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Kinetics of CO₂ exchange with carbonic anhydrase immobilized on fiber membranes in artificial lungs

D. T. Arazawa^{1,2}, J. D. Kimmel^{1,2}, and W. J. Federspiel^{1,2,3,4}

¹McGowan Institute for Regenerative Medicine, University of Pittsburgh, 3025 East Carson Street, Pittsburgh, PA 15203, USA

²Department of Bioengineering, University of Pittsburgh, Pittsburgh, PA, USA

³Department of Chemical and Petroleum Engineering, University of Pittsburgh, Pittsburgh, PA, USA

⁴Department of Critical Care Medicine, University of Pittsburgh Medical Center, Pittsburgh, PA, USA

Abstract

Artificial lung devices comprised of hollow fiber membranes (HFMs) coated with the enzyme carbonic anhydrase (CA), accelerate removal of carbon dioxide (CO₂) from blood for the treatment of acute respiratory failure. While previous work demonstrated CA coatings increase HFM CO₂ removal by 115 % in phosphate buffered saline (PBS), testing in blood revealed a 36 % increase compared to unmodified HFMs. In this work, we sought to characterize the CO₂ mass transport processes within these biocatalytic devices which impede CA coating efficacy and develop approaches towards improving bioactive HFM efficiency. Aminated HFMs were sequentially reacted with glutaraldehyde (GA), chitosan, GA and afterwards incubated with a CA solution, covalently linking CA to the surface. Bioactive CA-HFMs were potted in model gas exchange devices (0.0119 m²) and tested for esterase activity and CO₂ removal under various flow rates with PBS, whole blood, and solutions containing individual blood components (plasma albumin, red blood cells or free carbonic anhydrase). Results demonstrated that increasing the immobilized enzyme activity did not significantly impact CO₂ removal rate, as the diffusional resistance from the liquid boundary layer is the primary impediment to CO₂ transport by both unmodified and bioactive HFMs under clinically relevant conditions. Furthermore, endogenous CA within red blood cells competes with HFM immobilized CA to increase CO₂ removal. Based on our findings, we propose a bicarbonate/CO₂ disequilibrium hypothesis to describe performance of CA-modified devices in both buffer and blood. Improvement in CO₂ removal rates using CA-modified devices in blood may be realized by maximizing bicarbonate/CO₂ disequilibrium at the fiber surface via strategies such as blood acidification and active mixing within the device.

Correspondence to: W. J. Federspiel.

Conflict of interest W. J. Federspiel has an equity interest in Alung Technologies which has licensed portions of the technology described in this manuscript. For all other authors, there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

1 Introduction

Removal of CO₂ from circulating blood using extracorporeal gas exchange devices is a promising treatment for acute respiratory failure [1–7]. Current respiratory assist devices used for extracorporeal carbon dioxide removal (ECCO₂R) can require blood flow rates in excess of 1 L/min to extract a clinically therapeutic 50 % of metabolic CO₂ production (~100 mL/min), necessitating larger cannula inserted by surgeons, rather than the intensivists typically treating these patients. This has been one reason ECCO₂R has seen limited widespread use in ICUs [1, 7]. New technologies that facilitate accelerated CO₂ removal may lead to the development of smaller, more efficient respiratory assist devices that can be implemented by intensivists and other nonsurgical clinicians. We recently developed strategies to covalently immobilize the enzyme carbonic anhydrase (CA) to the surface of hollow fiber membranes (HFMs), as a means to increase CO₂ removal efficiency in respiratory assist devices. CA plays a critical role in blood carbon dioxide transport by catalyzing the reaction $\text{CO}_2 + \text{H}_2\text{O} \xrightleftharpoons{\text{CA}} \text{HCO}_3^- + \text{H}^+$. Most of blood CO₂ (>90 %) is carried as bicarbonate (HCO₃⁻), which is dehydrated during respiration to gaseous CO₂ by CA within red blood cells (RBCs). Immobilizing CA on the surface of HFMs locally catalyzes dehydration of HCO₃⁻ to CO₂, increasing the CO₂ partial pressure (PCO₂) at the fiber surface and increasing CO₂ flux through the membrane. Previous studies demonstrated bioactive CA-HFMs increase CO₂ removal by 115 % in phosphate buffered saline (PBS) and 37 % in blood, compared to unmodified fibers [8, 9].

