

Some experimental studies [5-8] found that nerve damage was slight, reversible and acceptable after IRE; and the epineurium, endoneurium and perineurium were intact with axonal regeneration and Schwann cell hyperplasia, which benefits nerve regeneration. Injury of recurrent laryngeal nerve is an important complication in thyroid surgery, though nerve monitoring devices have been used in thyroid surgery. Recurrent laryngeal nerve is very tiny, closely related with voice and breathing, which is located next to dorsal part of thyroid gland and difficult to be identified. Until now there were no reports about safety of IRE for tissue near tiny nerve, so we aimed to investigate potential nerve damage, pathological changes and regeneration of thyroid gland after IRE, by ablating most part of thyroid gland in this study.

### Materials and Methods

Approval for the use of the animal in this study was obtained from the Animal Care Committee of Sun Yat-sen University Cancer Center. Our institution’s guide for the care and use of laboratory animals was followed. All pigs were provided by the mini pig incubator in Yunfu City, Guangdong Province. The average weight was 18 ± 2 kg.

12 animals were divided into sham treatment group (4 pigs), control group (2 pigs) and IRE group (6 pigs). Sham treatment was given to 4 pigs with 2 electrodes being inserted in the thyroid gland, and surgery was performed in one of them to injury left recurrent laryngeal nerve as positive control. Two pigs were used as blank control.

Skin preparation, sterilization and draping were finished before the procedure. All pigs were fast 12 hours before the procedure. Each procedure started with premedication with Telazol (4.4 mg/kg) intramuscularly and after intubation, general anesthesia was maintained via venous injection of 3% pentobarbital sodium (1 mL/kg). All pigs were given buprenorphine 0.01 mg/kg subcutaneously before the procedure and every 12 h for 72 h as needed. Each pig also received succinylcholine (2 mg/kg) during IRE. Pancuronium (0.15 mg/kg) was administered intravenously 10 min before IRE to reduce muscle contraction.

The percutaneous placement of the electrode was guided with Computed tomography (CT). We used CT as guidance imaging, and ultrasound for monitoring possible bleeding and electrode dislocation. A multifrequency probe (5–10 MHz, Logiq E9/GE, MyLab™ GOLD Platform, ESAOTE S.P.A., Italy) was used.

The NanoKnife® system (software version 2.2.0) (Angiodynamics®, Latham, NY, USA) was used with the following system parameters: two electrodes, electrocardiogram (ECG) synchronization, with 90 pulses, a pulse length of 70 μs, an electrode spacing of 1.3–1.5 cm, 1,500 V/cm, tip exposure 1.5 cm.

The two electrodes were inserted between the dorsal part of thyroid gland membrane and anterior vertebral body, which were lined up longitudinally beside the nerve (Fig. 1), with the electrode tip 3–5 mm from one side of the trachea-esophageal groove, where the recurrent laryngeal nerve were located in or near (Figs. 2, 3). We aimed to ablate most part of the thyroid gland. Because the thyroid gland is not large in volume [9], we do not need to move the electrodes during the procedure. When the electrode spacing is 15 mm, the ablation volume given by an ablation zone estimator within the NanoKnife® Software is about 7.5 cm³: 25 mm (transverse diameter) × 25 mm (anteroposterior diameter) × 12 mm (superoinferior diameter); while the thyroid size of the experimental pig is small, and the diameter of its trachea is about 13–15 mm. The transverse diameter of its thyroid gland is nearly the same to the trachea diameter, the anteroposterior diameter is about 10 mm, and the superoinferior diameter is about 15–20 mm. So the thyroid volume is less than 4 cm³, that is: 20 mm (transverse diameter) × 20 mm (superoinferior diameter) × 10 mm (anteroposterior diameter), consistent with reported results [9]. The estimated ablation volume is larger than the pig’ thyroid size, and the estimated ablation volume can cover most part of the thyroid volume. During the procedure, we can rotate the transverse diameter by a small angle to further increase the actually ablated volume. According to the procedure voltage/current graph, we can know if the estimated ablation volume has been completely ablated; besides, contrast enhanced CT is performed immediately to make sure whether most part of thyroid gland was ablated according to no enhancement zone in the thyroid gland. We can give another IRE if no more than half of the thyroid volume was ablated. However, most part of thyroid volume was covered, confirmed by the first postoperative CT scan.

