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# The preventive effect of decellularized pericardial patch against Frey's syndrome following the superficial parotidectomy

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## ABSTRACT

**Purpose:** This study aimed to investigate the feasibility of adopting the ThormalGEN surgical patch (a porcine decellularized pericardial patch) in the superficial parotidectomy to prevent postoperative Frey's syndrome in patients with benign parotid tumor.

**Materials and methods:** A total of 59 patients with benign parotid tumor undergoing superficial parotidectomy between April 2016 and January 2017 were enrolled, and divided into the ThormalGEN group (n = 37) and the control group (n = 22) based on their willingness to have the ThormalGEN surgical patch used in the superficial parotidectomy. At 6 months postoperation, the incidences of postoperative complications and Frey's syndrome were assessed by subjective assessment (patient self-assessment) and objective assessment (starch-iodine test), respectively. The risk factor of Frey's syndrome was analyzed by the multivariate logistic regression model.

**Results:** The starch-iodine test, for objective assessment, showed that the ThormalGEN group had a significantly lower incidence of Frey's syndrome than the control group (8.11% vs. 40.91%, p = 0.06). However, in the patient self-assessment, there was no significant difference in the incidence of Frey's syndrome between groups (8.11% [3/37] vs. 13.64% [3/22], p = 0.4968). The incidences of postoperative complications were not significantly different between group (all p > 0.05). Two patients (5.41%) in the ThormalGEN group had salivary fistula. Multivariate logistic regression analysis showed that the ThormalGEN group had a significantly lower risk of Frey's syndrome than the control group (odds ratio = 0.11, 95% confidence interval = 0.02–0.51, p < 0.01).

**Conclusion:** These results suggest that the ThormalGEN surgical patch can effectively reduce the incidence of Frey's syndrome following superficial parotidectomy in patients with benign parotid tumor, and that this patch has a good safety and biocompatibility profile.

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## 1. Introduction

Salivary gland tumors are relatively uncommon diseases, accounting for 3–6% of all head and neck neoplasms (Wang et al., 2017), with an annual global incidence ranging from 0.4–13.5 per 100,000 individuals (Ma'aita et al., 1999; Tian et al., 2010; Jaber, 2006). Parotid tumors represent about 80% of all salivary gland tumors, which are the most common type (Bussu et al., 2011).

Approximately 75%–80% of parotid tumors are benign (Bussu et al., 2011). Currently, the surgical treatments for parotid tumors include superficial or total parotidectomy with preservation of the facial nerve and enucleoresection of the tumor (Orabona et al., 2014).

Frey's syndrome is a common postoperative complication following parotid gland surgery, characterized by flushing or sweating at the site of parotidectomy in response to salivary stimulation immediately after eating, seeing, thinking, or talking about certain food (Hayashi et al., 2016). Frey's syndrome was first described by Kastremsky in 1740 and in which the role of the auriculotemporal nerve was identified by Lucja Frey in 1923 (Blanc et al., 2016). It is known that the pathological mechanism of Frey's syndrome is the aberrant reinnervation of parasympathetic nerve fibers and auriculotemporal postganglionic nerve fibers to the

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sweat glands and subcutaneous vessels (Witt and Pribitkin, 2013). Frey's syndrome has been reported following salivary gland surgery and less commonly neck dissection, facelift procedures, and trauma (Motz and Kim, 2016). It has been shown that the incidence of Frey's syndrome varies among different treatments. A meta-analysis including 14 cohort studies with 3194 patients demonstrated that the incidence of Frey's syndrome following superficial parotidectomy was significantly higher than that of extracapsular dissection (Xie et al., 2015).

Several procedures have been developed to prevent the incidence of Frey's syndrome following superficial parotidectomy, such as use of autologous sternocleidomastoid muscle flap (Guntinas-Lichius et al., 2006), dermofat graft (Fasolis et al., 2013), acellular human dermal matrix (Sinha et al., 2003) and botulinum toxin (Jansen et al., 2017). The rationale for these treatments is based mainly on insertion of a barrier between the skin flap of the cheek and the facial nerve to prevent aberrant reinnervation. These methods have varying degrees of therapeutic efficacy, but are incapable of completely preventing Frey's syndrome. In addition, there are some disadvantages in these methods, such as prolonging the time of the operation, increasing the surgical trauma, increasing the scar of the patient and requiring repeated injection of botulinum toxin.

