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Abstract. Ischemia-reperfusion injuries are a critical determinant of lung transplantation success. The endogenous production of carbon monoxide (CO) is triggered by ischemia-reperfusion injuries. Our aim was, therefore, to assess the feasibility of exhaled CO measurements during the *ex vivo* evaluation of lungs submitted to ischemia-reperfusion injuries. Five pigs were euthanized and their lungs removed after pneumoplegia. After cold storage (30 min, 4°C), the lungs were connected to an extracorporeal membrane oxygenation circuit, slowly warmed-up, and ventilated. At the end of a 45-min steady state, CO measurements were performed by optical-feedback cavity-enhanced absorption spectroscopy, a specific laser-based technique for noninvasive and real-time low gas concentration measurements. Exhaled CO concentration from isolated lungs reached 0.45 ± 0.19 ppmv and was above CO concentration in ambient air and in medical gas. CO variations peaked during the expiratory phase. Changes in CO concentration in ambient air did not alter CO concentrations in isolated lungs. Exhaled CO level was also found to be uncorrelated to heme oxygenase (HO-1) gene expression. These results confirm the feasibility of accurate and real-time CO measurement in isolated lungs. The presented technology could help establishing the exhaled CO concentration as a biomarker of ischemia-reperfusion injury in *ex vivo* lung perfusion. © *2014 Society of Photo-Optical Instrumentation Engineers (SPIE)* [DOI: 10.1117/1.JBO.19.4.047001]

Keywords: carbon monoxide; ischemia-reperfusion; lung; laser spectroscopy; cavity-enhanced absorption.

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1 Introduction

Laser spectroscopy appears to be a very promising technique for exhaled breath analysis. Real-time, noninvasive measurements can be conducted with very high sensitivity and good selectivity of the species to be quantified.[1] Indeed, several laser spectroscopy methods have already been applied for breath analysis.[1–5] Among those techniques, optical-feedback cavity-enhanced absorption spectroscopy (OF-CEAS) offers many advantages in medical diagnostics: it allows real-time measurements with a small sampling volume, it does not require any calibration with certified gas mixtures, its response time is faster than a single respiratory cycle, and it enables the development of compact and robust instruments to be operated by nonspecialists in a medical environment. OF-CEAS absolute concentration measurements rely on cavity ring-down spectroscopy (CRDS) measurements a well-established technique which sensitivity and accuracy competes with laboratory traditional gas chromatographs and mass spectrometers.[6,7] Successful intercomparison of an OF-CEAS instrument and a mass spectrometer has been reported.[8] The OF-CEAS technique has been validated previously in humans to monitor exhaled carbon monoxide (eCO) and methane (CH_4).[5] The ability of the OF-CEAS technique to measure eCO could be of particular interest in the light of the recent findings about the physiology of CO.

Carbon monoxide is produced endogenously by the degradation of heme to biliverdin and iron.[9] This reaction is catalyzed by the rate-limiting enzyme family of heme oxygenases (HO). The expression of HO-1, the inducible isoform, is dramatically increased under many sources of stress including ischemia-reperfusion.[9] ischemia-reperfusion injury (IRI) can be defined as the tissue damage following blood flow restoration after organ ischemia. The induction of HO-1 during ischemia-reperfusion leads to an increase in CO production which, in turn, exerts anti-inflammatory, anti-apoptotic, and anti-proliferative effects and limits IRI consequences.[9] These physiological properties of CO are potentially of strong interest during lung transplant procedure whose success depends closely on the amount of IRI.[10,11] For example, the inhibition of HO activity after experimental lung IRI increases alveolar cell damage, recruitment of inflammatory cells, and edema.[12] In contrast, CO inhalation reduces apoptosis, excretion of inflammatory mediators and pulmonary edema caused by IRI.[13–17] Several studies have also demonstrated that the increase of endogenous CO production subsequent to ischemia-reperfusion can be detected. High eCO levels in lung transplant recipients have been correlated to graft dysfunction, the leading cause of morbidity and mortality after lung transplantation.[18–20]