After implanting two electrodes, we gave another CT scan with three-dimensional reconstruction to confirm
the electrode position. If the electrode is in a predetermined position without displacement, we began IRE ablation directly and did not move the electrodes during ablation. Because the thyroid gland of the experimental pig is small, the set IRE ablation area can cover most part of the thyroid gland and the purpose of ablation can be achieved, so the ablation can be completed at one time, no need to move the electrodes. The lasting time of pulse delivery in IRE ablation is less than 2 min every procedure, and the detailed information can be acquired from the procedure report (in attachment).

Therapeutic evaluation was assessed according to con-
trast enhanced CT imaging and procedure voltage/current graph immediately after IRE, and procedure success was judged by attenuation changes of targeted region and regular procedure voltage and current in 6 pigs. The initial voltage is 1,500 V/cm, and when the electrode spacing is 13 mm–15 mm, the initial voltage is between 1,950 V and 2,250 V, with the current between 20–30 Amp. The detailed data can be understood with the provided procedure report after IRE ablation. The attached graphs are used to demonstrate the voltage and current during the procedure (in attachment). If the current is lower than 20 Amp, another circle of IRE was given. One pig received IRE ablation twice in the same location, while the other five pigs received once.

**Imaging, pathology examination and blood test**

Before the procedure, blood test, contrast-enhanced CT were performed as baseline examination. Blood test was performed after 1, 3, 7, 150 days in all pigs of IRE group. Contrast enhanced CT was performed at 2 hours, day 1, 7, 14 and 60 postoperatively. Resection or CT guided thyroid biopsy was finished to perform pathologic examination at 2 hours, day 1, 3, 7, 14, 35, 60, 90 and 150 after IRE, and recurrent laryngeal nerve was harvest in 4 pigs at 14 days, two months and 3 months after IRE separately for pathological examination. Thyroid tissue was gained by biopsy in all pigs, and thyroid resection was performed when the pig was sacrificed. Hematoxylin-eosin (HE) staining and terminal deoxynucleotidyl transferase-mediated dUTP-biotin nick end labeling (TUNEL) assay for the thyroid tissue were performed to detect apoptosis and other pathologic changes. Precise sample has been achieved by CT guided biopsy, so continuous observation can be realized.

The number of pigs at each time point after IRE was as following: immediately after IRE (sacrifice of one pig), 3 days after (sacrifice of one pig), 14 days after (sacrifice of one pig), 2 months after (sacrifice of one pig), 3 and 5 months after (sacrifice of the left two pigs separately). In the sham treatment group, two pigs were sacrificed after 3 days and the other 2 pigs after 2 months.

Voice change was recorded with digital audio to detect if there is any hoarseness and weak voice. Serum concentrations of thyroid stimulating hormone (TSH), free T3 (free Triiodothyronine, fT3), free T4 (free thyroxine, fT4), serum parathyroid hormone, phosphorus and calcium were measured before and 2 hours and 3 days after treatment. Serum TSH, fT3 and fT4 were also measured at 1-, 3- and 5-month follow-up in the left pigs.

Apoptosis examination (TUNEL) was finished 2 hours, 24 hours and 3 days after IRE, by two technologists in pathology department with more than 5 years’ experience. The major reagents used were In Situ Cell Death Detection Kit, POD (Cat. No. 11 684 817 910, Hoffmann-La Roche Inc. USA), 3,3’-diaminobenzidine and Streptavidin peroxidase immunohistochemistry staining kit (Fuzhou Maixin Biotechnology Development Co., Ltd.).