The ThormalGEN surgical patch (Grandhope Biotech Co., Ltd, Guangzhou, China) is a new type of decellularized pericardial patch manufactured from porcine pericardial tissue, which has been successfully applied in thoracic surgery (Zhang et al., 2006) and esophageal cancer surgery (Shao et al., 2013), exhibiting good safety and biocompatibility. Therefore, we proposed that the ThormalGEN surgical patch could be applied as a barrier in parotidectomy to prevent the incidence of postoperative Frey's syndrome. In this study, we conducted a prospective trial to assess the feasibility of adopting the ThormalGEN surgical patch in the superficial parotidectomy to prevent the incidence of postoperative Frey's syndrome in patients with benign parotid tumors.

## 2. Materials and methods

### 2.1. Patients

A total of 61 patients with benign parotid tumor undergoing superficial parotidectomy between April 2016 and January 2017 in the Department of Maxillofacial Surgery of Union Hospital Affiliated to Fujian Medical University were enrolled into this study. Patients were included who were diagnosed with parotid tumor and who were receiving a preoperative health status evaluation, salivary computed tomography (CT) scan and physical examination, with the results showing that superficial parotidectomy was the optimal treatment. The exclusion criterion was malignancy found on intraoperative frozen biopsy. Two patients were excluded due to malignant tumor. The final number of participants was 59.

All patients were informed about the details of ThormalGEN surgical patch, including its origin, safety, cost and surgical application. The patients were randomized to the ThormalGEN group ( $n = 37$ ), in which the ThormalGEN surgical patch in the superficial parotidectomy was used, or to the control group ( $n = 22$ ) undergoing superficial parotidectomy alone. This study was approved by the institutional review board of our hospital. Written informed consent was obtained from each patient.

### 2.2. Preparation of ThormalGEN surgical patch

The ThormalGEN surgical patch was purchased from the Grandhope Biotech Co., Ltd and prepared as previously described (Wang et al., 2017) with some modification. Briefly, porcine

pericardial tissue was washed up and immersed in benzalkonium bromide for 15 h, followed by a 3% acetone solution for 24 h to remove adipose tissue and impurities. The tissues were decellularized using 0.1% Triton-100 solutions with 0.1% pepsin and ultrasonically digested under 30 kHz for 48 h to remove cells and debris. After that, the tissues were reacted with amide under pH 7 for 48 h and guanidine hydrochloride solutions under pH 8 for 24 h to become an antigen-free matrix. The matrixes were then stirred in a 0.5% polypropylene oxide solutions for 2 days to crosslink the collagen molecules in the matrix. The resulting decellularized pericardial patch was sterilized with gamma-ray and stored at room temperature until further use.

### 2.3. Surgical procedure

The standard superficial parotidectomy was performed by the same senior attending surgeons. After complete removal of the tumor and the affected parotid tissue, the pathology of the tumor was immediately confirmed by the frozen biopsy. In the ThormalGEN group, the ThormalGEN surgical patch was trimmed to completely cover the surgical area, and the absorbable 3/0 polyglactin stitches were used to fix the ThormalGEN surgical patch in case of loosening and displacement. The suction tubes were inserted between the remaining parotid beds, and local pressure bandaging was used to prevent hematomas or seromas (Fig. 1).

### 2.4. Postoperative assessment

The assessment of Frey's syndrome included subjective evaluation (patient self-assessment) and objective evaluation (starch-iodine test). The subjective assessment was used to evaluate if the patients experienced postoperative complications such as Frey's syndrome, wound infection, facial numbness, salivary fistula and satisfaction with the facial contour at 6 months postoperation. The objective assessment for Frey's syndrome was performed by the starch-iodine test in a yes-or-no-manner (Fig. 2) at 3, 6, and 12 months postoperation. Frey's syndrome was defined as a positive result of the starch-iodine test at the 3-month or 6-month follow-up.