The difference in CA-HFM catalyzed CO₂ removal between PBS and blood is not well understood. Compared to our current 30–40 % increase in blood CO₂ removal, we anticipate at least 100 % increase will be necessary for the development of next-generation minimally invasive devices. Since both fluid systems operate under the same PCO₂ of 50 mmHg, other predominant blood factors, such as RBCs or plasma proteins, must limit the CA coating performance in whole blood. The presence of endogenous CA within RBCs may compete with immobilized CA, reducing the coating efficacy [10]. Additionally, adsorption of plasma proteins and platelets are well known contributors to thrombus formation on the surfaces of polymeric materials, decreasing transport across the fibers and performance of respiratory assist devices [11]. Our use of immobilized CA on the surface of blood contacting gas exchange membranes is a new development in the field. Examining the kinetics of CO₂ transport within CA-modified gas exchange devices in both PBS and blood is an important step towards elucidating strategies for improved CO₂ removal efficiency.

In this study we examined the mass transport phenomena governing CO₂ removal using bioactive CA-HFMs in gas exchange modules. The roles of immobilized CA activity, liquid flow rate and blood cells and proteins were clarified. Using either high activity human or low activity bovine enzyme sources, CA II was immobilized onto HFMs. The CA modified HFMs were fabricated into model gas exchange devices to assess CO₂ removal performance from fluids containing individual blood components, PBS, PBS + 5 % Albumin, PBS + CA, PBS + RBC and whole blood under a mass transfer environment similar to those of commercially available oxygenators. The results of this study will be used to develop

respiratory assist devices in which bioactive CA-HFMs can be employed and more substantially increase CO₂ exchange in blood.

2 Methods

2.1 Materials

Carbonic anhydrase II from bovine erythrocytes, glutaraldehyde (GA), chitosan (MW = 50–190 kD, based on viscosity) and glacial acetic acid were purchased from Sigma-Aldrich (St. Louis, MO). Purified recombinant human carbonic anhydrase II was provided by Dr. Silverman and Dr. McKenna from University of Florida (Gainesville, FL) [12]. Commercial microporous polypropylene HFMs (Celgard™; OD 300 μm, ID 240 μm, Membrana GmbH, Wuppertal, Germany) were obtained from ALung Technologies, Inc. (Pittsburgh, PA) with an aminated-siloxane coating. Bovine blood with Naheparin anticoagulation (1:100 dilution of 1000 U/mL) was purchased from Lampire Biological Laboratories (Pipersville, PA). All other reagents were purchased from Sigma-Aldrich and were of analytical grade or purer.

2.2 In vitro CO₂ exchange in scaled down modules of CA-HFM

CA immobilization onto HFMs was performed as previously detailed [9]. A scaled-down gas exchange module was fabricated and an in vitro recirculating test loop was used to assess CO₂ gas exchange rates using unmodified HFMs and CA-immobilized HFMs (Fig. 1) [9]. CO₂ removal was assessed with PBS, PBS + 5 % Albumin, PBS + CA, PBS + RBC and whole blood. For all testing fluids, the system was maintained at 37 °C, ~50 mmHg PCO₂, and liquid flow rate was 45, 90, or 200 mL/min. Under these conditions the HFM CO₂ removal efficiency (mL/min/m²) matches commercially available devices. The rate of CO₂ removal ($\dot{V}_{CO_2}$) for each model oxygenator device was calculated using the sweep gas flow rate (Q_{OUT}^{STP}) and CO₂ fraction (F_{CO_2}) exiting the scaled-down gas exchange module and then normalized to 50 mmHg to correct for small deviations in the inlet

PCO₂: $\dot{V}_{CO_2} = Q_{OUT}^{STP} F_{CO_2} \frac{50}{PCO_2}$. For each device the fluid inlet/outlet PCO₂ and resulting

F_{CO_2} were measured in triplicate. The $\dot{V}_{CO_2}$ for each model gas exchange device is reported as an average of these measurements. The difference in PCO₂ from the device inlet to the outlet (PCO₂ inlet–PCO₂ outlet) is reported as PCO₂ drop.

