A MEMS-Based Renal Replacement System

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ABSTRACT

Over the last decade, medicine and the biological sciences have benefited from advances in microfabrication technologies and microdevices originally developed for consumer and industrial electronics, automobiles and aerospace, and national defense. Real-time miniaturized diagnostic tests, implantable drug delivery and minimally invasive imaging technologies are on the verge of revolutionizing the delivery of health care. Beyond these products lies the field of regenerative medicine, a merger of cell biology, computational biology, microfabrication and biomaterials, aimed at replacing the function of failing tissues and/or organs in patients with end stage organ failure. One of the most critical applications is in the field of renal dialysis, in which waste products in the blood are filtered in an intermittent, invasive and costly manner, with very poor patient outcomes. It is the aim of this work to develop a minimally invasive, continuous hemodialysis capability that utilizes a combination of advances in computational fluid dynamics, MEMS fabrication and biomaterials. Here, early ultrafiltration results are reported, using a microfabricated biopolymer blood processing unit, designed, built and tested in a model system containing a single vascular and dialysate layer.

BACKGROUND

End-Stage Renal Disease (ESRD) is a significant cause of morbidity and mortality in the United States, with 72,000 ESRD deaths reported annually. Standard clinical care for most of the 250,000 renal failure patients is three, four-hour hemodialysis sessions per week, costing nearly \$12 billion each year. However, these treatments only provide intermittent filtration and reabsorptive function, and patients are at higher risk for further complications. Moreover, life expectancy drops to less than 20% for patients on renal dialysis treatments for periods of five years or more. Recent advances have been reported in bioartificial kidney technology [1], nanofabricated ultrafiltration membranes [2], and MEMS-based micro-degassing devices for dialyzers [3]. In this work we report early design, fabrication and ultrafiltration results for a novel MEMS-based system for renal replacement that incorporates fractal microvascular network designs and micromolded flow chambers. This microfabricated device could ultimately provide a smaller, less invasive and less expensive therapy for ESRD. Preliminary studies show that the high surface area and precision of this MEMS-based hemofiltration system offers a significant improvement in the clearance efficiency of uremic wastes over current hemodialysis technologies. Additionally, the miniature aspect of this system enables it to be portable and potentially wearable. Most importantly, however, it will provide for *continuous* renal replacement therapy.

DESIGN AND ANALYTICAL APPROACH

The kidney is essentially a microfluidic device that processes and removes wastes while maintaining the proper balance of electrolytes including sodium, potassium, calcium and magnesium, as well as the retention of sugars and blood proteins such as albumin. Consequently, hemodialysis requires high rates of blood filtration and well-controlled processing of the blood in order to precisely regulate critical levels of electrolytes, sugars and other blood constituents. Dialysis membranes serve as the engine for hemodialysis, filtering out large molecules, which must remain in the bloodstream while removing smaller molecules such as urea and creatinine and routing them to the excretory system. Because electrolytes and sugars are also filtered from the blood, they must be reabsorbed into the bloodstream rapidly and in a highly regulated fashion. Renal proximal tubular cells, endothelial cells and other cells work in concert to provide this exquisite regulation of the full spectrum of blood constituents processed by the kidneys. For this early demonstration MEMS ultrafiltration device, however, cells have not been incorporated into the construct, since the focus has been the development of an acellular construct capable of separating blood components by size at high densities and with suitably biocompatible and hemocompatible materials.

Development and testing of a finite element based algorithm for simulation of intra- and extra-vascular transport within the kidney is critical. The governing partial differential equation for mass transport is as in Eqn. (1):

$$\frac{\partial c}{\partial t} + \vec{V} \cdot \nabla c = D \nabla^2 c \quad (1)$$

Here, $c(x,y,z,t)$ is concentration of the transported species e.g. ions (sodium, potassium, magnesium, phosphorous, calcium), blood proteins (albumin), D is the diffusion coefficient for these species, and $\vec{V}$ is the time and space dependent velocity field. The mass transfer in the intravascular and extravascular regions of the device is expected to be characterized as advective and diffusive, respectively. The advective transport will be treated using a characteristic Galerkin algorithm, while the diffusive region will be solved using standard Galerkin methods. This novel mass transfer algorithm will be used to evaluate the mass transport and filtration efficiency in the device.

Design of the vascular portion of this device is accomplished through the generation of a model network that mimics the requisite physiological features of the renal microcirculation. The computational fluid dynamic model utilized to generate this design has been described previously [4]; it is a fractal-based approach that produces ordered structures amenable to assembly by photomasking and microfabrication techniques. Among the physiological parameters most critical to successful operation of

the device are the blood flow rates, pressure losses, and the distribution of hematocrit (red blood cells) within the network. In Figure 1, the distribution of hematocrit across one such model network is illustrated, showing regulation within a narrow range.

Figure 1. Distribution of hematocrit across a model microvascular network, illustrating the ability of the model to provide for uniform spreading of mixed-phase flow within a network.