The nerve near the ablation region was fixed in 10% formalin, cut into 4-μm-thick slices, and then stained with hematoxylin-eosin and Masson’s trichrome method. Sections of nerve were also investigated with immunohistochemistry for Soluble protein-100 (S100) and neurofilaments. Masson’s trichrome method, immunohistochemistry staining for S100 using monoclonal rabbit anti-S100a antibody (Z0628; Dako, Carpinteria, CA, USA), and for neurofilaments (NF, axonal marker) using monoclonal mouse anti-pan-axonal neurofilaments antibody (SMI-312R; Covance, Princeton, NJ, USA) were performed for marking collagen, Schwann cells and axons, respectively, by a pathologist with 6 years of experience. All nerves were assessed for axonal swelling, axonal fragmentation, inflammatory reactions, Schwann cell proliferation and other injuries.

**Statistical analysis**

The statistical analysis was performed with SPSS 18.0 (SPSS, Inc., Chicago, IL). Quantitative data are presented as mean ± SD. Two-tailed p value of <0.05 was considered statistically significant. The Analysis of Variance (ANOVA) test was used for the comparisons between the numerical data.

**Results**

**Imaging**

Immediately after IRE, hypo-attenuation was shown in the plain and contrast enhanced CT imaging (Fig. 1). There was a little bleeding around thyroid gland in 1 pig. The thyroid gland after IRE was soft and swelling, but it did not cause compression to trachea and esophagus (Fig. 2).

Seven days after IRE, the ablation zone was mixed with high and low density zone on CT imaging, and the thyroid gland was still swelling, with heterogeneous enhancement (Fig. 3). Two weeks after IRE, the bleeding around thyroid gland was totally absorbed and invisible on CT imaging. The swelling zone disappeared after 2 weeks with normal size, and there was heterogeneous enhancement in the ablation zone. Tracheal, esophageal and muscle around the thyroid gland were not injured.

Five weeks after IRE, heterogeneous enhancement was still demonstrated in the ablation zone. Two months after IRE, the thyroid gland appeared normal on contrast enhanced CT.
Hypoechoic zone was seen in the ablation tissue of thyroid gland immediately after IRE on ultrasound (Fig. 4).

There was a little bleeding in pigs of sham treatment group on CT imaging. The thyroid gland in the blank control group was normal, and no hoarseness was observed in these animals.

Pathology

Thyroid gland was normal in sham treatment group and control group. No injury of trachea and esophagus was observed by bare eye, and there was no injury of muscle and trachea cartilage around thyroid gland in all pigs.

In gross examination, a small amount of bleeding in needle tract was shown and tissue in the needle tract was brown in IRE group. Thyroid gland had moderate edema and bleeding and it was soft in texture. Under optical microscopy, 2 hours, 1 day and 3 days after IRE, mild follicle broke and it was partly empty in IRE group with exfoliative cells, cell vacuolization and karyorrhexis, but the follicle structure existed (Fig. 5). Thyroid colloid in follicle broke obviously with karyorrhexis in IRE group. Most follicle epithelial cells showed vacuolization and apoptosis on HE staining (Fig. 5). Inflammatory infiltration was found near thyroid gland and they are mainly neutrophils and lymphocytes. All the tissue structure was complete; no abnormal follicular epithelial cells were seen. 7 days after IRE, some follicles were still empty with karyorrhexis in it.

From two to five weeks after IRE, follicle and its epithelia regenerated, with only a few collagenous fibers in the needle tract. There were no fibrotic capsule around the ablation zone. The number of empty follicles decreased and the number of full follicles increased. And more and more follicles were fulfilled with colloid as time went by. One month later, only a few empty follicles without colloid were seen in the ablation zone. Some fragmentations of cell nucleus were still shown in the colloid of the follicle 5 weeks after IRE, follicular epithelial cells were normal. 2 months after IRE, follicles with different size were seen in the ablation zone, but no karyorrhexis and empty follicle were found on HE staining. Three and five months after IRE, the structure of thyroid gland was normal (Fig. 5) with even follicles full of colloid, and no apoptosis was detected on HE staining and apoptosis test at that time. There was no fibrous formation during thyroid regeneration except the needle tract. On HE staining, there was no thrombus and no obvious damage to large vessel.

Apopotosis was shown in IRE group 2 hours, 24 hours and 3 days after IRE, by TUNEL assay. No apoptosis was detected one week later. There were more and more apoptotic cells as time last within 3 days after IRE (Fig. 6).

