

6-1-2000

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Kaur, Shubjeet; Cortiella, Joaquin; and Vacanti, Charles A., "Identifying a site for maximum delivery of oxygen to transplanted cells" (2000). *Anesthesiology Publications and Presentations*. 124.

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## Identifying a Site for Maximum Delivery of Oxygen to Transplanted Cells\*

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### ABSTRACT

For *in vivo* cell implantation techniques to be successful, the energy and metabolic substrate requirement of the cells being grown must be met. Certain cells with high-energy requirements (*e.g.*, hepatocytes, pancreatic island cells) experience a high degree of cell death after implantation due to a limited supply of oxygen. We proposed that the pleural cavity might be an oxygen-rich environment and hence an excellent site for cell implantation. To test the hypothesis that the delivery of oxygen to the pleural cavity is directly proportional to the inspired oxygen concentration we measured the  $pO_2$  of saline instilled in the pleural cavity as compared to that of the peritoneal cavity. We postulated that the physiologic basis for any difference was the result of direct diffusion of oxygen into the pleural space across the alveoli. The study was conducted on sheep ( $n = 6$ ), after induction of general anesthesia, in two phases, control and experimental. Saline was instilled into the peritoneal and pleural cavities via catheters. after equilibration at given  $FiO_2$ , the  $pO_2$  of the saline aspirated from the two cavities was compared. In the experimental group, animals were sacrificed (no circulation) and ventilated. The same sequence of steps as in the control phase were repeated. In the control group, the  $pO_2$  of saline aspirated from the pleural cavity approached the arterial  $pO_2$  at all  $FiO_2$  levels. The  $pO_2$  of the peritoneal saline aspirate fell over time. In the experimental phase (no circulation), the  $pO_2$  of the pleural cavity saline rose to  $>400$  mmHg. We conclude that this is a result of direct diffusion and is a potential source of unlimited oxygen supply not dependent on vascular supply.

### INTRODUCTION

As applications for engineered tissues develop, one barrier to cell survival is optimization of the supply of oxygen to cells immediately upon implantation. Some cell types have a very low metabolic rate and will survive in a relatively hypoxic environment until a vascular supply can be established. Other cell types with high-energy requirements (*e.g.*, hepatocyte, pancreatic islet cells) may have a better survival if they are immediately implanted into an oxygen-rich environment. These cell types may experience a high degree of cell death if this oxygen requirement is not met. We tested the hypothesis that the pleural cavity

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\*Presented in part at the Annual Meeting of the Association of University Anesthesiologists, Washington DC, April 17–20, 1997.

**TABLE 1. GAS ANALYSIS OF SALINE SAMPLES ASPIRATED FROM THE ABDOMINAL CAVITY AS A FUNCTION OF TIME (FiO<sub>2</sub> 0.70) IN CONTROL GROUP ANIMALS**

<i>Time</i>	<i>0</i> ( <i>mean ± SEM</i> )	<i>5 min</i> ( <i>mean ± SEM</i> )	<i>25 min</i> ( <i>mean ± SEM</i> )	<i>35 min</i> ( <i>mean ± SEM</i> )	<i>45 min</i> ( <i>mean ± SEM</i> )
pH	6.85 ± 0.070	6.8 ± 0.071	6.58 ± 0.047	6.51 ± 0.040	6.47 ± 0.028
pCO <sub>2</sub>	20.16 ± 4.96	22.33 ± 5.68	74.33 ± 3.78	118.66 ± 5.23	154.83 ± 3.4
pO <sub>2</sub>	102.83 ± 12.26	130.16 ± 17.74	9.66 ± 2.20	3.16 ± 0.401	2.66 ± 0.422

pO<sub>2</sub> and pCO<sub>2</sub> values reported are in mm of Hg.

may provide such an oxygen-rich milieu. Furthermore, it was previously believed that gas delivery to any body cavity is a function of its vascular supply.<sup>1,2</sup> We proposed that this is not the case, that instead oxygen diffuses directly through the lung tissue into the pleural space and its delivery is independent of vascular supply. If indeed this were true, the concentration of oxygen delivered to cell implants in the chest cavity (pleural space) could (1) be easily manipulated by adjusting the concentration of inspired oxygen, and (2) its supply would be virtually unlimited, similar to the environment in an incubator, with oxygen diffusing down a concentration gradient to the cells, as it is metabolized, rather than being dependent on blood supply that is disrupted and seriously impaired when the cells are implanted elsewhere in the body.

