ENGINEERING A BIOARTIFICIAL LIVER DEVICE

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Abstract

Fulminant hepatic failure is a clinical syndrome associated with a high mortality rate. Orthotopic liver transplantation is the only clinically proven effective treatment for patients with end-stage liver disease who do not respond to medical management. A major limitation of this treatment modality is the scarcity of donor organs available, resulting in patients dying while waiting for a donor liver. An extracorporeal bioartificial liver (BAL) device containing viable hepatocytes has the potential to provide temporary hepatic support to liver failure patients, serving as a bridge to transplantation while awaiting a suitable donor. In some patients, providing temporary hepatic support may be sufficient to allow adequate regeneration of the host liver, thereby eliminating the need for a liver transplant. Although the BAL device is a promising technology for the treatment of liver failure, there are several technical challenges that must be overcome in order to develop systems with sufficient processing capacity and of manageable size. In this overview, the authors describe the critical issues involved in developing a BAL device. They also discuss their experiences in hepatocyte culture optimization within the context of a microchannel flat-plate BAL device.

NOMENCLATURE

Symbol	Description	Unit
H or h	Channel height	μm
C_o	Inlet oxygen concentration	nmole/cm^3
Pe	Peclèt number	No Unit
Q	Volumetric flow rate	mL/min
W	Channel width	cm
D	Oxygen diffusion coefficient	cm^2/sec
γ	Cell seeding density	cells/cm^2
Da	Damköhler number	No Unit
V_{max}	Oxygen Uptake Rate	nmole/s/10^6 cells
τ	Shear stress	dynes/cm^2

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1. INTRODUCTION

The liver is the largest solid organ in the body and performs numerous functions which are vitally important to maintaining metabolic homeostasis. These functions include synthesis of serum proteins, regulation of nutrients, production of bile, and metabolism and conjugation of compounds for excretion in the bile or urine. Hepatocytes, which are the predominant cell type within the liver, account for two-thirds of the liver mass. The liver is normally able to regenerate after acute injury and regain its function under appropriate physiological stimuli [3]. However, liver failure occurs when the normal regenerative process is compromised and the residual functional capacity of the damaged liver is unable to sustain life.

Over 30,000 patients die annually in the United States from liver disease. The two principal causes of liver failure are cirrhosis and fulminant hepatic failure. Liver cirrhosis, whose etiology includes alcoholism and chronic hepatitis, is an irreversible process that occurs

when fibrotic tissue replaces normal liver tissue as a result of chronic injury. Fulminant hepatic failure is a clinical syndrome defined by impaired mental and neuromuscular function whose etiology includes chemical and viral hepatitis. It occurs as a result of massive hepatocyte necrosis and is the most severe manifestation of end-stage liver disease with mortality rates greater then 80%. Although there is currently no cure for liver failure, orthotopic liver transplantation is the only clinically proven effective treatment for patients with end-stage liver disease [4]. In the year 2003, there were 5670 liver transplants performed in the United States and 1829 patients died while waiting for a liver transplant (Based on UNOS OPTN data as of January 28, 2005). As can be seen from these statistics, a major limitation of this treatment is the scarcity of donor organs which results in patients dying while on the waiting list. There are currently over 17,500 patients on the waiting list to receive a donor liver.

In this article, we give a succinct overview of approaches to liver function replacement, addressing the critical issues in bioartificial liver (BAL) development, and summarize our experience using a microchannel flat-plate BAL device with an internal membrane oxygenator.

2. REPLACING LIVER FUNCTIONS

Over the past four decades, many attempts have been made to develop artificial liver systems to support patients with liver failure. The goal of these systems is to provide temporary hepatic support for patients with fulminant hepatic failure who are awaiting orthotopic liver transplantation. Since the liver has tremendous regenerative capacity, some liver failure patients may spontaneously recover if provided temporary hepatic support, thereby averting a liver transplant and the associated life-long immunosuppressive therapy. Duplicating the liver's complex metabolic functions that are essential for survival has been a significant challenge. There are two general categories of artificial liver devices that have been developed, nonbiological and biological. Nonbiological methods, including hemodialysis and hemoperfusion, have had minimal patient survival benefit due to their inability to replace synthetic and metabolic functions [5]. These results demonstrated the inability of treating liver failure using solely mechanical, nonbiological-based processes. Biological-based approaches such as cross-circulation, extracorporeal liver perfusion with crosshemodialysis, and liver tissue hemoperfusion were all shown to provide limited functional support on a short-term basis. The problems associated with these approaches were due to the difficulty in maintaining tissue viability and immunologic complications.