Recently, a technique named the *ex vivo* lung perfusion (EVLP) has been developed for the evaluation of isolated lungs before transplantation.[21] The innovative concept of this

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technique is to assess lung grafts that were initially rejected for transplantation in order to requalify them for clinical use. The ultimate goal of this EVLP technique is to increase the number of available lungs for transplantation and therefore limit the organ shortage. However, EVLP is still lacking well-defined lung acceptance criteria during the *ex vivo* reperfusion phase.[22] To be a useful criterion for lung acceptance, a physiological marker should be noninvasive, sensitive, reproducible, and quantifiable in real time.[22] In this perspective, eCO measurements could help to explore IRI during EVLP procedures and therefore discriminate suitable lungs for transplantation. However, such measurements face several problems: the technique has to be very sensitive because expected concentrations of CO are under one part per million in volume (ppmv); the measured CO should originate from the lungs; finally, the measurements should not be polluted by ambient gas. We report here a pilot study addressing these issues and validating the measurement of eCO with the OF-CEAS technique in the specific setting of EVLP in pig lungs.

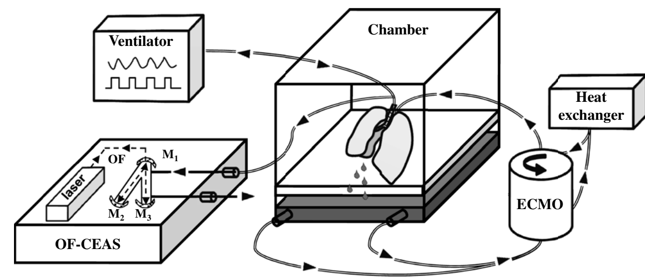

Fig. 1 Schematic of overall experimental system. Lungs are placed in a hard shell chamber and connected to an extracorporeal membrane oxygenation pump and a ventilator for reperfusion and ventilation. A small fraction of the gas sample is driven directly from the trachea to the spectrometer (optical-feedback cavity-enhanced absorption spectroscopy) where the laser absorption allows highly sensitive and highly specific real-time CO (and CH_4) measurements. M_1, M_2, M_3: mirrors of the V-shaped cavity. OF: optical feedback.

2 Materials and Methods

2.1 *CO Spectrometer*

CO was quantified according to the OF-CEAS technique, which was described in detail in the previous publications.[23–25] Spectroscopic measurements of very low gas concentrations (<1 ppmv for endogenous expired CO) require a large light absorption path. As other laser spectroscopy techniques, an OF-CEAS device is based on a resonant optical cavity inside which the gas sample continuously flows. In our spectrometer, the cavity composed of high-reflective mirrors (mirror reflectivity: $R = 99.9947\%$) allows a 19 km effective absorption length with a compact setup: the cavity is only 1-m long (folded to 50 cm) and its internal sample volume does not exceed 18 cm^3.[23–25] The difficulty of using very high-reflective mirrors consists in injecting a sufficient amount of laser light into the cavity, since the spectral width of the laser emission is usually orders of magnitude larger than the spectral profile of the cavity resonance (which produces a very narrow peak in transmission). To overcome this problem, the optical cavity is made of three mirrors placed in a V-shaped configuration (Fig. 1).[25] In this way, a fraction of the light trapped in the optical cavity is directed back to the laser, giving rise to an OF effect. By narrowing the laser emission spectrum to below the spectral width of light trapped in the cavity, OF provides strong transmission signal strength.[25] Finally, OF-CEAS provides the absolute concentration measurements without any periodic calibration with certified gas mixtures. Indeed, a real-time absorbance normalization procedure is realized continuously, based on the cavity optical loss determination by CRDS, a technique well known for absolute calibration.[6,24] One ring-down measurement is performed at the end of each OF-CEAS spectra. Absolute concentrations are calculated in real time by fitting absorption spectra acquired over a small spectral region ($\sim$1 cm^{-1} in wave number) by scanning the laser frequency several times per second ($\sim$5 Hz). The smallest detectable absorption coefficient is typically on the order of 10^{-10}/cm. Data acquisition on a given spectral region allows high selectivity and simultaneous measurements of different molecules. This is a key point in breath analysis since exhaled air is a highly complex gas mixture.