No apoptosis and inflammatory infiltration was detected in control group and sham group on HE staining or TUNEL assay. There were a few red blood cells in the needle tract.

No damage of recurrent laryngeal nerve was detected in IRE group. There was also no axon damage, demyelination or inflammatory infiltration on histopathology (Figs. 7, 8). The architecture of epineurium, endoneurium and perineurium were intact, and the collagen in the nerve was intact with Masson trichrome method.

No evidence of large vessel injury was found on CT images nor histopathology.

Comparison CT images with HE images, we have found the uneven density zone on CT images was consistent with thyroid tissue that had empty follicles and full follicles at the same section on HE images after IRE. The density of ablation zone in thyroid gland after IRE decreased on CT imaging, but it was hyper-dense in contrast to the muscle around thyroid gland because of slight

Fig. 4 a hypoecho area was seen after IRE (circle); b: Needle tract bleeding was seen after IRE on ultrasound (white arrow).
Before IRE, the density of thyroid gland on CT imaging was even, and the follicles were full of colloid. Blood test For serum parathyroid function, thyroid stimulating hormone, free T3, free T4, calcium, phosphorus, there were no significant differences among given time points (Table 1). There was no obvious rise for TSH in each pig after IRE. Three months after IRE, the thyroid function, PTH, calcium and phosphorus were normal for the 2 left pigs.

Complications
No procedure related death occurred. No obvious changes of pathology in blank control group and sham treatment group. In sham treatment group, there was a little bleeding in the needle tract. The electrodes near tracheoesophageal groove did not result in edema and hoarseness. Voice change appeared in the pig experiencing nerve transection, and no voice changed in other pigs (Supplementary data).

There were no cough, dyspnea, dysphagia, weakness, bucking and hoarseness in IRE group. One pig had fever and red eye. Foot moving and wag tailing were observed during IRE. No arrhythmia and other cardiovascular events were observed in the IRE group. In particular, there were no supraventricular tachycardia and no atrial fibrillation during IRE. Decreased appetite was observed in two pigs for 5 days.

Fig. 6 Normal tissue control with blue nucleus, no apoptosis was detected (left ×100); After IRE, yellow nucleus was found (TUNEL), which indicated cell apoptosis (right ×200)
Discussion

The efficacy of IRE was indicated by loss of thyroid colloid, empty follicle, cell vacuolization, karyorrhexis and cell apoptosis, and most part of thyroid gland was ablated. Thyroid regeneration is benefit for preservation of structure and function of thyroid gland, while selective ablation does good to nearby tissues.

Injury of recurrent laryngeal nerve is a major complication in thermal ablation and surgery for thyroid disease, which may cause hoarseness and dyspnea. Voice change after RFA is uncommon, nevertheless, it is the most serious complication. It is likely caused by thermal injury to recurrent laryngeal nerve. Tracheal injury, esophageal injury, or permanent voice changes have also been reported after thermal ablation of thyroid nodule.

Selective ablation will provide protection for certain tissue, such as nerve. Former studies indicated the sparing effect of IRE for nerve, with preservation of endoneurium and nerve sheath which allows nerve regeneration [7], and the nerve damage was limited as II degree, which means successful regeneration of nerve. And the nerve injury is limited and its function recovered eventually, which is consistent with other studies [5, 8].

The number of experimental pigs used in this study is not enough to judge nerve damage only by sound changes, so we mainly study nerve injury with pathological examination. Because recurrent laryngeal nerve (RLN) is very tiny, nowadays it is really hard to image it by using Magnetic Resonance (MR) imaging. We used

Fig. 7 Two months after IRE, transverse section of nerve showed no signs of nerve injury. (×100, left), (×200, right). a and b: The nerve structure was normal on HE staining. c and d: Collagen is blue and extracellular matrix has a normal architecture; endoneurium, perineurium and epineurium are intact. (Masson trichrome method). e and f: Immunohistochemistry for neurofilaments revealed no axon swelling and other injuries. g and h: Immunohistochemistry for S100 revealed no demyelination.
HE staining, Masson trichrome method, immunohistochemistry for neurofilaments and immunohistochemistry for S100 to demonstrate possible changes of nerve structure, extracellular matrix (endoneurium, perineurium and epineurium), axon and myelination. We did not find obvious injury from these examinations, in the aspect of nerve structure.