The hybrid BAL device, in which functional hepatocytes are housed within a man-made synthetic device, can overcome some of the problems seen in other forms of liver support. These devices, with their metabolically active hepatocytes, can provide a broader range of liver-specific functions compared to non-biological or other biological-based systems [6]. For these devices to function optimally, novel designs are needed which allow the maintenance of the high cell densities required of a clinical device, with minimal mass transfer limitations to the hepatocytes.

3. DESIGN ISSUES FOR A BIOARTIFICIAL LIVER DEVICE

For a BAL device to function optimally, it must maintain the hepatocytes in an environment that mimics the in vivo environment as close as possible. In order to do this, there are certain design criteria that must be met including: 1) to use a sufficient number of well-differentiated hepatocytes that can maintain long-term function, 2) to reduce mass transfer resistances and eliminate substrate limitations so that the device can function at maximum efficiency, and 3) to minimize the dead volume within the device thereby reducing plasma dilution effects in the patient. Various design configurations have been utilized in an effort to achieve these design criteria. In general, a bioreactor is inoculated with hepatocytes and the patient's blood or plasma circulates through the device. The ideal bioreactor design would maximize mass transfer to the hepatocytes thereby allowing nutrients, including oxygen, and toxins from the patient's blood or plasma to reach the hepatocytes. The treated blood or plasma, including metabolites and synthetic products, is then returned to the patient's circulation. Achieving this task requires a large surface area for cell attachment with uniform cell distribution and flow.

Several bioreactor designs incorporate membranes of different selectivity's to prevent direct blood or plasma contact with the hepatocytes. In these designs, mass transfer is determined by the molecular weight cutoff (i.e., pore diameter) of the membrane for a given pressure and flow rate, which can influence the performance of the bioreactor. The idea is to select a molecular weight cutoff which allows transport of

proteins (e.g., albumin) to the patient's circulation and toxins out of the patient's circulation while preventing immune-mediated injury to the hepatocytes. The properly selected membrane can also exclude the transport of xenogeneic substances, as well as cells, to the patient's circulation. Some designs use membranes with low molecular weight cutoff (70 – 100 kD) which allow passage of serum albumin but excludes proteins of higher molecular weights, such as immunoglobulins, thereby providing immunoprotection to the hepatocytes. Other designs use microporous membranes with large pore diameters (0.2 μm) which allow free passage of plasma proteins as well as large molecular weight proteins (e.g., clotting factors) and toxins (soluble or protein bound) between blood or plasma and the hepatocytes. These microporous membranes, however, do exclude passage of cells (e.g., blood cells and hepatocytes). The rational for using membranes with large pore diameters is that fluid convection is enhanced, thereby improving transfer of substrates and products to and from the hepatocytes.

Another important issue in the design of a BAL device is the maintenance of sufficient oxygen supply to the hepatocytes. Hepatocytes are highly metabolic with high oxygen uptake rates [7-10]. Therefore, they require adequate oxygenation to maintain viability and function. In order to oxygenate the circulating blood or plasma, some designs incorporate an oxygenator within the bioreactor itself, while other designs use an inline oxygenator within the extracorporeal perfusion circuit. Physiological temperatures are maintained via heat exchangers placed in the perfusion circuit.

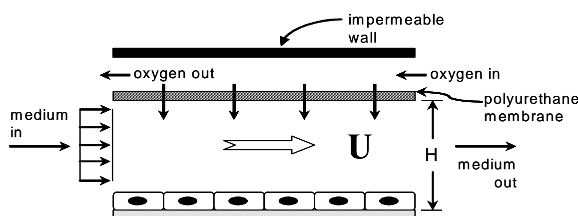

Figure 1: Bioreactor with internal membrane oxygenator.