In contrast to the somewhat complex physics underlying OF-CEAS, its optical layout consists of few basic optical elements allowing for a compact and robust device. The OF-CEAS instrument, including electronics for control and data acquisition, fits inside a 19-in. rack ($45 \times 59 \times 13$ cm^3) so it could be comfortably installed in the medical environment. A laptop personal computer connected to the control electronics runs user-friendly software for real-time data processing. The OF-CEAS device used here was first described in detail by Kassi et al.[23] Only minor improvements were implemented to this spectrometer, which is optimized for CO and CH_4 measurements by using a distributed feedback diode laser emitting around 2.3 μm. In this configuration, CO sensitivity is about 0.002 ppmv for an acquisition time of 0.2 s (with an absorption detection limit of $\sim 5 \times 10^{-10} cm^{-1}$). In the small spectral region scanned to record OF-CEAS spectra also lie CH_4 absorption lines as reported previously.[5] CH_4 is produced in the colon by the breakdown of carbohydrates and its concentration in exhaled air is thus not expected to vary in our *ex vivo* model.[26] CH_4 concentrations were monitored (accuracy 0.02 ppmv in 200 ms) as a reference background gas as compared to CO variation. The gas sample was flowed continuously through the optical cavity at a rate of 100 mL/min with a pressure servo-controlled to 50 mbar. These values were set to optimize the response time imposed by the gas exchange rate inside the measurement volume (18 cm^3), while still requiring a modest sample flow in order not to interfere with the lungs ventilation. The effective response time is 1.3 s. It is estimated by considering the rising and falling edges of recorded time series of CO measurements when the inlet is switched between exhaled CO by the lung to ambient air CO measurements (Fig. 3). This response time could still be significantly lowered by using a larger flow rate or by decreasing the pressure in the cavity. Also, we have recently demonstrated that the cavity volume in the OF-CEAS instrument can be significantly reduced down to 12 cm^3.

2.2 *Animal Protocol*

Our experiments were performed in compliance with institutional animal care committee guidelines and were approved by the Grenoble Animal Care Committee. Five pigs (*sus scrofa*) (19.5 ± 5 kg) were anesthetized with pentobarbital and suxamethonium after intramuscular premedication by azaperone. Tracheae were intubated and lungs were mechanically ventilated (AS/3 ADU, Datex-Ohmeda, Louisville, Colorado). After a 15-min steady state allowing *in vivo* eCO measurements by the

same OF-CEAS instrument, we undertook EVLP as described extensively by the other authors.[21,27,28] Briefly, the animals were euthanized by deep anesthesia (pentobarbital and potassium chloride) and lungs were surgically harvested by sternotomy. During the organ procurement, lungs were manually inflated with the pressure limiting valve of the respirator circuit set to 10 cm H_2O, and the tracheal tube was clamped. The pulmonary vasculature was rinsed with 1 L of cold Perfadex (4°C, Vitrolife, Sweden) buffered with trometamol. The collected lungs were stored at 4°C for 30 min. After cold ischemia, lungs were placed in a hard-shell chamber (Fig. 1; Vitrolife). The pulmonary artery was connected to an extracorporeal membrane oxygenator circuit, including a heat exchanger. The trachea was intubated and connected to a mechanical ventilator. Lungs were progressively rewarmed by a perfusion of Krebs solution added with 5% of bovine serum albumin (colloid osmotic pressure of 20 mm Hg),[29,30] up to the theoretical cardiac output of 70 mL/kg/min. Oxygen (2 L/min) and CO_2 (7% mixed with 93% of N_2, 2 L/min) were added through the oxygenator. When the lung temperature reached 32°C, the tracheal tube was connected to the respirator, and the ventilation was increased slowly (respiratory rate = 12/min, positive end expiratory pressure = 5 cm H_2O, fraction of inspired oxygen $FiO_2 = 50\%$) up to 7 mL/kg of tidal volume. At full perfusion and ventilation, when the lung temperature reached 35°C, O_2 supply from the oxygenator was stopped, and the lungs were maintained in this steady state for 45 min. Ventilation parameters at steady state lead to a constant flow rate of 80 mL/min. Finally, the fresh gas flow to the trachea was set to 2.5 L/min in order to avoid rebreathing.