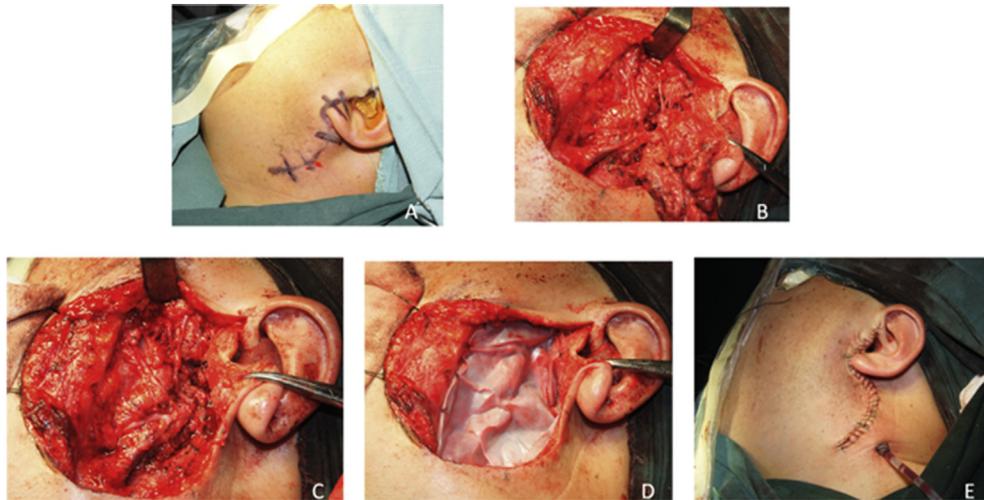
### 2.5. Statistical analysis

Continuous data were presented as the mean  $\pm$  standard deviation (SD), whereas categorical variables were presented as number and percentage. The Mann–Whitney U test was used to compare the difference between means while normality of age was not assumed. The Chi-square test and Fisher exact test were used to analyze categorical data. Univariate and multivariate logistic regression models were used to investigate the association of independent variables with Frey's syndrome. The statistical significance level for all the tests was set at a P-value  $< 0.05$ , two-tailed. All analyses were performed using IBM SPSS Version 20 (SPSS Statistics V20, IBM Corporation, Somers, NY, USA).

## 3. Results

### 3.1. Patients' demographic and clinical characteristics

A total of 59 patients was included and divided into the ThormalGEN group ( $n = 37$ ) the control group ( $n = 22$ ). The mean age was  $52.90 \pm 14.56$  years of all 59 patients and did not differ between groups ( $51.09 \pm 16.87$  [ThormalGEN group] vs.  $52.90 \pm 14.56$  years [control group],  $p = 0.530$ ). As shown in Table 1, there were 26 (70.27%) and 14 (63.64%) male patients in the ThormalGEN group and the control group, respectively ( $p = 0.774$ ).



**Fig. 1.** Procedure for superficial parotidectomy. (A) Surgical incision planning; (B) dissection of facial nerve; (C) removal of tumor; (D) surgical patch was fixed; (E) incision was sutured.

### ThormalGEN group



### Control group



**Fig. 2.** The objective assessment for Frey's syndrome was performed by the starch-iodine test at 6 months postoperation. The sweating area (red arrows) was visualized when the patient was chewing vitamin C.

**Table 1**  
Patients' demographic and clinical characteristics and postoperative complications.

Parameters	ThormalGEN group (n = 37)	Control group (n = 22)	All	p Value
Gender				0.774
Male	26 (70.27)	14 (63.64)	40 (67.80)	
Female	11 (29.73)	8 (36.36)	19 (32.20)	
Pathological types				0.202
Pleomorphic adenoma	12 (32.43)	12 (54.55)	24 (40.68)	
Warthin tumor	12 (32.43)	6 (27.27)	18 (30.51)	
Others	13 (35.14)	4 (18.18)	17 (28.81)	
Postoperative complications				
Frey's syndrome (starch test)	3 (8.11)	9 (40.91)	12 (20.34)	0.006
Frey's syndrome (patient self-assessment)	3 (8.11)	3 (13.64)	6 (10.17)	0.4968
Facial numbness	10 (27.03)	5 (22.73)	15 (25.42)	0.767
Unsatisfied with facial contour	1 (2.70)	1 (4.55)	2 (3.39)	1.000
Wound infection	1 (2.70)	0 (0.00)	1 (1.69)	1.000
Salivary fistula	2 (5.41)	0 (0.00)	2 (3.39)	0.524

Data was presented as n (%).