The testing fluids were created as described. PBS: A PBS solution was prepared with a final pH 7.4 and PCO₂ of 50 mmHg by diluting 780 mL PBS to 1 L with deionized water and adding 30 mMol sodium hydroxide. The basic PBS solution was introduced to the gas exchange loop and upon solvation of CO₂ gas, carbonic acid is formed, decreasing PBS pH to physiologic 7.4. PBS + 5 % albumin: PBS was prepared as described, and once PCO₂ equilibrated to 50 mmHg, lyophilized bovine serum albumin was dissolved to 5 % (w/v). PBS + carbonic anhydrase: PBS was prepared as described, and once PCO₂ equilibrated to 50 mmHg lyophilized bovine carbonic anhydrase II was added to 16 μM. This concentration was selected to mimic physiologic RBC CAII concentration [13]. PBS + RBC: RBCs were isolated from heparinized whole bovine blood and suspended in PBS. Whole bovine blood was centrifuged at 2500 RCFs for 10 min and the buffy coat discarded. Packed RBCs were rinsed in PBS under gentle shaking for 10 min, centrifuged again and repeated, for a total of

three wash steps. Packed RBCs were suspended in PBS to 35 % hematocrit, glucose was adjusted to 100–300 mg/dL prior to use by the addition of 5 % dextrose solution and allowed to incubate for 1 h [14]. Bovine Blood: Heparinized bovine blood was purchased from Lampire Biological Labs (Pipersville, PA), glucose was adjusted to 100–300 mg/dL by the addition of 5 % dextrose solution and hematocrit to 35 %, and allowed to incubate for 1 h [14].

2.3 Carbonic anhydrase esterase activity assay

Immobilized CA enzyme activity was measured spectrophotometrically by monitoring the hydrolysis of *p*-nitrophenyl acetate (*p*-NPA) to *p*-nitrophenol (*p*-NP) at 412 nm. CA-modified fibers were fabricated into model oxygenators as described in Sect. 2.2 and a recirculating loop of 15 mL, 100 mM phosphate buffer pH 7.5, 80 μ M *p*-NPA was setup to measure the esterase activity under 45, 90 and 200 mL/min flow rate. Absorbance measurements at 412 nm were recorded every minute over a 5 min period, and plotted as a function of time. One activity unit was defined as the amount of enzyme required to generate 1 μ mol pNP per minute.

2.4 Statistical analyses

All data are presented as a mean with standard deviation. Statistical significance between sample groups was determined using ANOVA followed by post hoc Tukey testing of specific differences. All other data was compared using a Student's *t* test assuming equal sample variance. Differences were considered statistically significant for $P < 0.05$.

3 Results

3.1 Immobilized CA-HFM activity: bovine versus human

Human and bovine CAII HFMs were characterized for both immobilized esterase activity (μ mol/min) and CO₂ removal rate (mL/min/m²) in PBS buffer. Human CA-HFMs demonstrated a fivefold increase in immobilized esterase activity compared to bovine CA-HFMs, 8.7 and 1.7 U respectively (Fig. 2). Flow induced shear under clinically relevant mass transport conditions did not affect immobilized CA esterase activity, as increasing fluid flow rate between 45 and 200 mL/min had negligible effects on immobilized CA activity. In contrast to the esterase activity assay, bovine and human CA devices demonstrated similar CO₂ removal rates. At 45 mL/min PBS flow rate, unmodified HFMs removed 85 mL/min/m² while bovine and human CA-HFMs removed 135 and 143 mL/min/m² respectively (Fig. 3). Human CA devices attained marginally higher (<10 %) removal rates over bovine CA devices with increasing PBS flow rate. This result indicates that the current level of immobilized bovine CA activity is not a limiting factor in the CO₂ removal rate of bioactive CA-HFMs. Increasing *p*-NPA activity from 1.8 (bovine) to 8.8 U (human) did not dramatically increase gas exchange.

3.2 Blood component effects on CA-HFM CO₂ removal rates

CO₂ removal rates were assessed on unmodified and human CA-HFMs in PBS, PBS + 5 % Albumin, PBS + CA, PBS + RBC, and whole blood under a mass transfer environment similar to those of commercially available oxygenators. Albumin solution had no negative

effects on CO₂ removal performance by unmodified or CA-HFMs. In PBS and PBS + 5 % albumin, unmodified HFMs removed 75 and 88 mL/min/m² respectively, while CA-HFMs removed 154 and 187 mL/min/m² respectively, for a 106 % (PBS) or 112 % (PBS + 5 % albumin) increase in CO₂ removal rate (Fig. 4). Albumin adsorbing onto HFMs did not impede CA coating performance. Conversely, solutions containing CA significantly decreased the enhancement of CA coatings over unmodified HFMs. Unmodified HFMs CO₂ removal increased from 75 in PBS to 172 mL/min/m² with PBS + CA, 236 mL/min/m² with PBS + RBC and 222 mL/min/m² with whole blood. Free CA increased unmodified HFM CO₂ removal rate, which limited immobilized CA contribution to CO₂ removal. CA-HFMs increased CO₂ removal over unmodified fibers by 7 % (PBS + CA), 27 % (PBS + RBC) and 37 % (whole blood). This substantial decrease in CA coating performance as compared to 106 % in PBS is attributed to competition between immobilized and free solution CA to catalyze bicarbonate dehydration to CO₂.