Fig. 8  Histopathologic slide obtained 3 months after IRE ablation, longitudinal section of nerve showed no signs of nerve injury. (×100, left), (×200, right). a and b: The nerve structure was normal on HE staining. c and d: Collagen is blue and extracellular matrix has a normal architecture; endoneurium, perineurium and epineurium are intact. (Masson trichrome method). e and f: Immunohistochemistry for neurofilaments revealed no axon swelling and other injuries. g and h: Immunohistochemistry for S100 revealed no demyelination.

Table 1  Serum parathyroid function, thyroid function in 5 pigs which received IRE

<table>
<thead>
<tr>
<th></th>
<th>fT3 (ng/dL)</th>
<th>fT4 (μg/dL)</th>
<th>PTH (ng/mL)</th>
<th>Calcium (mg/dL)</th>
<th>Phosphorus (mg/dL)</th>
</tr>
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<tr>
<td>Before IRE</td>
<td>5.18 ± 2.97</td>
<td>14.93 ± 6.75</td>
<td>22.93 ± 11.15</td>
<td>2.37 ± 0.81</td>
<td>2.05 ± 0.47</td>
</tr>
<tr>
<td>2 h after IRE</td>
<td>12.47 ± 9.89</td>
<td>18.36 ± 10.45</td>
<td>13.00 ± 9.28</td>
<td>2.56 ± 0.26</td>
<td>2.11 ± 0.24</td>
</tr>
<tr>
<td>3 days after IRE</td>
<td>6.68 ± 4.18</td>
<td>13.27 ± 2.20</td>
<td>19.07 ± 14.37</td>
<td>2.40 ± 0.16</td>
<td>2.20 ± 0.34</td>
</tr>
<tr>
<td>F value</td>
<td>1.794414</td>
<td>0.634118</td>
<td>0.900645</td>
<td>0.115124</td>
<td>0.194687</td>
</tr>
<tr>
<td>p value</td>
<td>0.208</td>
<td>0.547</td>
<td>0.432</td>
<td>0.892</td>
<td>0.826</td>
</tr>
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Two side p value < 0.05 was considered as significant difference.
pathology. The injury of bilateral recurrent laryngeal nerves demonstrated transient hoarseness, weak cough and severe dyspnea. The IRE ablation volume and parameters were well controlled to avoid possible thermal damage to nerve in our study. Our ablation zone was near recurrent laryngeal nerve- the electrode tip was reached dorsal part of the thyroid capsule, about 3–5 mm far from the groove. If recurrent laryngeal nerve was compressed or tracked by postoperative fibrous tissue, voice change may also occur. Non-thermal IRE will not induce formation of massive fibrosis, so the risk of fibrous traction to nearby nerve was decreased. IRE can achieve cell apoptosis with certain parameters setting, and thermal injury of nerve can be avoided. On HE staining, there was no thrombus and obvious damage to large vessel wall. There were no obvious damage of recurrent laryngeal nerve function and structure in our study. Masson’s trichrome method for collagen and immunohistochemistry for S100 and neurofilaments indicated that the endoneurium and nerve sheath were intact, without neuron apoptosis or demyelination, which may be related to the following reasons: Firstly, the time we investigated nerve ranged from 14 days to three months after IRE. It might give the nerve enough time to recover. Secondly, the recurrent laryngeal nerve was near the boundary part of thyroid gland rather than in the center of the ablation region, so the injury could be subclinical and electroportion of the cell membrane was reversible; apoptosis of neurons didn’t occur in that situation. Also, the preservation of endoneurium and perineurium help to nerve regeneration [7]. Thirdly, the surrounding tissues of the recurrent laryngeal nerve are complicated and heterogeneous, so the corresponding impedance and current may be uneven, which will be beneficial for nerve preservation. Nerve in former studies were large and in relatively even electrical field [5-7], composed mainly by muscle.