2.3 *CO Measurements in Airways and Ambient Air*

For the measurements of the CO exhaled by the lungs (eCO), the spectrometer OF-CEAS was connected to the tracheal tube so that a small fraction of the ventilation gas sample (∼2%) was driven directly to the spectrometer during both the exhalation and inhalation phases (Fig. 1). Periodically, the inlet was switched from eCO to ambient air in the hard-shell chamber using an electrovalve.

Baseline concentrations of CO in medical gas cylinders (air and oxygen) were quantified three times with three different gas tanks. These measurements were made at ambient room temperature at the beginning of each experiment by direct sampling of the gas delivered by the ventilator at different FiO_2 levels (21%, 50%, or 100%). After steady states of both *in vivo* and EVLP procedures, we measured CO concentrations in exhaled air under several FiO_2 conditions (21%, 50%, or 100%). In the same time, CH_4 concentrations were monitored as a reference gas (i.e., not modified by the variations of FiO_2). Finally, we artificially polluted the ambient air in the chamber by gas mixtures containing high (9 ppmv) or low (≤0.015 ppmv) CO concentrations in order to evaluate a possible external pollution of our EVLP measurements.

2.4 *Physiological Measures*

During the EVLP procedure, "blood gases" [i.e., partial pressures of O_2 (PaO_2) and of CO_2 ($PaCO_2$)] were measured in the perfusion fluid collected directly from pulmonary veins (ABL5, Radiometer America, Westlake, Ohio). Pulmonary arterial pressures were measured due to a pressure probe (Baxter, Uden, Holland, Netherlands) placed into the pulmonary artery. We measured the hemoglobin concentration in the perfusion fluid at a steady state. We used a hemoglobin colorimetric assay kit for pigs according to the manufacturers' recommendations (DetectX, Arbor Assays, Ann Arbor, Michigan). The detection limit was 20 μg/mL of hemoglobin.

At the end of the experiment, the superior lobe of the left lung was excised and rapidly weighed. After 48 h at 90°C, the sample was weight again and the weight-to-dry ratio was calculated.

2.5 *HO-1 Transcript Detection*

Transcripts of HO-11 and beta-actin (βACT) were evaluated by quantitative real time polymerase chain reaction (RT-q-PCR) analysis, as described previously.[31] Briefly, 100 mg *sus scrofa* lung were sonicated and total RNA extraction was performed with NucleoSpinRNA (Macherey Nagel, Duren, Germany). cDNA was reverse transcribed from 1 μg of total RNA with the SuperScript III First-Strand Synthesis (Invitrogen, Saint Aubin, France), and RNase H treatment was added. Real-time RT-q-PCR was conducted using the QuantiTect SYBR Green RT-PCR kit (Qiagen) and a Stratagene Mx 3005P (La Jolla, California). One reference genes were used to normalize: βACT sense-ATC AAG ATC ATC GCG CCT C and antisense-TAC TCC TGC TTG CTG ATC CAC (T_m 60°C, 400 nM), against HO-1: sense-ATGTGA ATG CAA CCC TGT GA and antisense-GGA AGC CAG TCA AGA GAC CA (T_m 60°C, 400 nM). Primers were synthesized by Life Technologies (Saint Aubin, France). Gene expression was quantified using the comparative threshold cycle (C_t) method.[32] The amount of target gene was normalized to the endogenous reference genes (delta C_t).