3.3 Mass transport limitations by CA-HFMs in blood CO₂ removal

Bioactive CA-HFM CO₂ removal rate and fluid PCO₂ were assessed in whole bovine blood with varying flow rates. Increasing fluid flow rate adversely affects CA-HFM performance, with a 37, 22 and 13 % increase in CO₂ removal at 45, 90 and 200 mL/min, respectively (Fig. 5). The difference in PCO₂ from device inlet to outlet (PCO₂ drop) decreased from 13.9 mmHg at 45 mL/min to 11.0 mmHg at 90 mL/min and 5.0 mmHg at 200 mL/min (Fig. 6) ($P < 0.01$). This PCO₂ drop behavior was similar between unmodified and CA-HFMs within each flow rate. Increasing fluid flow decreased CO₂ removal per unit volume passing through the scaled-down gas exchange module, and adversely affected CA coating contribution to total CO₂ removal rate. Increasing PCO₂ drop improves bioactive CA-HFMs performance.

4 Discussion

The goal of this study was to examine the kinetics of CO₂ transport within CA-modified gas exchange devices in PBS and blood, as a means to elucidate strategies for improved blood CO₂ removal using CA-HFM devices. We employed two forms of CA to empirically evaluate the kinetics and mass transport processes limiting HFM CO₂ removal in prototype gas exchange devices. The CO₂ removal performance by CA-HFMs is not influenced by immobilized CA activity or adsorption of blood plasma proteins (albumin). In blood, endogenous CA within RBCs participate along with surface immobilized CA to catalyze bicarbonate/CO₂ equilibrium, in effect throttling immobilized CA.

The mass transport processes for CO₂ removal by CA-modified gas exchange devices is comprised of four distinct physicochemical processes. Overall mass transport in the system is governed by the individual resistances of each process in series. The first resistance in HFM CO₂ removal is interconversion between bicarbonate and CO₂ within the bulk fluid, catalyzed by endogenous CA in blood or uncatalyzed in PBS. Second is diffusion of bicarbonate and CO₂ from the bulk fluid into the liquid diffusional boundary layer adjacent to the HFM surface. Third is the catalyzed dehydration of bicarbonate to CO₂ by immobilized CA on the HFM surface. Last is the diffusion of gaseous CO₂ through the

membrane and into the HFM sweep gas stream. Previous work demonstrated the rate limiting step in CO₂ removal for unmodified HFMs is diffusion of CO₂ through the liquid-side diffusional boundary layer, not diffusion through the HFM itself [15, 16]. In this application we must additionally consider the impact of immobilized CA catalytic rates, and equilibrium between bicarbonate/CO₂ on system-wide CO₂ transport.