The histologic types were not significantly different between groups ( $p = 0.202$ ) (Table 1). There were 12 (32.43%) and 12 (54.55%) pleomorphic adenoma in the ThormalGEN group and the control group, respectively, which was the most common histological type. Twelve (32.43%) cases in the ThormalGEN group and 6 (27.27%) cases in the control group were found to have Warthin tumor. The other types included basal cell adenoma, oncocytic metaplasia and lymphoepithelial sialadenitis.

### 3.2. Outcomes and complications

According to the starch-iodine test, the incidence of Frey's syndrome was significantly lower in the ThormalGEN group than in the control group (8.11% [3/37] vs. 40.91% [9/22],  $p = 0.006$ ) (Table 1). However, in the patient self-assessment, there was no significant difference in the incidence of Frey's syndrome between groups (8.11% [3/37] vs. 13.64% [3/22],  $p = 0.4968$ ). One patient in ThormalGEN group complained about the feeling of flush and sweat at the edge of the flap, which affected his daily life. Three patients in the control group complained about the feeling of flush and sweat, which severely affected their daily life.

According to the subjective assessment, the incidences of postoperative complications were not significantly different between groups (all  $p > 0.05$ ). Almost all the patients were satisfied with the facial contour after the surgery, except for 1 (2.70%) patient in the ThormalGEN group and 1 (4.55%) patient in the control group, who complained slight asymmetry at the surgical side. Ten (27.03%) patients in the ThormalGEN group and 5 (22.73%) in the control group had facial numbness at 6 months postoperation, but the symptom disappeared at 1 year postoperation. Two patients (5.41%) in the ThormalGEN group had salivary fistula for 2 weeks, which was resolved by removal of effusion and bandage compression dressing.

### 3.3. Risk factors associated with Frey's syndrome

To analyze the risk factors associated with Frey's syndrome, logistic regression analysis was performed. As shown in Table 2, the treatment method was the only risk factor associated with Frey's syndrome in the univariate logistic regression models ( $p < 0.01$ ). In addition, after adjustment for gender, age and tumor pathological subtype, the multivariate logistic regression model consistently showed that the ThormalGEN group had a significantly lower risk of Frey's syndrome as compared with the control group (odds ratio [OR] = 0.11, 95% confidence interval [CI] = 0.02–0.51,  $p < 0.01$ ).

## 4. Discussion

In this study, we investigated the effect of ThormalGEN surgical patch on preventing Frey's syndrome following superficial parotidectomy in patients with benign parotid tumor. The results showed that the patients receiving the ThormalGEN surgical patch treatment had a significantly lower incidence of Frey's syndrome (by the starch test) than the control patients. In addition, the incidences of postoperative complications were comparable between groups. Furthermore, multivariate logistic regression analysis showed that the patients with the ThormalGEN surgical patch treatment had a significantly lower risk of Frey's syndrome as compared with the control group. Taken together, these results suggested that the ThormalGEN surgical patch was capable of effectively preventing Frey's syndrome following superficial parotidectomy, and had a good safety and biocompatibility profile.

In this study, the assessment of the incidence of Frey's syndrome included subjective evaluation (patient self-assessment) and

objective evaluation (starch test). In the subjective assessment, a positive result was reported only when the patient had significant manifestations of Frey syndrome. The starch test was performed in a yes-or-no-manner, and the result was not dependent on the severity of the symptoms. Therefore, we observed a significant difference between groups in the starch test but not in the patient self-assessment.

Our results and multivariate logistic regression analysis consistently suggested that the ThormalGEN surgical patch can effectively reduce the incidence of Frey's syndrome. Although there were still 3 (8.11%) patients in the ThormalGEN group who had Frey's syndrome, in all cases the symptoms developed at the edge of the flap. Hence, we proposed that the symptoms might be attributed to the fact that the space between the flap and the isolated parotid was difficult to perfectly cover by the ThormalGEN surgical patch. Therefore, if we can carefully trim the surgical patch to make it completely cover the edge of the flap, Frey's syndrome might be thoroughly prevented. However, this notion should be further validated in a subsequent study.