## MATERIAL AND METHODS

The study design was reviewed and approved by our Institutional Animal Studies Review Committee in accordance with APS/NIH guidelines. A total of six sheep (average weight 50 kg) were studied in two phases; a control group was studied as described below. The same animals were then allowed to reequilibrate and then sacrificed and studied in an identical manner as the experimental group. In the control group, general anesthesia was induced with intramuscular xylazine and ketamine and the animals paralyzed with pancuronium. After endotracheal intubation (9.0 i.d.), the animals were placed on controlled ventilation (Ohmeda 7000 Ventilator) and anesthesia was maintained with isoflurane. A 14-gauge catheter was placed in the femoral artery and baseline arterial blood gas analysis was performed (Instrumentation Laboratories 16/40 pH, Blood Gas, Electrolytes Analyzer, Lexington, MA) while the animals were ventilated with 100% oxygen. A 20-gauge catheter (PerFix Epidural Anesthesia Set, Braun Medical Inc., Bethlehem, PA) was then advanced into the peritoneal cavity just lateral to the umbilicus. A similar catheter was placed in the right pleural cavity in the fifth intercostal space in the midaxillary line. The blood gas analysis was repeated to document that there was no change from baseline reflecting lung injury secondary to pleural catheter placement. Animals were then ventilated with an FiO<sub>2</sub> of 0.25%, and 50 cc of normal saline was injected via the previously placed catheters into each cavity. After allowing 10 min for equilibration (based on prior preliminary experiment data, not reported), saline samples (0.5 to 1 cc) were aspirated from each body cavity as well as the arterial catheter for analysis of pH, pO<sub>2</sub>, and pCO<sub>2</sub>. The sheep were then ventilated with increasing inspired oxygen concentrations (0.25, 0.45, 0.70, 100%) in a stepwise manner (control phase),

**TABLE 2. GAS ANALYSIS OF SALINE ASPIRATED FROM PLEURAL SPACE AS A FUNCTION OF TIME (FiO<sub>2</sub> 0.70)—CONTROL PHASE**

<i>Time</i>	<i>0</i> ( <i>mean ± SEM</i> )	<i>5 min</i> ( <i>mean ± SEM</i> )	<i>25 min</i> ( <i>mean ± SEM</i> )	<i>35 min</i> ( <i>mean ± SEM</i> )	<i>45 min</i> ( <i>mean ± SEM</i> )
pH	6.9 ± 0.121	6.91 ± 0.034	6.88 ± 0.03	6.91 ± 0.047	6.91 ± 0.016
pCO <sub>2</sub>	19.25 ± 1.662	20 ± 4.139	35.66 ± 2.34	40.66 ± 0.98	43 ± 0.955
pO <sub>2</sub>	180.16 ± 17.98	157 ± 7.47	202 ± 2.37	349 ± 6.6	431.50 ± 3.46

pCO<sub>2</sub> and pO<sub>2</sub> values reported are in mm of Hg.

**TABLE 3. COMPARISON OF pO<sub>2</sub> (MMHG) OF PLEURAL VERSUS PERITONEAL ASPIRATES AT VARYING FiO<sub>2</sub> DURING CONTROL PHASE (VENTILATION PLUS CIRCULATION)**

FiO <sub>2</sub>	100% (mean ± SEM)	70% (mean ± SEM)	45% (mean ± SEM)	25% (mean ± SEM)
Pleural aspirate				
pO <sub>2</sub>	217.66 ± 20.36	209.00 ± 19.37	170.00 ± 13.69	188.60 ± 19.09
Peritoneal aspirate				
pO <sub>2</sub>	62.50 ± 7.93	57.20 ± 8.93	77.40 ± 19.10	68.00 ± 13.30
<i>p</i> Value	<0.001*	<0.001*	<i>p</i> = 0.004*	<0.001*

\* *p* value statistically significant.

allowing 5–10 min at each stage for reequilibration. The analysis of pH, pO<sub>2</sub>, and pCO<sub>2</sub> was repeated at each inspired oxygen concentration. At an FiO<sub>2</sub> of 70% the pH, pO<sub>2</sub>, and pCO<sub>2</sub> of both the pleural and peritoneal saline aspirate was analyzed at time points of 5, 25, 35, and 45 min. The animals were ventilated on room air with an FiO<sub>2</sub> of 0.25% until equilibration.

At this time the experimental phase of the study began. The animals were sacrificed by intravenous pentobarbital injection. Ventilation was continued with a stepwise increase in inspired oxygen concentration after cardiac arrest (no circulation). Saline samples were aspirated from each body cavity and analyzed for pH, pCO<sub>2</sub>, and pO<sub>2</sub> for this phase of the study.