The importance of immobilized CA activity on CO₂ removal was studied through human and bovine CA. Immobilized human CA has fivefold increase in *p*-NPA activity over bovine CA, likely due to greater purity of the recombinant human CA solution as compared to the lyophilized bovine CA containing contaminants and denatured proteins. In previous studies, increasing immobilized CA activity through more efficient conjugation strategies increased CO₂ removal rates [9]. We hypothesized higher activity human CA-HFMs would increase CO₂ removal over bovine CA-HFMs. In contrast to the *p*-NPA assay, CO₂ removal rates were comparable between human and bovine CA-HFMs (Fig. 3). Gas exchange was performed in PBS to avoid effects from protein adsorption on the fiber surface or endogenous CA. Chitosan immobilized CA resulted in sufficient activity such that increasing immobilized CA activity with human CA no longer increased CO₂ removal rate. This suggests diffusive resistance within the liquid boundary layer, as with unmodified HFMs, is the rate limiting step for CO₂ transport rather than immobilized enzyme activity. CA catalyzes bicarbonate/CO₂ and *p*-NPA/*p*-NP using the same active site, however the rate constant (*k*) is 1 million times greater for CO₂, $1 \times 10^6 \text{ s}^{-1}$ for CO₂ versus 1 s^{-1} for *p*-NPA [17, 18]. CA catalyzed conversion of bicarbonate/CO₂ is fast enough such that diffusion through the boundary layer dominates transport resistance [19, 20]. Decreasing boundary layer resistance by increasing fluid flow rate, increases delivery of *p*-NPA to the fiber surface, but does not increase *p*-NPA catalysis by immobilized CA (Fig. 2). Differences in immobilized CA activity are measurable with the *p*-NPA assay because CA *p*-NPA catalysis is the rate limiting resistance compared to the fluid boundary layer diffusional resistance. Conversely for bicarbonate or CO₂ as the enzyme substrate, the boundary layer resistance dominates as the rate limiting step over the CA CO₂ catalytic rate. Divergence in the human and bovine CA curves with increasing flow rate in Fig. 3 is interpreted as reduction in boundary layer thickness (resistance) with increasing flow rate. This hypothesis is supported by regression fits to the data resulting in exponents approaching 0.5, which is the theoretical relationship between boundary layer thickness and liquid flow rate [15]. Due to the extremely fast catalytic rate of CA (among the highest known) [17], strategies to improve CO₂ removal efficacy through increased CA loading or activity on the fiber surface are not productive avenues unless a corresponding decrease in diffusional boundary layer resistance can be achieved.

The performance of CA-HFMs were examined in whole blood at 45, 90 and 200 mL/min and compared with unmodified HFMs. Increasing blood flow rate increased CO₂ removal rate for unmodified and CA-HFMs, by decreasing the fluid boundary layer resistance. A decrease in CA coating enhancement from 37 to 13 % was observed with increasing blood flow rate from 45 to 200 mL/min (Fig. 5). Shear-induced enzyme activity loss as a consequence of increased liquid flow rate is a potential hypothesis. Data from *p*-NPA activity assay suggest fluid induced shear did not negatively impact CA under these flow rates (Fig. 2). Additionally, CO₂ removal at 45 mL/min is repeatable after exposure to 200

mL/min flow rate (data not shown), indicating permanent shear-induced effects are unlikely, although transient shear effects under clinically relevant conditions still need to be explored. While increasing blood flow rate increases total CO₂ removal, fluid residence time within the device decreases and consequently the CO₂ removal per fluid volume declines (Fig. 6). In PBS, HFMs remove CO₂ faster than bicarbonate can dehydrate to CO₂ (uncatalyzed), resulting in a pool of bicarbonate as PBS is depleted of gaseous CO₂. As a drop in bulk PCO₂ is observed, disequilibrium between bicarbonate/CO₂ is created. Approximately 10³ CO₂ molecules are removed per second by CA-HFMs at a liquid flow rate of 45 mL/min, while the uncatalyzed bicarbonate to CO₂ conversion rate is ~1 s⁻¹. Bioactive CA-HFMs facilitate the dehydration reaction to restore CO₂ pressure in the fluid boundary layer. In blood, endogenous CA within RBCs catalyzes bicarbonate/CO₂ conversion in the bulk fluid, but cannot maintain equilibrium within the fluid boundary layer, evidenced by the impact of CA coatings on HFM CO₂ removal. The fluid PCO₂ drop can be considered a metric of disequilibrium between bicarbonate and CO₂ where the higher the fluid flow rate, the smaller the PCO₂ drop, and the smaller the impact of bioactive CA coatings on HFM CO₂ removal.

We consistently observed a significant reduction in CA coating efficacy in blood compared to PBS. CO₂ removal by bioactive CA-HFMs was assessed in solutions containing individual blood components: plasma protein albumin, free CA and RBCs. Protein adsorption onto CA-HFMs did not decrease CO₂ removal by unmodified or CA-HFMs. In contrast, endogenous CA within circulating RBCs reduced enhancement of the immobilized CA coating on CO₂ removal. Blood maintains bicarbonate/CO₂ equilibrium more effectively than PBS and directly limits the CA-HFM CO₂ removal enhancement, evident by the 106 % increase in CO₂ removal (PBS) versus 37 % increase (blood). RBC CA solutions increased CO₂ removal rates for both unmodified and CA-immobilized devices compared to PBS solution (Fig. 4). Free CA spiked in PBS decreased CA coating efficacy to only 7 % improvement over non CA-HFM devices. Since CA-HFM performance increases when CA is localized to RBCs (7 % free CA vs. 27 % RBC CA), diffusion of bicarbonate/CO₂ across the RBC membrane potentially contributes to the resistance limiting bulk fluid equilibrium from restoring gaseous CO₂ as it is depleted by HFMs. Free CA spiked in PBS may localize closer to the HFM surface similar to immobilized CA, whereas RBCs tend to migrate away from stationary surfaces due to hydrodynamic forces, a phenomenon known as the Fahraeus effect for blood flow in small tubes [21].

