Towards Improved Artificial Lungs Through Biocatalysis

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Statement of Purpose: In this study, we have developed a "bioactive" hollow fiber membrane (HFM) that could significantly improve CO_2 removal in respiratory assist devices, which are being developed for acute therapy or bridge-to-lung transplant therapy. Carbonic anhydrase was covalently immobilized to the surface of conventional HFM, which by catalyzing the dehydration of bicarbonate in blood, may facilitate diffusion of CO_2 towards the fiber membranes, essentially mimicking the function of the enzyme on lung capillary surfaces. Specifically, we have: 1) examined the impact of enzyme attachment on the diffusional properties of the HFM and 2) assessed the extent to which CO_2 removal was improved using the bioactive HFM in a model respiratory assist device.

Methods: Carbonic anhydrase (CA) from bovine erythrocytes was purchased from Sigma-Aldrich (St. Louise, MO) and used without further purification. Poly(methyl pentene) HFMs (Oxyplus, Type PMP 90/200, OD: 380 μm, ID: 200 μm) were obtained from Membrana GmbH (Wuppertal, Germany).

CA-immobilized HFMs were prepared by initially modifying HFM with surface active hydroxyls via plasma deposition. The plasma modified HFMs were activated with cyanogen bromide and subsequently reacted with CA. The diffusional properties of the modified HFM were characterized via scanning electron microscopy and by measuring gas permeance. The extent of CA loading was determined by assaying esterase activity of the enzyme-modified HFM using the substrate *p*-nitrophenyl acetate. Rate of CO_2 exchange using the bioactive HFM was measured in a model respiratory assist device. The device was fabricated by inserting conventional or conventional or CA-immobilized HFM into a tubular module. Carrier gas (O_2) was passed through the fibers while sodium bicarbonate was circulated around the shell-side compartment of the module. The concentration of CO_2 in the gas effluent was measured potentiometrically using a CO_2 selective electrode (Analytical Sensors Instruments, Sugar Land, TX). In experiments employing free CA, the enzyme was added directly to the shell-side compartment of the device containing conventional HFM.

Results/Discussion: Gas permeance of the surface modified HFMs increased significantly with combinations of increased plasma discharge power and lengthened exposure times, which was a result of surface cracking as verified by SEM. However, when discharge power and exposure time were limited to 25 W and 30 s respectively, no change in gas permeance of modified HFM was observed. Using these conditions, CA was immobilized at up to 88 % of theoretical monlayer surface coverage based on enzyme activity. The effect of increased plasma discharge power and exposure time on enzyme loading was negligible while gas permeance studies showed enzyme attachment did not impede CO_2 or O_2 diffusion. When employed in a model respiratory assist device, the bioactive HFMs improved the rate of CO_2 removal by as much as 75 % from physiological bicarbonate solution (**Figure 1**). Similar rate enhancements were measured when free CA was added to the device containing conventional HFM as with equivalent amounts of immobilized esterase activity. Moreover, no CA leaching from modified HFMs upon extensive washing with buffer was detected.

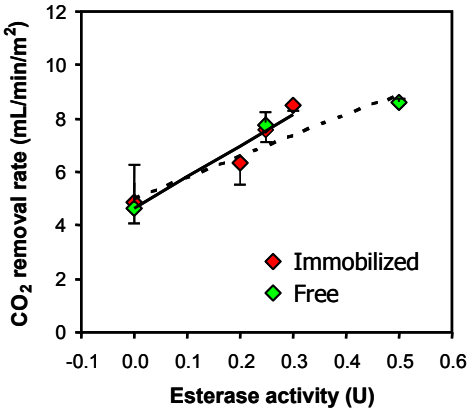

Figure 1. CO_2 exchange from circulating buffer in model respiratory assist device increases with immobilized CA activity

Conclusions: We have covalently immobilized CA to the surface of HFMs and demonstrated facilitated diffusion of CO2 using the bioactive HFMs in a model respiratory assist device. These findings represent a significant advancement towards the design of new respiratory assist devices with enhanced CO_2 elimination capability, requiring an effective membrane area substantially smaller than that in current conventional devices. Further studies are required to determine the degree to which immobilization of CA on HFMs facilitates CO_2 diffusion in whole blood and to assess the full potential of bioactive HFM to reduce the critical membrane area constraint of current respiratory assist devices.

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