2.6 *Statistical Analysis*

Data are expressed as mean ± SD. CO concentrations in ambient air and medical gas were compared to eCO using a Student's *t*-test. Various experimental conditions were compared by multifactor analysis of variance with the Tukey *post hoc* test. Statistical significance was accepted at $p < 0.05$. Analyses were performed with SPSS (v.17, Chicago, Illinois).

3 Results

CO concentration was 0.30 ± 0.06 ppmv in ambient air, and 0.006 ± 0.002 ppmv (FiO_2 100%), 0.092 ± 0.006 ppmv (FiO_2 50%), and 0.129 ± 0.009 ppmv (FiO_2 21%) in medical gas supplies. CO concentrations in medical gas were subtracted from all the CO measurements to calculate the exact CO amount exhaled from the lungs, especially when the tests were performed with different FiO_2 levels.

3.1 *In Vivo Measurements*

The mean PaO_2/FiO_2 ratio was 435 ± 95 mmHg, mean $PaCO_2$ was 37 ± 2 mmHg, and pH was 7.38. Online eCO quantification in the air exhaled from lungs disclosed an oscillating pattern with maximal CO levels during the expiration phase (Fig. 2). eCO patterns mirrored the respiratory cycle, whereas as expected no cyclic variation was detected in the baseline ambient or medical gases measurements. Exhaled CO concentration (eCO) was consequently calculated as mean CO peak values over 1 min. eCO was significantly higher than the values in ambient air and medical gases (3.2 ± 1.3 ppmv; $p < 0.001$ for all comparisons). When the animals were ventilated with

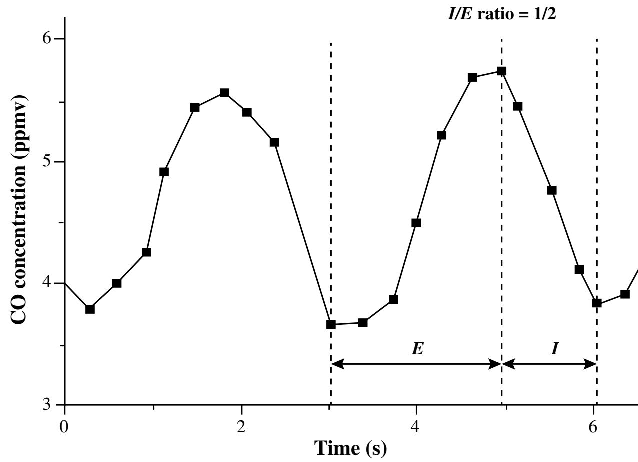

Fig. 2 Example data recorded from a pig (*in vivo* part of the study). Exhaled CO exhibits an oscillatory pattern mimicking ventilation. Ventilation parameters are tidal volume = 7 mL/kg, respiratory rate 20/ min; $FIO_2 = 50\%$, positive end expiratory pressure = 5 mm H_2O, inspiration(I)/expiration(E) ratio = 1/2.

FiO_2 100%, eCO increased significantly while the exhaled eCH_4 concentration did not vary (Table 1).