In this study, we observed that 15 (25.42%) patients developed facial numbness at 6 months after parotidectomy, but that the symptoms disappeared at 1 year postoperation. It has been documented that Frey's syndrome develops mainly at 6–18 months after parotidectomy (Li et al., 2013; Sharma, 2014). Therefore, we proposed that the aberrant reinnervation of the sympathetic and parasympathetic nerve may be completed at least after 6 months postoperation, which is also in agreement with the timeline of the symptom of facial numbness starting to disappear in our observation. ThormalGEN patch is a decellularized pericardial patch manufactured from porcine pericardial tissue. A rabbit model of duraplasty showed that the ThormalGEN surgical patch could be maintained at least 1 year (Liu et al., 2004). Therefore, the surgical patch may have the potential to prevent aberrant reinnervation at 6 months postoperation, in turn preventing Frey's syndrome. This may contribute to the preventive efficacy of ThormalGEN patch on Frey's syndrome in superficial parotidectomy. However, the detailed mechanism needs to be further investigated.

Given that the ThormalGEN surgical patch is manufactured from porcine pericardial tissue, its safety and biocompatibility should be concerned. On the other hand, some patients may refuse to use due to their religious beliefs. In this study, some patients refused to use the surgical patch due to its xenogeneic source. However, the ThormalGEN surgical patch can be absorbed at 1 year after implantation, and surgical removal is not required. In contrast, some unabsorbable synthetic surgical patch may increase the incidence of wound infection and rejection (Singh et al., 2011). Two patients (5.41%) in the ThormalGEN group had salivary fistula after the patch implantation. One patient was cured after the bandaging treatment, whereas another patient did not strictly follow the medical advice and developed an infection of the wound. The wound surface remained unhealed for a long time, and therefore the surgical patch was taken out after 2 months postoperation. No rejection and allergic reaction cases were found in our study. These findings suggest that the ThormalGEN patch has good safety and biocompatibility. Another limitation of applying the ThormalGEN surgical patch in superficial parotidectomy is that it increases the time and cost of the operation. The cost of the ThormalGEN surgical patch is about USD \$430 to USD \$730. As a foreign implant, the ThormalGEN surgical patch may induce concerns about its adverse effects on the quality of ultrasound examination and revision surgery. So far, however, although some patients had effusion under the ThormalGEN surgical patch, we did not observe that the quality of ultrasound was affected by the surgical patch. In addition, there were no patients receiving revision surgery in this study. Therefore the

**Table 2**

Univariate and multivariate logistic regression of the risk factor associated with Frey's syndrome.

Parameters	Univariate		Multivariate <sup>a</sup>	
	OR (95% CI)	p Value	OR (95% CI)	p Value
Group				
The control group	ref	-	ref	-
The ThormalGEN group	0.13 (0.03–0.55)	0.006	0.11 (0.02–0.51)	0.005
Gender				
Male	ref	-	ref	-
Female	1.07 (0.28–4.11)	0.925	1.20 (0.22–6.66)	0.835
Age	1.01 (0.96–1.05)	0.819	1.01 (0.97–1.06)	0.601
Pathological diagnosis		0.942		0.809
Pleomorphic adenoma	ref	-	ref	-
Warthin tumor	1.09 (0.25–4.79)	0.914	1.72 (0.25–11.93)	0.585
Others	0.81 (0.17–3.99)	0.800	1.70 (0.26–10.94)	0.576

<sup>a</sup> The patients' gender, age and tumor pathological subtype were adjusted in the multivariate logistic regression model.

effect of the ThormalGEN surgical patch on revision surgery remains to be further evaluated.

There are still some limitations to this study. Since it has been demonstrated that the incidence of Frey's syndrome increases over time (Wolber et al., 2018) and that the size and location of the affected area change over time (Steffen et al., 2012), the short follow-up period was a major limitation of this study. Our patients are still being followed-up, and long-term outcome will be reported in a later follow-up publication. For a later follow-up publication, the affected area of Frey's syndrome should be compared between the two groups and the assessment should be conducted in a blinded manner. The sample size of this study was relatively small. In addition, the procedure should be further optimized to reduce the incidences of Frey's syndrome and other postoperative complications. All these limitations should be addressed in a subsequent study.

## 5. Conclusion

In conclusion, our results suggest that the ThormalGEN surgical patch can effectively reduce the incidence of Frey's syndrome following superficial parotidectomy in patients with benign parotid tumor and that it has good safety and biocompatibility and can thus be used as a strategy for preventing Frey's syndrome.

## Conflicts of interest

The authors declare that there are no conflicts of interest.

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