The unit cell for this hemodialysis approach is a bilayer device with lateral flows of blood and waste in a countercurrent fashion, separated by a thin ultrafiltration membrane. Each bilayer device contains a planar network of blood-carrying vessels and a compartment for waste (dialysate), sandwiched together with an intervening ultrafiltration membrane (Figure 2.) Replacement of current dialysis therapy in a wearable device will require a microfabricated device with large filtration capacity. Calculations show that a device of approximately 100 layers is necessary to provide sufficient blood processing capacity on a wearable, continuous device. Thus, current designs that are produced on a 100 mm diameter wafer platform, will be vertically integrated to produce a 100-layer device by stacking individual bilayer devices together. Experiments reported here are based on single bilayer devices. However, current efforts are aimed at scaling the system to 35 – 100 layers for large-scale *in vitro* and *ex vivo* studies.

Figure 2. Experimental design for bilayer ultrafiltration device.

EXPERIMENTAL DETAILS

The computational algorithm described above was developed to generate a fractal microvascular network that mimics proper anatomical and physiological conditions for flow and mass transport in the device (Figure 3.) This algorithm has been used to design vascular compartments for vital organ tissue engineering, in which the density of small blood vessels must be maximized to provide sufficient oxygenation of growing tissue. In the current application, these algorithms remain useful because the ultrafiltration device must process large volumes of blood rapidly, requiring a very high density of blood vessels and maximal surface area between the vascular network and the dialysate network. No such algorithms were used to design the dialysate layer, which carries waste blood products back out of the device. Consequently, for the first generation system, the dialysate layer has been represented by a simple microfluidic compartment rather than a network.

Figure 3. Design process for generation of microvascular networks, with capillary beds (right inset) arranged in rectangular arrays in each device layer. Bottom photograph: Replica-molded PDMS layer cast in the inverse image of the designs shown.

Based on these algorithms, bilayer devices have been fabricated using silicon micromachining and polymer replica molding processes [5-6]. Vascular and dialysate layer designs were generated using L-Edit (Tanner Research), and produced as transparencies on high-resolution 5080 dpi printers (PageWorks). Next, transparency masks were used to pattern silicon wafers, which were then etched to a depth of 35 microns using an isotropic silicon etching recipe in a standard plasma etcher (STS). Passivation layers were deposited in a deep reactive ion etcher (STS) to promote release of replica-molded polymer films. Thin PolyDiMethylSiloxane (PDMS) films were repeatedly produced using solvent casting against the silicon wafer masters. Thicknesses of PDMS films as low as 100 microns were routinely produced from these molds, consistent with high packing densities for multilayer devices. For the single bilayer device demonstrations, however, PDMS thicknesses of ca. 2 mm were targeted for ease of handling during flow studies.

For the early ultrafiltration experiments in acellular constructs, properties of the membrane drive the performance of the device. Membrane materials such as polysulfone and polyethersulfone (PES) are commonly used as dialyzer membranes, because they are proven to be capable of effective ultrafiltration while possessing outstanding hemocompatibility with patients' blood. Furthermore, these membranes are preferred over other membrane materials for their uniform pore size control,

durability, and ability to be chemically modified. The hemodialysis system design for this device consists of a PDMS structure with membranes interspersed between alternating layers of the vascular and dialysate bed. Since the incorporation of PES membranes into MEMS-based polymeric constructs has required substantial process development and is not yet completed, initial device fabrication and testing has incorporated polycarbonate (PC) membranes (Millipore), which are more amenable to current fabrication processes. Moreover, PC membranes have similar ultrafiltration properties to PES membranes; thus, they were used as an alternative initial model. However, PES membranes will be used in these devices once the process development is completed. Multilayer microfluidic constructs with PC and PDMS membranes have been reported elsewhere [7-9]. In this case, the PC membrane is readily incorporated through the use of a thin adhesive layers, which provides adequate connectivity between layers without adversely affecting the porosity of the interfacial layer.

To study the ultrafiltration capabilities of our devices, we examined clearances of urea and creatinine from the vascular stream into the dialysate stream at varying flow rates under single-pass countercurrent conditions. Fluid samples from each flow stream were taken at various time points up to five hours and analyzed for final concentration levels. These concentration values were used to calculate individual solute clearances. Finally, clearance values for urea and creatinine were normalized with respect to current hemodialysis parameters (surface area and flow rate) and compared for overall efficiency.

