**Novel thermographic detection of regional malperfusion caused by a thrombosis during ex vivo lung perfusion**

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**Abstract**

**OBJECTIVES:** Although ex vivo lung perfusion (EVLP) has been clinically applied as a novel rig to evaluate marginal donor lungs, no parameters have been reported to objectively detect regional lung damage during EVLP. The aim of this study was to investigate whether regional donor lung malperfusion-related damage caused by a thrombus could be detected by thermography during EVLP.

**METHODS:** Lewis rats were divided into two groups: the Thrombosis group and the Control group (n = 6 in each group). All rats were heparinized and the lungs were flushed with 20 ml of Steen solution. In the Thrombosis group, a 30 mg artificial thrombus was inserted into the left main pulmonary artery. All the lungs were perfused and ventilated using the EVLP system. Perfusion flow was increased every 2 min up to 10 ml/min. The lungs were evaluated by collecting thermographical and physiological data during EVLP.

**RESULTS:** Pulmonary artery pressure was higher and lung compliance was lower in the Thrombosis group compared with those in the Control group (P = 0.0005 and <0.0001, respectively). Macroscopically, no differences were seen between the perfused area and the malperfused area, whereas significant differences were detected between them by thermography. The surface temperature of both lungs in the Control group and the right lungs in the Thrombosis group rose with increasing perfusion flow, whereas the surface temperature of the left lungs in the Thrombosis group did not rise (P < 0.0001).

**CONCLUSIONS:** Although physiological data could possibly imply the existence of thrombi in the Thrombosis group, it could not reveal which area was obstructed by thrombi; however, thermography could detect a malperfused region. Thermographical evaluation may become a promising strategy to detect regional damage in donor lungs.

**Keywords:** Lung transplantation • Marginal donor • Ex vivo lung perfusion • Thrombosis

**INTRODUCTION**

Lung transplantation has become an established treatment for end-stage respiratory diseases. However, donor shortage still remains the limitation for the utilization of this treatment. To overcome this issue, donation after cardiac death donor lung is used to expand the donor pool [1, 2]. In Japan, although the number of brain-dead donors is increasing after the amendment of the brain-dead bills, the shortage of donor lungs is still serious. We use over 50% of potential donors, including marginal donors, to ameliorate this shortage [3].

*Ex vivo* lung perfusion (EVLP) was developed to assess the suitability for safe transplantation of potentially damaged lungs, such as marginal donor lungs or lungs donated after donor cardiac death [4–6]. Although EVLP has been clinically applied as a novel rig to evaluate whole lungs, no parameters have been reported to objectively detect regional lung damage during EVLP. All physiological data including lung compliance, pulmonary artery pressure and PaO2 reflect the condition of the whole lung. There are no current methods for the detection of regional information. For example, if a thrombus exists locally in the right lower lobe, this cannot be determined during EVLP. Thrombi are detected in 35% of declined lungs and unexpected thrombi are found in donor lungs, which are associated with primary graft dysfunction [7, 8]. Thus, if these thrombi can be detected prior to transplantation, we may make a more objective judgement concerning declining the lung, resecting the damaged region, using an intact region or reconditioning the lung with appropriate methods [9–12]. This may reduce not only acute injury after reperfusion but also chronic lung allograft dysfunction, which closely correlates with primary graft dysfunction [10]. We hypothesized that the lung surface temperatures of malperfused areas are lower than those of normal perfused areas. The aim of this study was to investigate...
whether regional donor lung malperfusion caused by a thrombus could be detected by thermography during EVLP.

**MATERIALS AND METHODS**

**Animals**

Lewis rats weighing 290–345 g (Japan SLC, Hamamatsu, Japan) were used. All animals received humane care in compliance with the ‘Principals of Laboratory Animal Care’, formulated by the U.S. National Society for Medical Research, and the Guide for the Care and Use of Laboratory Animals, prepared by the U.S. Institute of Laboratory Animal Resources and published by the National Institutes of Health (NIH Publication 85-23, revised 1996). The study was approved by the ethics committee of the Faculty of Medicine at Kyoto University, Japan.

