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Acidic sweep gas with carbonic anhydrase coated hollow fiber membranes synergistically accelerates CO_2 removal from blood

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ABSTRACT

The use of extracorporeal carbon dioxide removal ($ECCO_2R$) is well established as a therapy for patients suffering from acute respiratory failure. Development of next generation low blood flow (<500 mL/min) $ECCO_2R$ devices necessitates more efficient gas exchange devices. Since over 90% of blood CO_2 is transported as bicarbonate (HCO_3^-), we previously reported development of a carbonic anhydrase (CA) immobilized bioactive hollow fiber membrane (HFM) which significantly accelerates CO_2 removal from blood in model gas exchange devices by converting bicarbonate to CO_2 directly at the HFM surface. This present study tested the hypothesis that dilute sulfur dioxide (SO_2) in oxygen sweep gas could further increase CO_2 removal by creating an acidic microenvironment within the diffusional boundary layer adjacent to the HFM surface, facilitating dehydration of bicarbonate to CO_2. CA was covalently immobilized onto poly (methyl pentene) (PMP) HFMs through glutaraldehyde activated chitosan spacers, potted in model gas exchange devices (0.0151 m^2) and tested for CO_2 removal rate with oxygen (O_2) sweep gas and a 2.2% SO_2 in oxygen sweep gas mixture. Using pure O_2 sweep gas, CA-PMP increased CO_2 removal by 31% (258 mL/min/m^2) compared to PMP (197 mL/min/m^2) ($P < 0.05$). Using 2.2% SO_2 acidic sweep gas increased PMP CO_2 removal by 17% (230 mL/min/m^2) compared to pure oxygen sweep gas control ($P < 0.05$); device outlet blood pH was 7.38 units. When employing both CA-PMP and 2.2% SO_2 sweep gas, CO_2 removal increased by 109% (411 mL/min/m^2) ($P < 0.05$); device outlet blood pH was 7.35 units. Dilute acidic sweep gas increases CO_2 removal, and when used in combination with bioactive CA-HFMs has a synergistic effect to more than double CO_2 removal while maintaining physiologic pH. Through these technologies the next generation of intravascular and paracorporeal respiratory assist devices can remove more CO_2 with smaller blood contacting surface areas.

Statement of Significance

A clinical need exists for more efficient respiratory assist devices which utilize low blood flow rates (<500 mL/min) to regulate blood CO_2 in patients suffering from acute lung failure. Literature has demonstrated approaches to chemically increase hollow fiber membrane (HFM) CO_2 removal efficiency by shifting equilibrium from bicarbonate to gaseous CO_2, through either a bioactive carbonic anhydrase enzyme coating or bulk blood acidification with lactic acid. In this study we demonstrate a novel approach to local blood acidification using an acidified sweep gas in combination with a bioactive coating to more than double CO_2 removal efficiency of HFM devices. To our knowledge, this is the first report assessing an acidic sweep gas to increase CO_2 removal from blood using HFM devices.

1. Introduction

In patients suffering from acute respiratory failure, extracorporeal carbon dioxide removal ($ECCO_2R$) is a powerful alternative or adjuvant therapy to avoid mechanical ventilation (MV) induced lung injury. High tidal volume MV can initiate and often exacerbate lung injury, increasing patient morbidity and mortality [1–3].

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Delivery of low tidal volumes and airway pressures mitigates these deleterious effects, as demonstrated by the acute respiratory distress syndrome (ARDS) Network trial where low tidal volume MV at 6 mL/kg vs. 12 mL/kg reduced lung injury and improved survival [4]. Recent data suggests even more ultra-protective MV settings may further improve outcomes, as alveolar over-distention is still observed at 6 mL/kg [3,5,6]. Clinicians are often unable to apply lung protective ventilation (LPV) strategies, reporting hypercapnia and acidosis as significant barriers to implementation [7]. In consequence, mortality rates remain between 40% and 45% for ARDS ICU patients [8]. For the chronic obstructive pulmonary disease (COPD) population, concerns over hypercapnia and severe acidosis can be mitigated through $ECCO_2R$, enabling LPV, weaning of patients off MV, or avoiding intubation altogether [9–12]. $ECCO_2R$ in combination with LPV has not seen widespread application as current devices require surgical placement of cannula 19 Fr or larger to facilitate blood flow rates up to 1 L/min or higher, in order to remove a significant fraction (50%) of total adult CO_2 production [6,13]. Large diameter arterial cannulation systems have shown complication rates as high as 24% comprising of vein tearing, limb ischemia, compartment syndrome and intracranial hemorrhage, in part due to their demand for approximately 25% of cardiac output [14,15]. A clinical need exists for low blood flow $ECCO_2R$ devices (<500 mL/min) that require less invasive cannulation and can regulate blood CO_2 independent of alveolar ventilation in patients suffering from acute lung failure [3,16,17].