RESULTS

Clearances (K_i in ml/hr) of urea and creatinine from a vascular stream into a dialysate/parenchymal stream were determined at varying flow rates under *single-pass countercurrent* conditions. For each ultrafiltration experiment performed, the vascular fluid consisted of ultra-pure water and dissolved urea and creatinine each at a concentration of 200 mg/dl, while the dialysate fluid consisted of only ultra-pure water initially. Thus, the urea and creatinine solutions are pumped through the vascular layer containing the microvasculature and the ultra-pure water/"dialysate" is pumped through the open dialysate chamber. Three separate flow-rate ratios of vascular flow (Q_B) to dialysate flow (Q_D) were examined:

1. $\approx 2:1$ ($Q_B:Q_D$) at 0.9 and 2.0 ml/hr respectively
2. 1:1 ($Q_B:Q_D$) with both fluids at 1.0 ml/hr
3. 1:1 ($Q_B:Q_D$) with both fluids at 2.0 ml/hr

Each experiment was performed in an incubator at 37°C and a syringe pump was used to administer each fluid into its respective layer. Samples from each flow stream were taken at various time points, for up to five hours, and analyzed for final concentration levels. Concentrations of urea and creatinine from each fluid stream were tracked versus time; each showed slightly different trends with respect to the dialysate-side clearance values. However, both sets of data indicate that an approximate 2:1 vascular:dialysate flow rate ratio – with $Q_B = 0.9$ ml/hr and $Q_D = 2.0$ ml/hr – is the optimal ratio for the clearance of both solutes. This corresponds directly to the ratio used in conventional hemodialysis. A higher concentration gradient of solute is established between the vascular and dialysate networks when the vascular fluid is allowed to remain in the device longer, relative to the dialysate. The equation used to calculate dialysate-side clearance is:

$$K_D = Q_{D_o} * (C_{D_o} / C_{B_i}) \quad (2)$$

where K_D is the dialysate-side clearance, Q_{D_o} is the flow rate of the dialysate at the outlet, C_{D_o} is the solute concentration in the dialysate outlet, and C_{B_i} is the solute concentration at the vascular inlet. Finally, the calculated clearance values for urea and creatinine obtained from our experiments were normalized with respect to current hemodialysis parameters from a Preset Multiflow M-60 dialyzer, including overall total surface area for filtration (6.0 m²) and flow rates used (500-2500 ml/hr). Our normalized values for urea and creatinine clearance were then compared with published clearance values for overall efficiency [10] (Brunet *et al.*) The results of this comparison are summarized in Figures 4a and 4b. This normalized data is extremely useful because it enables us to determine the maximal solute clearances our devices can achieve in accordance with the minimal total surface area and flow rates that are needed for a fully functional, continuous, and wearable renal replacement device based on the number of 'stacked' layers of such a device.

Figure 4a. Experimental urea clearance at various flow rate conditions: Comparison with published data (Q_D and Q_B denote dialysate and blood flow rates, respectively, K_i is the solute clearance, and A is the total surface area for mass transport).

Figure 4b. Experimental creatinine clearance at various flow rate conditions using the same nomenclature as in Figure 4a.

Figure 5. Diffusion study conducted using PES membrane.

These early results, obtained in a device constructed using a PC membrane, indicate that the filtration efficiency in the microfabricated device is quite high. However, the PC membrane does not offer optimal ultrafiltration performance with regard to hemocompatibility and molecular selectivity. Current efforts are focused on the construction and testing of microfabricated devices with a PES membrane for ultrafiltration. Initial diffusion studies with these membranes indicate that solute concentrations behave appropriately. Furthermore, PES membranes are invariant to surface treatments applied to the membrane for bonding purposes. Figure 5 illustrates the results of one such diffusion study, in which untreated and treated PES membranes (GE Osmonics) exhibited excellent urea filtration efficiency. Untreated PES membranes denote those obtained directly from the vendor, without any surface preparation aimed at promoting adhesion to PDMS. However, the surface chemistry of PES is such that weak bonding to PDMS is expected, and this has been observed in preliminary device assembly experiments. Therefore, PES membranes have been treated with various processing recipes designed to promote strong, permanent surface adhesion to PDMS. The PES membrane treatments in these cases included oxygen plasma exposure and various related processes.

CONCLUSIONS AND FUTURE OUTLOOK

Fractal microvascular networks designed using our computational models exhibit appropriate anatomical and physiological conditions, including uniform flow and hematocrit distribution, optimized flow resistance, and maximal surface area for exchange of gases, nutrients, and wastes. Microfabricated bilayer devices built based on these microvascular designs have been assembled using polycarbonate nanoporous membranes as a model system for a hemodialysis unit cell. Initial results of our ultrafiltration experiments show that when normalized and compared to conventional hemodialysis parameters, our bilayer devices are roughly twice as effective at removing uremic solutes from incoming vascular flow under optimized flow conditions.

Therefore, current *in vitro* data supports our first goal of creating an ultrafiltration unit capable of efficiently filtering uremic metabolites from incoming vascular flow. Next, we will incorporate renal proximal tubular cells into these MEMS-based

devices to examine the re-adsorption of electrolytes, a critical hemostatic function not performed by standard hemodialysis treatment. Our microfabricated renal replacement system promises dramatic improvements over current hemodialysis technologies, including the potential for our system to be wearable and continuous.

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