3.2 *Measurements During EVLP*

At steady state, the basic physiologic parameters of *ex vivo* lung preparations were PaO_2/FiO_2 ratio $= 410 \pm 90$ mmHg, $PaCO_2 = 36 \pm 2$ mmHg, pH $= 7.12 \pm 0.04$, peak airway pressure $= 17 \pm 3$ cm H_2O, mean pulmonary arterial pressure $= 11.2 \pm 0.7$ mmHg, pulmonary capillary pressure $= 6.0 \pm 0.8$ mmHg, and pulmonary vascular resistances $= 8.7 \pm 0.6$ mmHg/L/ min. eCO from isolated lungs displayed the same oscillatory pattern than in live animals. The concentration ranges were lower than those of eCO *in vivo* (0.44 ± 0.19 ppmv versus 3.2 ± 1.3 ppmv; $p < 0.001$) but remained higher than the CO values in medical gases ($p < 0.001$). Conversely to *in vivo* values, FiO_2 modifications did not evoke any significant variation of eCO or eCH_4 from *ex vivo* lungs (Table 1). When the CO concentration in ambient air of the perfusion chamber was artificially increased or depleted by flushing the chamber with CO-enriched air (9 ppmv) or CO-depleted air (≤ 0.015 ppmv), eCO did not present any significant change (Fig. 3 and Table 2). Finally, hemoglobin concentration in the perfusion solution at steady state was 34 ± 5 μg/dL and the weight-to-dry ratio was 5.4 ± 0.7.

Table 1 Exhaled CO and CH_4 concentrations according to different inspired O_2 fractions.

	FiO2 21%	FiO2 50%	FiO2 100%
		In vivo measurements Lungs eCO (ppmv)	*Ex vivo* measurements Lungs eCO (ppmv)
Lungs eCO (ppmv)		3.2 ± 1.3	7.4 ± 2.3^a
Lungs eCH_4 (ppmv)		330 ± 80	280 ± 80
Lungs eCO (ppmv)	0.45 ± 0.30	0.45 ± 0.19	0.41 ± 0.26
Lungs eCH_4 (ppmv)	5.9 ± 0.6	6.2 ± 0.6	6.5 ± 0.6

Note: *In vivo* eCO measurements were not conducted at FiO_2 21% according to the Grenoble Animal Care Committee policy. Values are expressed as mean ± SD of values obtained from the five pigs. FiO_2: inspired O_2 fraction; ppmv: parts per million in volume.
[a]$p < 0.001$.

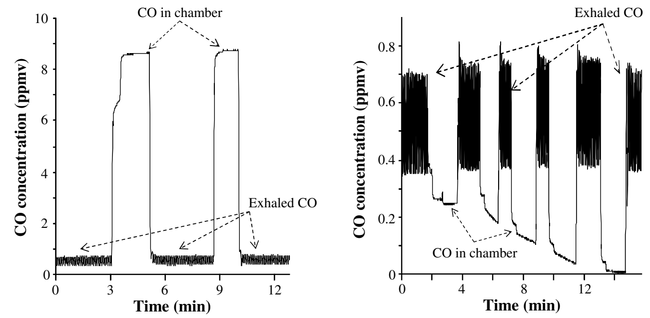

Fig. 3 Example of exhaled CO concentrations recorded in isolated lungs (*ex vivo* part of the study) submitted to high (top, 9 ppmv) or low (bottom, <0.015 ppmv) CO environments in the sealed chamber. Exhaled CO exhibits the respiratory pattern (resembling to noise on this scale, see zoomed view in Fig. 2) contrary to the chamber air. Peak expiratory exhaled CO remains stable while chamber air CO concentration is progressively modified.

3.3 *HO-1 Detection*

HO-1 transcript was detected in all *sus scrofa* lungs. Its levels were stable between the animals (mean delta $C_t = 6.5 \pm 0.6$) and no significant correlation was found between the eCO concentrations and HO-1 mRNA levels.

4 Discussion

Our experiments demonstrate that OF-CEAS allows accurate measurement of eCO from *ex vivo* lungs. Endogenous eCO is detected at a ppbv level and OF-CEAS provides a consistent real-time monitoring.