**Artificial thrombus preparation**

A 1-ml sample of rat blood was obtained from the inferior vena cava of Lewis rats without heparinization. Aliquots of 0.2 ml of blood, 0.1 ml (8 mg) of fibrin and 0.1 ml (30 U) of thrombin (Beriplast® P, CSL Behring, Tokyo, Japan) were injected into a silicone tube (Masterflex®, ID 1.6 mm, OD 4.9 mm, Masterflex AG, Gelsenkirchen, Germany). After 10 min, the blood, fibrin and thrombin complex formed a clot, which was forced out of the silicone tube. A clot of 30 mg was measured and placed in a 16-G catheter (SURFLO®, Termo, Tokyo, Japan).

**Heart–lung bloc preparation**

The Lewis rats were divided into two groups: the Thrombosis group and the Control group (n = 6 in each group). All rats were anaesthetized with an intraperitoneal injection of sodium pentobarbital (50 mg/kg), intubated after tracheotomy and ventilated during surgery. All rats were heparinized with 500 U of heparin, and an intravenous cannula was inserted into the main pulmonary artery and their lungs were flushed with 20 ml of cold Steen solution (Vitrolife, Uppsala, Sweden) with drainage taking place through an incision of the left ventricle. In the Thrombosis group, after the flushing period, a 30-mg artificial thrombus was placed in the 16-G catheter and gently injected into the left main pulmonary artery with 0.5 ml of 4°C Steen solution. Thereafter, in both groups, a drainage cannula was inserted into the left atrium. Finally, the heart and lungs were excised en bloc and the lung was perfused and ventilated in the EVLP system. This method was used to create the malperfused lungs (left lungs in the Thrombosis group), the normal perfused lungs (both lungs of the Control group) and overperfused lungs (right lungs in the Thrombosis group).

**Ex vivo lung perfusion model**

To evaluate lung function, we used the isolated rat lung perfusion set-up (Model 829; Hugo-Sachs Electronic Harvard Apparatus, Holliston, MA, USA). The isolated heart–lung bloc was secured in an artificial thorax and ventilated with ambient air at positive pressure under the following conditions: respiratory rate = 60 cycles/min, peak inspiratory and expiratory chamber pressures of +8 and +4 cm H₂O, respectively. The lungs were perfused with Steen solution at 37°C using a roller pump. Subsequently, 300 U of heparin was added to the perfusate. EVLP was started with a low flow and the flow rate was gradually increased every 2 min in a stepwise manner (3 → 5 → 7 → 10 ml/min). The artificial thorax, the perfusate circuit and the airway were maintained in a water jacket at 37°C throughout the experiment. The perfusate, which was deoxygenated in a glass column filled with a gas mixture of nitrogen (92%) and carbon dioxide (8%), was pumped into the pulmonary artery of the isolated lung. The inner surface of the deoxygenator was spherical to maximize the surface area allowing the perfusate to be deoxygenated while it dribbled along the inner surface. The perfusate draining from the left atrium was recirculated to the deoxygenator. Using a pressure equilibration vessel, the pulmonary venous pressure was kept constant at +2 cm H₂O against hilum. PVR [(cm H₂O/ml/min), defined as pulmonary arterial pressure minus pulmonary vein pressure/perfusate flow], weight gain of the lung (mg) and dynamic airway compliance (ml/cm H₂O) were monitored continuously and recorded at 10 min intervals during EVLP. Physiological data from each group were continuously recorded and analysed after the experiment.

**Monitoring of lung surface temperature**

We used an InfRec Thermo Gear G100® (Nippon Avionics Co., Ltd, Japan) thermometer to detect lung surface temperature. This thermovision camera contains an uncooled focal plane array detector with a geometric resolution of 76,800 pixels per picture (320 × 240) and can efficiently serve different applications with a wider measurement range (−40 to 1500 ider) and finer temperature resolution (0.004°C) for common ambient temperature (−20 to 60°C). All thermovision data from the infrared camera were stored in a memory card. All lung surface temperatures were measured at every flow rate step-up period and at the end of EVLP evaluation (Fig. 1). Thermal data were recorded at each dot and analysed using the InfRec Analyser NS9500® standard. The lung
was traced using the software and the thermal data recorded for the dots in the traced line were extracted. The average temperature on each lung surface was calculated (Fig. 2).