Because thyroid gland is hypervascular, postoperative bleeding is a major complication after thyroid ablation. Ultrasound is useful for monitoring bleeding and changes of blood supply. Moreover, avoiding puncturing vessels directly is also very important for bleeding prevention. The two electrodes were inserted on the edge of the target tissue instead of in the center of it, which may be helpful for decreasing the chance of bleeding and tumor implantation. At least two electrodes are needed to finish IRE, so the risk of vessel injury increases as the puncturing time is doubled. However, the IRE electrode is very tiny and the puncture damage may be small. Postoperatively, edema and bleeding of thyroid gland may cause possible compression to trachea, and even dyspnea. The obvious swelling may be due to the following reasons: bleeding, exudation and dysfunction of cellular energy metabolism, which lead to further intracellular sodium and water retention.

Nowadays, there are some ablation modalities for thyroid nodules [10], while radiofrequency ablation for thyroid nodule is the most widely used technique, with certain complications [1, 11]. There were cases of recurrent laryngeal nerve injury and tracheal chondronecrosis after microwave ablation [4]. Four of 21 patients developed hoarseness after experiencing microwave ablation for early thyroid carcinoma.11. Thyroid function became normal one month after laser ablation for cold thyroid nodules in 122 patients [2]; there were only 2 hypothyroidism, 2 hypothyroidism and 2 vocal cord disorders. Percutaneous ethanol injection was mainly used for cystic nodule [1, 2], and transit pain may occur. After thyroid resection, there were reports of hypoparathyroidism, parathyroidectomy [12], hypocalcemia [13-15] and recurrent laryngeal nerve injury [3, 16]. There were no obvious function changes after RFA for thyroid nodule [16, 17], even for those who experienced previous lobectomy [16]. Patients often have to take medicine orally to maintain thyroid function after total or subtotal thyroidectomy. There was report of thyroid mass rupture after radiofrequency ablation [18]. Extracellular matrix was well preserved after IRE and there was no heat effect, so rupture of nodule may not easily occur.

After IRE, the ablation zone resolve fast in some reported studies [19-22]. Because preservation of follicle structure and cell regeneration in situ, there was no fibrous formation except in the needle tract. Fibrous capsule can inhibit the blood supply and tissue regeneration in the ablation region. Also, the patent and intact vessels, fast resolution of the ablation region and hypervascularity of thyroid gland, benefit its regeneration. Eventually in our study the thyroid gland was normal. During the process of regeneration, we can find the evidences of ablation region shrinking, colloid loss and new-born follicle epithelial cells. During thyroid regeneration, uneven follicles may be a sign of function compensation, as it is in nodular goiter.

In our study, there were no obvious changes in thyroid function and these changes were reversible, partly due to precise CT guidance and tiny electrode. IRE benefits preservation of follicle structure and thyroid regeneration; there was no contraction of nearby nerve and no need to differentiate residual tumor from inflammatory mass. Though RFA do not affect thyroid function in patients experienced partial thyroidectomy, the compression of postoperative edema and hematoma to trachea and recurrent laryngeal nerve, or contraction of fibrous tissue, may affect the function of surrounding tissue or organ. While IRE cause cell apoptosis and subsequent regeneration, and this merits help recovery of thyroid...
function because of regeneration of thyroid follicle and colloid. Besides, IRE did not result in formation of massive fibrosis as well as severe edema which would reduce compression to the adjacent organs. IRE may be a promising alternative in the treatment of thyroid nodules. Because of anatomy difference between swine and human beings, further clinical validation is needed.

Conclusion

IRE was shown to be both effective and safe with complete regeneration of thyroid tissue and preservation of the function and structure of the recurrent laryngeal nerve.

Data Availability

The data analyzed during this study have been provided in the manuscript and any further information can be made available upon request to the corresponding author.

Conflict of Interest

The authors declare that they have no conflict of interest.

References


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Supplementary Materials

Our supplementary material was audio clips showing normal voice of pig (with IRE) and hoarseness of pig voice (without IRE).

Procedure Voltage/Current graph has also been provided to better demonstrate that intraoperative voltage and current and how to judge if complete ablation was achieved after IRE (Supplementary Materials).