The sensitivity required to measure CO during EVLP avoids the use of commercial, hand-held CO sensors based on electrochemical measurements. These devices can be suitable for high (several ppmv) concentrations in breath, e.g., to detect smoker patients. With sensitivity of 1 ppmv, they are perfectly suited for the detection of air quality and CO poisoning. More complex commercial but still portable systems allow for real-time CO measurements with sensitivity lower than 1 ppmv but still inadequate for the application presented in this work (e.g., Logan

Table 2 Exhaled and hard shell chamber air CO concentrations in various CO environments in the sealed chamber.

	Ambient air	High CO	Low CO
Chamber air CO (ppmv)	0.29 ± 0.06	8.78 ± 0.05^a	$0.010 \pm 0.002^{a,b}$
Ex vivo lungs eCO (ppmv)	0.50 ± 0.30	0.45 ± 0.35	0.45 ± 0.32

Note: Values are expressed as mean ± SD derived from CO measurements of 1 min in ambient air or in five lungs. Various CO environments correspond to ambient air (baseline), high CO (pollution with 9 ppmv CO-mixed gas) and low CO (dilution with <0.015 ppmv CO-mixed gas).
[a]$p < 0.001$ compared to baseline.
[b]$p < 0.001$ compared to high CO environment.

Research, LR4000, 0.2 ppmv in 5 s). Even more sensitive instruments are based on gas chromatography or mass spectrometry, but they do not provide online real-time detection in medical environments because they are voluminous, heavy, and require sample collection.[33] For real-time measurements at sensitivity lower than 0.2 ppmv, very few instruments based on different laser spectroscopy techniques have been developed and applied for real-time CO quantification in breath.[5,34–36] As demonstrated in these works, in addition to provide a required sensitivity, laser spectroscopy is the sole method that enables response time faster than the respiratory cycle. However, the work reported in Ref. 34 is based on a CO laser and constitutes a bulky laboratory setup not adapted for *in-situ* measurements. The spectroscopic technique employed in Refs. 35 and 36 is not based on the use of a resonant cavity but on a multiplass cell, resulting in much larger sampling volume (respectively, 6 L and 0.5 L) than in OF-CEAS (18 mL). As a consequence, multipass cell-based technique cannot be used for experiments such as those reported in this paper that require online analysis without perturbing the ventilation line whose tidal volume is as small as 140 mL/ min. In addition, it is clear that measuring faster than a breathing cycle allows us to easily discriminate between the background CO levels present in the inhaled air and the endogenous CO levels present as an excess in the expired air. Without the fast response on a small flow, an average value can be obtained from which one has to subtract the background value which has then to be maintained constant during a whole measurement session. In our study, the challenge was to demonstrate that OF-CEAS combines all requirements for the real-time assessment of endogenous CO produced by *ex vivo* lung preparations (i.e., on a ppbv scale recorded faster than the respiratory cycle on a small gas sample and by a compact, robust device without calibration with certified gas mixtures). To our knowledge, OF-CEAS is the only technique that offers such capabilities at present.

