A TWO COMPARTMENT MICRODIALYSIS MICRODEVICE FOR CONTINUOUS PROTEIN EXTRACTION FROM WHOLE BLOOD

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ABSTRACT

This work presents the design of a continuous microdialysis based blood protein extraction system to be used for clinical monitoring of inflammatory responses in patients undergoing cardiac surgery. The microdialysis system consists of a two compartment mass exchanger with two sets of polydimethylsiloxane (PDMS) microchannels separated by a porous polycarbonate membrane. Whole blood flows through channels on one side of the membrane (reservoir channels), while an isotonic perfusion fluid flows on the other side (perfusion channels). The membrane has a pore size of 400 nm, allowing free diffusion of small molecules and proteins across the membrane while excluding blood cells and platelets to obtain a clean, cell-free perfusate for analysis.

KEYWORDS: microdialysis, cardiopulmonary bypass, anaphylatoxin

INTRODUCTION

Cardiopulmonary bypass (CPB) can provoke a severe inflammatory response resulting in postoperative complications. The damaging effects of CPB are most attributed to prolonged interaction of blood with synthetic surfaces of the extracorporeal circuit. However, other factors, such as shear stress generated by blood pumps, tissue ischemia/reperfusion injury and the types of oxygenators used during the CPB procedure can play an important role in tissue injury and inflammatory response which may continue long after the discontinuation of CPB. Even though postoperative morbidity and mortality following CPB have been declining, they are still significant [1,-3]. Real-time dialysis and analysis of blood proteins will allow researchers to precisely pinpoint which parts of the CPB procedure are most critically associated with complement activation and inflammatory response in order to minimize its adverse effects. However, current technology is not sufficient to provide timely measurement of cellular activation. This work presents a new technology capable of continuous extraction of blood proteins from whole blood during extracorporeal circulation while maintaining high protein recovery for further analysis in patients undergoing cardiac surgical operations.

THEORY

The microdialysis device has been fabricated as a porous membrane sandwiched between and separating two compartment of the device (Figure 1). A semipermeable polycarbonate membrane was bonded to both side of the device using low viscosity two-part epoxy (EPO-TEK 301, Epotek, Billerica, MA).Whole blood flows through channels on one side of the membrane (reservoir channels), while an isotonic perfusion fluid flows on the other side (perfusion channels).The device was designed to balance transmembrane hydrodynamic pressure with osmotic pressure which prevents flow leakage from the perfusion channel into the reservoir channel.

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The device was designed with 32 parallel dialysis channels in both the reservoir and perfusion layers. This parallel design maximizes the transport area to allow a sufficient perfusion flow rate so that ~50-100µl of fluid could be collected at the device outlet (minimum volume required for immunoassays) in a reasonable period of time while maintaining a high recovery of protein.

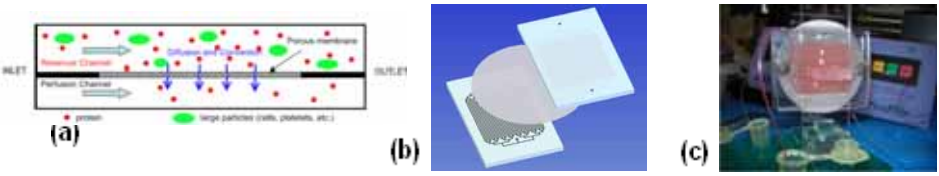

Figure 1: (a) Schematic of the two compartment microdialysis device. The top compartment is a reservoir channel where blood flows from the CPB circuit. The bottom channel is a perfusion channel where proteins diffuse from the reservoir channels into the perfusion fluid. (b)Microdialysis device including the two PDMS microchannels with a thin porous membrane sandwiched in between (C) Photo of the device being perfused from the CPB machine.