A clinically desirable low flow $ECCO_2R$ device would encompass minimally invasive vascular access (11–15%Fr cannula) with low blood flow rates (200–500 mL/min) [6,18] and eliminate up to 100 mL/min of CO_2 to meet 50% of the metabolic needs of an adult patient [19]. Current devices including the Quadrox D (Maquet, Rastatt, Germany), Hilite 7000LT (Medos Medizintechnik AG, Stolberg, Germany) and Affinity NT (Medtronic, Eden Praire, USA) require an external blood pump, blood flow rates greater than 1%L/min and large surface areas greater than 1.3 m^2 [20]. The PALP (Maquet, Rastatt, Germany), iLA Active (Novalung, Baden-Württemberg, Germany) and Hemolung RAS® (ALung Technologies, Pittsburgh, USA) devices have taken steps towards low blood flow CO_2 removal, enabling partial CO_2 removal support at blood flow rates less than 1 L/min [21–23]. Achieving clinically significant CO_2 removal at blood flow rates less than 500 mL/min remains a challenge. New technologies such as active blood mixing within gas exchange fiber bundles have improved CO_2 removal efficiency at low blood flow rates [23–25], but gas transport in $ECCO_2R$ devices is ultimately limited by the blood CO_2 partial pressure (PCO_2) gradient across hollow fiber membranes (HFMs) [26]. Our lung tissues face the same diffusional challenges as HFMs, however they employ the enzyme carbonic anhydrase (CA) within red blood cells and on the endothelial surfaces of lung capillaries to accelerate diffusion by catalyzing the reversible dehydration of HCO_3^- (bicarbonate) to gaseous carbon dioxide: $CO_2 + H_2O \overset{CA}{\Longleftrightarrow} HCO_3^- + H^+$. We reported development of CA immobilized bioactive HFMs which converts bicarbonate to CO_2 directly at the HFM surface, restoring the trans-HFM CO_2 gradient as it is depleted in the diffusional boundary layer, and increasing CO_2 removal rates from blood by 36% in model gas exchange devices [27–29]. The main impediment to CO_2 removal by bioactive HFMs is diffusional boundary layer resistance which restricts transport of CO_2 and bicarbonate from the bulk fluid to the HFM surface, not the CA catalyzed conversion of bicarbonate to CO_2 [28]. Further improvements in the trans-HFM CO_2 gradient and exploitation of CA coating activity could be realized through blood acidification, chemically shifting equilibrium from bicarbonate to CO_2.

Blood acidification was first described by Snider et al. in 1987, in which infusion 2–8 mEq/min of lactic acid infusion was used to chemically increase trans-HFM CO_2 pressure gradients by acidifying the blood entering the $ECCO_2R$ devices, shifting equilibrium from bicarbonate to favor gaseous CO_2 and increasing CO_2 removal by 120–170%, however visible hemolysis was present [30]. More recently, Zanella et al. have refined this approach to mitigate hemolysis concerns [31–34]. The resulting acidified blood increased PCO_2 from 56 to 136 mmHg, decreased pH from 7.39 to 6.91, and increased CO_2 removal up to 70% [31]. The pH drop is similar to the pH values measured in human capillaries during heavy exercise [35]. Additionally, blood acidification offsets respiratory alkalosis as the blood leaving $ECCO_2R$ devices without acidification have an increased pH [31].

In this study we hypothesized local blood acidification at the HFM surface would increase CO_2 removal while minimizing perturbations in whole blood pH. Since HFM CO_2 removal is driven by trans-HFM pressure gradients, it should not be necessary to acidify the bulk fluid, but instead only the diffusional boundary layer adjacent to the HFM surface. While lactic acid infusions increase blood PCO_2, this approach acidifies the entire blood volume passing through the device. By mixing dilute concentrations of acidic sulfur dioxide (SO_2) gas into the oxygen (O_2) sweep gas, we created an acidic HFM boundary layer, synergistically working with CA-HFMs to increase trans-HFM CO_2 gradients and accelerate CO_2 blood removal while preserving whole blood pH. The acidic byproduct sulfite naturally occurs in mammalian systems, and has been shown safe in animal models at doses similar to those which would be seen in clinical use of an acidic sweep gas device [36].

2. Methods

2.1. Materials

Allylamine, Glutaraldehyde, chitosan (MW = 50–190 kD, based on viscosity) and glacial acetic acid were purchased from Sigma–Aldrich (St. Louis, MO). Commercial poly (methyl pentene) (PMP) hollow fiber membranes (HFMs) (Oxyplus™; OD: 380 μm, ID: 200 μm) were obtained from Membrana GmbH (Wuppertal, Germany). Bovine blood with Na-heparin anticoagulation (1:100 dilution of 1000 U/mL) for gas exchange was purchased from Lampire Biological Laboratories (Pipersville, PA). Purified recombinant human carbonic anhydrase II was provided by Dr. Silverman and Dr. McKenna from University of Florida (Gainesville, Fl) [37]. Sulfur dioxide was purchased from Matheson Gas (Pittsburgh, PA). Sulfite assay kit was obtained from R-Biopharm (Darmstadt, Germany). All other reagents were purchased from Sigma–Aldrich and were of analytical grade or purer.

2.2. PMP amination

Allylamine was polymerized onto unmodified PMP through plasma enhanced chemical vapor deposition (PECVD) with the PVA TePla Ion 40 system to create amine functional groups for covalent CA immobilization. PMP HFMs samples (238 cm^2 surface area) were placed on the second shelf from the top. The chamber was evacuated to a pressure of 50 mTorr and then allylamine was continuously introduced to the chamber through a mass flow controller at 180 mL/min for a final chamber pressure of 350 mTorr. Pulsed power was applied for 5 min at 300 W, with a 20% duty cycle and a 150 Hz frequency. After deposition the samples were immediately rinsed with 100 mM Phosphate Buffer (PB) at pH 8.5, 3 times for 15 min each. This treatment results in a 5.6 nmol/cm^2 amine density as quantified through colorimetric technique [38].

2.3. Carbonic anhydrase immobilization

Carbonic anhydrase (CA) was immobilized onto PMP HFMs (CA-PMP) by secondary amine linkage through reaction of surface

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