A detailed statistical analysis and comparison using Student's *t*-test was performed between the mean value of the pO<sub>2</sub> of the saline aspirate from the pleural versus the peritoneal cavity at four different inspired oxygen concentrations (for 0.25%, 0.45%, 0.75%, 100%).

## RESULTS

The baseline values for arterial blood were a pH of 7.6 pCO<sub>2</sub> of 26 and a pO<sub>2</sub> of 148. For the pleural cavity saline aspirate, the pH was 7.15 pCO<sub>2</sub> 22.9 and pO<sub>2</sub> was 226. The peritoneal saline aspirate showed a pH 7.18, pCO<sub>2</sub> 18 pO<sub>2</sub> of 44.

In the control group (*i.e.*, animals with circulation and ventilation), the peritoneal saline aspirate analysis on an inspired FiO<sub>2</sub> of 70% was analyzed sequentially over a period of 45 min. The results are demonstrated in Table 1 and show that the pO<sub>2</sub> of the saline aspirated from the peritoneal cavity, during the control phase, decreased from a peak of 156 mmHg to 2 mmHg over 45 min. During the same time period of the control phase of the experiment, the pO<sub>2</sub> of saline aspirated from pleural space rose from 165 mmHg to 433 mmHg, as shown in Table 2. The pO<sub>2</sub> of the pleural cavity saline aspirate approximated or was greater than the arterial pO<sub>2</sub> at all FiO<sub>2</sub> levels. Comparison of the pO<sub>2</sub> of the saline aspirate from the pleural versus peritoneal cavity at various inspired oxygen concentrations during the control phase of the experiment is summarized in Table 3. The pleural saline pO<sub>2</sub> was consistently higher than the peritoneal aspirate and this difference was statistically significant at all levels of FiO<sub>2</sub> (*p* < 0.001).

In the experimental phase (ventilation without circulation) the pO<sub>2</sub> of the pleural aspirate, at an FiO<sub>2</sub> of .70% is shown (Table 4) in comparison to the pleural saline aspirate pO<sub>2</sub> at the same FiO<sub>2</sub> in the control phase (animal alive). The two values were comparable.

**TABLE 4. COMPARISON OF THE pO<sub>2</sub> OF PLEURAL CAVITY SALINE ASPIRATE AT AN FiO<sub>2</sub> OF 70% IN THE CONTROL VERSUS EXPERIMENTAL PHASE (*p* = 0.001)<sup>a</sup>**

	(mean ± SEM)
Control phase	209.00 ± 19.37
Experimental phase	367.00 ± 25.57

<sup>a</sup>Statistically significant.

## DISCUSSION

It has been previously believed that gas delivery to any body cavity is dependent on its vascular supply.<sup>1,2</sup> Our findings refute this long-held belief. Our results demonstrate that the partial pressure of oxygen in the pleural space is a function of the inspired oxygen concentration and independent of circulation (vascular delivery). The  $pO_2$  of the saline aspirated from the pleural cavity approached the arterial  $pO_2$  at all  $FiO_2$  levels. Increasing the inspired oxygen concentration can easily increase the concentration of oxygen in the pleural cavity. This can result in a virtually unlimited supply of oxygen in the pleural space. The pleural space may mimic the environment in an incubator and provide the ideal *in vivo* environment for growth of cells with a high metabolic need. This can result in a significantly higher survival ratio for cells implanted in this cavity.

In contrast to the pleural space, the  $pO_2$  of the saline aspirate from the peritoneal cavity fell over time (possibly secondary to local consumption or diffusion into the intestines). Furthermore, in the experimental phase, even after the animal was sacrificed (*i.e.*, no circulation/vascular delivery, but ongoing ventilation), the  $pO_2$  of the pleural cavity aspirate increased with increasing  $FiO_2$ . The high  $pO_2$  of the pleural cavity aspirate in the second phase of the experiment can only be accounted for by direct diffusion of oxygen from the alveoli to the pleural cavity across a concentration gradient.

In conclusion, our study has identified a high oxygen availability site for *in vivo* cell growth and established direct diffusion to be the mechanism responsible for transfer of oxygen from the alveoli to the pleural space. The size of the pleural space (in the clinical setting of an expanded lung) may be a limiting factor for the cell mass that may be grown in it. Further experiments are needed to define this issue. Cells may be grown in the pleural space for subsequent transplantation in other body sites or as a permanent implant, *e.g.*, hepatocytes in the right pleural space. This opens areas for further investigation and research.

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