Development of improved and more efficient respiratory assist devices will enable next generation, minimally invasive extracorporeal support of failing lungs at low blood flows. Carbonic anhydrase immobilized on HFMs is a novel approach to increase CO₂ removal rates, but the factors limiting the enhancement of CO₂ removal by CA-HFMs were not well understood. Our results demonstrate bioactive CA-HFMs operate under Le Chatelier's principle, catalyzing conversion of bicarbonate/CO₂ to preserve chemical equilibrium within the fluid boundary layer. In blood, endogenous CA within RBCs also catalyzes bicarbonate/CO₂ equilibrium, in effect throttling immobilized CA's contribution towards CO₂ removal. Mechanisms which reduce the diffusional boundary layer resistance and conditions which promote local bicarbonate/CO₂ disequilibrium, such as fluid longer transit times, will result in the largest contribution from the CA coating. Development is underway

on CO₂ removal devices which reduce the boundary layer resistance by increasing fluid velocity past HFMs through mechanical mixing independent of fluid flow rate through the device [22–24]. This approach increases delivery of bicarbonate/CO₂ to the HFM surface without decreasing blood transit time, which may take advantage of CA's rapid catalysis and boost CO₂ removal performance from increased substrate delivery. Active mixing and CA-HFMs can only restore equilibrium within the fluid boundary layer, but cannot augment fluid PCO₂ above the device inlet condition (around 50 mmHg). Since bicarbonate/CO₂ equilibrium is described by $\text{CO}_2 + \text{H}_2\text{O} \xrightleftharpoons{\text{CA}} \text{HCO}_3^- + \text{H}^+$, increasing the H⁺ concentration in the fluid boundary layer will create disequilibrium, forcing CA to catalyze dehydration of bicarbonate, increasing fluid PCO₂ beyond the inlet 50 mmHg, and driving further improvement in HFM CO₂ removal. By using an acidic gas within the oxygen sweep gas passed through the HFM lumens, an acid product can be introduced to the fluid boundary layer, creating local disequilibrium while minimizing acidification of the whole bulk fluid. In subsequent work we demonstrate dilute sulfur dioxide in oxygen sweep gas increases CO₂ removal, and when used in combination with bioactive CA-HFMs has a synergistic effect to more than double CO₂ removal while maintaining physiologic pH [25].

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Fig. 1.

Diagram of a model respiratory device and experimental setup for measuring CO₂ removal rates of unmodified and modified HFMs. Bovine blood or PBS solutions were perfused over the outside of the fibers while oxygen sweep gas was passed through the fiber lumens in the opposite direction. De-oxygenator employed the use of a heat exchanger to maintain blood temperature at 37 °C

Fig. 2.
Immobilized enzyme *p*-NPA activity is independent of flowrate at 45, 90 and 200 mL/min
(* $P > 0.90$)

Fig. 3.

CO₂ removal rates for gas exchanges devices in PBS modified with human or bovine CA, compared to unmodified HFMs. Human CA modified HFMs with 4× activity of bovine CA does not significantly increase CO₂ removal (* $P > 0.10$)

Fig. 4. CO₂ removal by unmodified and human CA immobilized HFMs in PBS, PBS + albumin, PBS + CA, PBS + RBC and bovine blood in a model respiratory assist device. In PBS and PBS + albumin, the modified fibers demonstrated a 106 and 112 % increase in CO₂ removal rate. In solutions containing carbonic anhydrase, PBS + CA, PBS + RBC and bovine blood, percentage increase in CO₂ removal rate decreases to 7, 27 and 37 % (**P*<0.05)

Fig. 5. CO₂ removal by unmodified and human CA immobilized HFMs in bovine blood at 45, 90 and 200 mL/ min. Increasing blood flow rate adversely affects the percentage increase by carbonic anhydrase coatings on HFMs (* $P < 0.05$)

Fig. 6. Drop in blood PCO₂ across testing module oxygenator, calculated from difference between inlet and outlet, decreases with increasing blood flow rate (* $P < 0.05$)