EXPERIMENTAL

In order to test device performance an experiment was performed using heparinized human blood within an in-vitro model normothermic cardiopulmonary bypass (CPB) circulation loop (Figure 2a) commonly used during cardiac surgeries. The CPB circulation loop was primed with 500 ml of heparinized blood, hemodiluted to 26% Hct in lactated Ringer's solution and circulated at a rate of 500 ml/min at an arterial circuit pressure of 100 mmHg, typical of neonatal surgical conditions. A small portion of the blood is redirected from the arterial port of the membrane oxygenator through the reservoir channels at a flow rate of ~40 µl /min, driven by pressure from the circulation circuit. The perfusion channel was infused at 4 µl/min with lactated Ringer's solution using a syringe pump (Figure 2b). The fluid fractions from both device outlets of the reservoir and perfusion channels were collected over a period of 15 minutes for a total circulation time of 2 hours. 1 ml discrete blood samples were collected from the arterial port of the membrane oxygenator as a control sample. Samples were analyzed for complement C3a, C4a and C5a concentrations using a commercially available anaphylatoxin cytometric bead kit (BD Biosciences, San Jose, CA, USA).

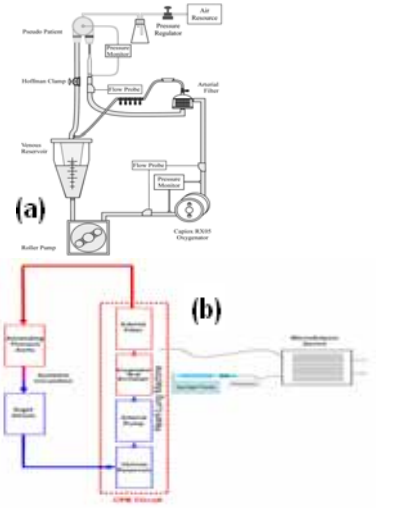

Figure 2 a): A schematic of the extracorpeal circuit. The blood from the blood reservoir, which represents the pseudo-patient with the systemic circulation resistance modelled by the tightening of a Hoffman clamp, is pumped across a heat exchanger to alter the temperature of the blood. Thereafter, the blood circulates across an oxygenator, where the blood is oxygenated. The oxygen rich blood then flows over an arterial filter that serves as filter and bubble trap. From here a small portion of the blood is redirected to the microdialysis device, while the rest of the blood is returned to the circuit. (b) A Schematic of CPB circuit and device connection.

RESULTS AND DISCUSSION

Figure 3 presents experimental results showing the ability of the microdialysis device to recover a high percentage of protein and track the complement concentrations within the circulating blood. Figure 3(a) shows complements C3a, C4a, and C5a relative recoveries. Figure 3(b) demonstrates temporal tracking by showing relative recovery of C4a versus circulation time for both the perfusion and reservoir outlets.

$$\text{Relative Recovery} = \frac{\text{(Perf or Rsrv Outlet Conc.)}}{\text{Control Concentration}}$$

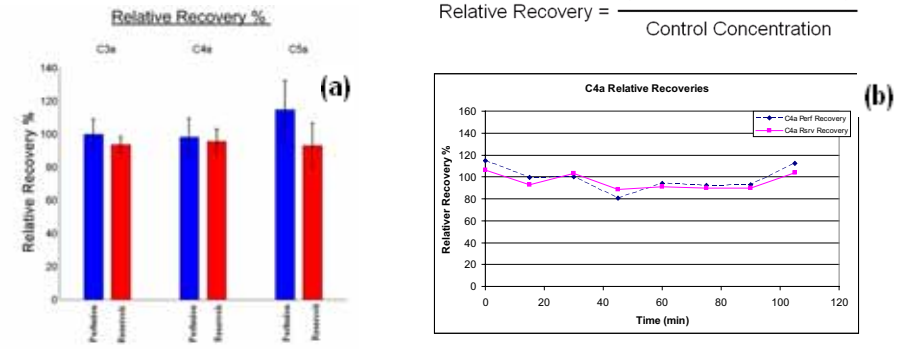

Figure 3: (a) Relative recovery of C3a, C4a and C5a, averaged over time, from the Perfusion and Reservoir Outlets. (b) Relative Recovery of C4a versus time for the Perfusion and Reservoir outlets.

CONCLUSIONS

These results demonstrate the ability of the microdialysis system described here to recover a high percentage of protein and track the complement concentrations within the circulated blood. Ultimately, this device will be modified to use a smaller number of microchannels at a lower flow rate with the outlet of the perfusion channels fed into a microimmunoassay module to continuously track changing inflammatory protein markers during cardiac surgery [4].

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