Beyond demonstrating the accuracy of the OF-CEAS device during EVLP, our experiments prove that measured CO originates from the lungs. Indeed, eCO was higher than the CO in ambient air, both in the *in vivo* and *ex vivo* parts of our study. Furthermore, CO measurements revealed a ventilation pattern, and external air with high or low CO concentrations did not pollute eCO levels. Besides these basic observations, our results are also consistent with physiological mechanisms of CO production and excretion. eCO is highly variable, depending on CO concentration in inspired air, blood-transport, and lung-diffusing capacities.[37] For these reasons, our experiments were conducted at a very controlled steady state to avoid variation of CO production or lungs capacity. Under such experimental conditions, *in vivo* eCO more than doubled when ventilation was switched to 100% of FiO_2. This effect has already been described and might be the consequence of increased CO blood elimination caused by the elevation in O_2 arterial tension and O_2 hemoglobin affinity.[38] Conversely, we did not observe any significant change in eCO when FiO_2 was modified in the *ex vivo* part of the study. During procurement surgery, the lungs are rinsed with a preservation solution and very few blood cells remain in the pulmonary vasculature. Hematocrit is <1% in the reperfusion solution and the concentration of free hemoglobin in the reperfusion solution is very low. Therefore, the vast majority of gases are carried in their soluble form and carboxyhemoglobin is very unlikely to have biased our results. These particular experimental conditions could account for the lack of eCO variation under different FiO_2 values in EVLP preparation. Moreover, as no CO is stored in the perfusion circuit, real-time eCO variations may only reflect changes in lung CO production.[37] The variation by a factor of 10 between the *in vivo* and the EVLP procedures leads us to the same conclusion. In fact, this discrepancy is unlikely due to a decrease in HO substrate (i.e., heme wash out in the perfusate) as CO concentrations remained stable during the whole 45-min steady state. Furthermore, PaO_2/FiO_2 ratio did not significantly change between the different parts of this study. eCO differences between the *in vivo* and the EVLP procedure may, therefore, not be explained by a change in the alveolar ventilation, PaO_2, or lung-diffusing capacity. An endogenous CO production limited to the lungs is the most plausible hypothesis to explain this 1/10th ratio. Therefore, in this EVLP model, eCO concentrations may provide a good appraisal of endogenous CO production in lungs submitted to IRI because it is less influenced by common errors related to *in vivo* measurements.[39]

Under ischemia-reperfusion stress, reactive oxygen species, inflammatory mediators, and damage-associated molecular patterns culminate in the activation of several transcriptional factors which, in turn, promote HO-1 expression.[9] This increase in HO activity leads to an amplified CO production.[40] In several models of IRI or inflammation, HO expression seems to be strongly correlated to the extent of histological injuries.[14,41–45] As a consequence, eCO has been widely studied as a biomarker of infection disease, chronic inflammatory disease and critical illness. These studies usually demonstrate higher eCO levels in the disease group but fail to determine a threshold value for diagnosis or severity assessment.[46–49] We believe that the weak predictive value of eCO in such conditions is mainly the consequence of both the ubiquitous production of endogenous CO and the ability of HO-1 to be stimulated under multiple stresses. In this paper, we describe a new application of HO-1 that is unlikely to be bias by systemic CO production or heterogeneous HO-1 induction as the *ex vivo* lung model provides a unique CO source. eCO monitoring could, therefore, be valuable in EVLP procedures with respect to lung reconditioning and evaluation before transplantation.[21,27,28] EVLP is now largely implemented by lung transplantation teams, and studies show that this technique is reliable, even after prolonged ischemia.[28] However, lung acceptance criteria for transplantation after EVLP are quite basic and principally based on vascular resistance and gas exchange parameters.[22] eCO measurement during EVLP could be studied as a biomarker of IRI to help physicians to evaluate the lung damage and select acceptable organs. However, we were not able to find a correlation between eCO concentration and HO-1 expression level. We believe that this result can be explained by two factors. First, our aim was to validate eCO measurement during EVLP and we purposely tried to perform the experiments with a high reproducibility. Therefore, eCO concentrations did not vary significantly between lungs and this could explain at least in part the lack of correlation with HO-1 expression levels, which were comparable between all animals. Second, eCO may indirectly reflect the various mechanisms induced by ischemia-reperfusion such as neutrophilic lung inflammation. Thus, other specific investigations, submitting lungs to different levels of IRI, would be necessary to determine if eCO could serve as a useful marker of lung IRI during EVLP.

In conclusion, the primordial finding of this study is demonstrating the feasibility of eCO measurements during EVLP. We

further demonstrate that eCO measurement during EVLP is unlikely to be bias by systemic CO production or heterogeneous HO-1 induction. These results could pave the way for a new lung assessment technique and could therefore lead, in the medium term, to the definition of new lung acceptance criteria before transplantation. However, before concluding to this technique usefulness, correlation between eCO concentrations and IRI severity has to be demonstrated during EVLP procedures and especially under varying conditions of IRI.

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