Wet-to-dry ratio

The lower part of the left lobe and the right lower lobe were resected to measure the wet-to-dry ratio after EVLP. The lung tissue samples were weighed to give the wet lung weight. The lung tissue was then placed in an oven at 180°C for 6 h, and reweighed to give the dry lung weight. The wet-to-dry lung weight ratio was calculated to evaluate insufficient penetration of perfusate solution or lung oedema.

Histological analysis

Lung tissue samples were collected from the left lobe after EVLP. They were fixed in 10% buffered formalin, embedded in paraffin and stained with haematoxylin and eosin.

Statistical analyses

All statistical analyses were performed using the StatView 5.0 software (Abacus Concepts, Berkeley, CA, USA). All values are presented as the mean ± standard error of the mean (SD). Data were evaluated using one-way analysis of variance (ANOVA), repeated-measures ANOVA and Student’s paired t-test. A probability (P)-value of < 0.05 was considered statistically significant.

RESULTS

Physiological data

Lung compliance was gradually decreased in the Thrombosis group (P < 0.0001), and pulmonary artery pressure was higher in this group throughout the EVLP period (P = 0.0005). No difference was observed in PaO₂ (P = 0.11; Fig. 3).

Changes in lung surface temperature during ex vivo lung perfusion

Macroscopically, we could not distinguish the difference between each group. The surface temperature of normal perfused lungs was gradually increased depending on the flow rate. However, the surface temperature of the left lungs of the Thrombosis group was lower compared with that of the other groups (Fig. 4). The surface temperatures were gradually increased depending on perfusate flow rate in each group (P < 0.0001; Fig. 5). The surface temperature of the malperfused lungs (the left lung in the Thrombosis
group) was significantly lower than that of the normal perfused lungs \( (P < 0.0001; \text{Fig. 5}) \).

### Wet-to-dry ratio

In general, high wet-to-dry ratios indicated lung oedema. Wet-to-dry ratios revealed that malperfused lungs were dried out compared with normal lungs (left lung in the Thrombosis group (malperfused lung) versus left lung in the Control group (normal perfused lung), \( P < 0.0001; \text{Fig. 6} \)). The wet-to-dry ratio of the right lung in the Thrombosis group was higher than that of the right lung in the Control group (right lung in the Thrombosis group (over perfused lung) versus right lung in the Control group (normal perfused lung); \( P = 0.0024; \text{Fig. 6} \)).

### Histological findings

Macroscopically malperfused lungs were dry compared with perfused lungs (Fig. 7A). After completion of EVLP, we histologically confirmed that a massive thrombus was still present in the left pulmonary artery (Fig. 7B).
DISCUSSION

There are several reports concerning thermography in the medical field, for instance, screening of mammary carcinoma, diagnosis of skin diseases and assessment of the indication for molars [13–17]. In the organ transplantation field, we were the first to demonstrate the use of thermography to detect regional malperfusion areas caused by a thrombus. Conventionally, physiological data have been used for evaluation during EVLP. In the present study, the physiological data except for PaO2 showed some meaningful information. No difference in PaO2 was found between the Thrombosis and Control groups. This is because the acellular solution is immediately saturated; therefore, PaO2 shows high levels even if the lung is damaged [18]. If there are significant shunts in a lobe, PaO2 from the drainage vein of the lobe may decrease. We may distinguish the shunt area and the intact area with this method. However, when a damaged region is not well-perfused because of thrombosis or severe oedema, the perfusate solution cannot go through the lobe. Thus, perfusate from the pulmonary vein of a lobe, which is not well-perfused, is almost the same as the back-flow perfusate from the left atrium. PaO2 in the perfusate is expected to be the same as that in the other pulmonary vein. This may mislead the evaluation of the damaged lobe. Thermography could resolve this issue without PaO2 measurement. Dynamic compliance was lower and pulmonary artery resistance was higher in the Thrombosis group. Although the ventilation was not obstructed, lung compliance decreased in the Thrombosis group. We interpret this finding as follows: the wet-to-dry ratio in the right lung was higher than that in the normally perfused lung. Therefore, the right lung became oedematous because of overperfusion. We believe that this change caused the decrease of the dynamic compliance in the Thrombosis group. At any rate, these data and macroscopic findings did not give us regional information, for example, on which side the thrombus existed and which side of the lung was malperfused. In contrast, thermography could detect that the thrombus was in the left main pulmonary artery in the Thrombosis group and contributed to the insufficiency of the perfusate solution. The surface temperature of the malperfused area was obviously lower than that of the normal perfused area. The rationale of the mechanism is as follows; the donor lung was flushed with Steen solution at 4°C, thus, cooling the lung to around 4°C. After starting EVLP, the lung was gradually warmed depending on the perfusion flow rate. The surface temperature of the lung was observed as the consequence of the mixed temperature of the perfusate and the air around or in the lung. The malperfused area was displayed as a blue area, because the warm perfusate could not reach the peripheral area and the lung surface temperature came close to the air temperature.

We considered several advantages for the use of thermography in ex vivo conditions. First, the surface temperature of the donor lung is hardly interfered with during EVLP because there are no structural objects.
Secondly, the contrast of the lung surface is vividly observed particularly in the step-up period. In order to detect carcinoma or inflammation in vivo conditions, thermography should discriminate tiny changes caused by neoangiogenesis or increased metabolic activity. However, this thermal change is too slight and is too affected by the surrounding tissue to be detected clearly by thermography. Thus, many studies hardly demonstrated a positive conclusion, and it is believed to be difficult to use thermography for the screening of breast cancer [19]. In contrast, the thermal change is extremely wide in EVLP. We could set the detection temperature range between 20 and 40°C. This contributed in reducing the interference of the circumstances and increasing the sensitivity, and allowed us to clearly detect a visual difference between the malperfused areas and the normal perfused areas.

Thirdly, thermography could evaluate both the initial status of the donor lung and the status after reconditioning. In our previous study, we have demonstrated that administration of plasmin could improve the donor lung damaged by a thrombus during EVLP [12]. To take the study to the next step, we have to select donor lungs that have been damaged by a thrombus. Thermography can detect the malperfused area and might make it possible to confirm the effect of plasmin administration during EVLP. According to the wet-to-dry ratio, the malperfused lung was lighter than the normal perfused lung. This indicated that the malperfused lung was insufficiently supplied with perfusate solution; thus, the lung had been dried out. The malperfused lungs were exposed to risk of damage during EVLP. From the clinical evidence, in the presence of a thrombus, the malperfused area is expected to be severely damaged after reperfusion, which leads to primary graft dysfunction [8]. Therefore, the malperfused lungs should be detected and treated or resected before lung transplantation to avoid primary graft dysfunction. Using thermography, we can then objectively decide which part of the graft lungs can be used. Finally, we did not use any drugs or molecular biological assays and did not touch the donor lungs. Furthermore, we could obtain the information instantaneously.

Therefore, thermography is a simple, low-cost, non-invasive and instantaneous method to obtain regional information. During a clinical transplant procedure, the malperfused area may appear red after flushing. If the lung is perfused with an acellular solution, the area remains red unless the thrombus is removed. This finding may help identify a malperfused area, just like thermography does. However, we are not sure whether this phenomenon always occurs. If the thrombus is floating during flushing, red blood cells are almost completely flushed. In that case, surface colour would be pale. The shades of the colour of the lung surface are very diverse; therefore, we think that the identification of a malperfused area based on the lung surface colour alone would be difficult. Furthermore, an acellular solution and a cellular (red blood cell-based) solution are used for clinical EVLP. When a lung is perfused with a cellular solution and the malperfused area is incompletely flushed, the malperfused area has a weaker colour contrast; however, when it is completely flushed, it yields a good colour contrast. In this study, to simplify as well as clarify these complicated issues, we inserted a thrombus into the pulmonary artery after flushing.

There is a limitation to this study. The wet-to-dry ratio of the right lung in the Thrombosis group suggested the existence of lung oedema due to overperfusion. In this study, thermography could not detect the oedema. Nevertheless, we plan to continue doing research to establish a method to detect regional lung oedema and more regional malperfusion (at least one lobe) using thermography. This study is the first to introduce thermography into the field of transplantation. Although physiological data could imply the possible existence of thrombi in the Thrombosis group, it could not reveal which area was obstructed by a thrombus; however, thermography could clearly detect the malperfusion area caused by a thrombus. The process was not invasive; it was a simple, low-cost method of obtaining regional information. Thermographical evaluation may become a promising strategy for the detection of regional damage in donor lungs.

Conflict of interest: none declared.

REFERENCES