3.1 BIOARTIFICIAL LIVER DEVICES UNDERGOING CLINICAL TRIALS

Most devices undergoing clinical trials are designed based on hollow-fiber technology, in which either porcine hepatocytes are attached to collagen-coated microcarriers [11] or cells from the human hepatoblastoma cell line (HepG2/C3A) [12] are typically loaded into the extraluminal compartment and patient plasma [11] or blood [12] is allowed to flow within the fiber lumina. In Phase I studies, these devices appeared to be well tolerated by the patients, but no survival advantage was demonstrated over standard of care in appropriately controlled settings. Recently, a clinical, multicenter Phase II/III randomized trial of a hollow-fiber BAL device was conducted at several United States and European sites. The results showed a trend toward improved survival in fulminant hepatic failure patients who received treatment with this BAL device [13]. It has been suggested that these hollow-fiber devices are subject to substrate limitations due to the relatively large diameter of the fibers as well as the transport resistances associated with the fiber wall [14-16]. Given the high oxygen utilization of hepatocytes and low solubility of oxygen in plasma, the adequate delivery of oxygen in hollow-fiber BAL devices has been problematic.

To improve oxygenation, some designs utilize hollow fibers as conduits for oxygen delivery. In one design, discrete bundles of woven capillary membranes enter and leave the bioreactor forming a three-dimensional structure. The hepatocytes are distributed in a collagen matrix on the membrane framework and the extracapillary space is perfused with plasma. The capillary bundles allow independent oxygen supply and plasma inflow and outflow. Using this design, it was demonstrated that hepatocytes could express differentiated functions over several weeks. In a Phase I clinical trial using this device with porcine hepatocytes, acute liver failure patients were successfully bridged to liver transplantation [17].

3.2 MICROCHANNEL FLAT-PLATE HEPATOCYTE BIOREACTOR DESIGN

In an effort to maximize oxygen availability to the hepatocytes and to reduce mass transport limitations, we recently developed a microchannel flat-plate bioreactor with an internal gas permeable membrane through which oxygen was supplied [1, 2] (Figure 1). The hepatocytes were attached to a collagen-coated glass substrate (25 x 75 mm) and were in direct contact with the perfusing medium. A gas permeable membrane separated the liquid compartment from the oxygenating gas compartment. This design allowed oxygen delivery to the hepatocytes to be decoupled from the medium flow, thereby allowing oxygen delivery and flow to be studied independently. In these studies, the bioreactor channel heights ranged between 50 and 500 μm and medium

flow rates ranged between 0.06 and 4.18 mL/min.

3.3 MATHEMATICAL MODELING

Flat-Plate Bioreactor Without Internal Membrane Oxygenation

In the case for a microchannel flat-plate bioreactor with a high aspect ratio between longitudinal length and height under fully-developed laminar flow (typically Reynolds number < 2000), one can neglect the axial diffusion, and the oxygen conservation can be expressed as

$$\frac{\partial \tilde{C}}{\partial \tilde{x}} = \frac{1}{Pe}\frac{\partial^2 \tilde{C}}{\partial \tilde{y}^2} \quad (1)$$

with dimensionless variables being $\tilde{x} = \frac{x}{h}$; $\tilde{y} = \frac{y}{h}$; $\tilde{C} = \frac{C}{C_0}$, where h is the height of the bioreactor compartment, C(x,y) is the oxygen concentration, C_o is the uniform inlet oxygen concentration (nmole/cm^3), Pe is Peclèt number (= Q/WD), Q is the volumetric flow rate, W is the channel width, and D is oxygen diffusion coefficient (2.0 x 10^{-5} cm^2/sec). Boundary conditions for the bioreactor without internal membrane oxygenation are $\tilde{C} = 1$ @ $\tilde{x} = 0$, $\frac{\partial \tilde{C}}{\partial \tilde{y}} = -\frac{\gamma \ (OUR)h}{C_o D} = -Da$ @ $\tilde{y} = 1$ and $\frac{\partial \tilde{C}}{\partial \tilde{y}} = 0$ @ $\tilde{y} = 0$, where γ is the cell seeding density (i.e., number of cells per unit area), Da is the Damköhler number, and OUR is the oxygen uptake rate of hepatocytes (nmole/sec/10^6 hepatocytes). Assuming OUR of hepatocytes is constant and equal to the maximum oxygen uptake rate, V_{max}, Damköhler number reduces to $Da = \frac{\gamma V_{max} h}{C_0 D}$, and Equation (1) can be solved to yield [18]:

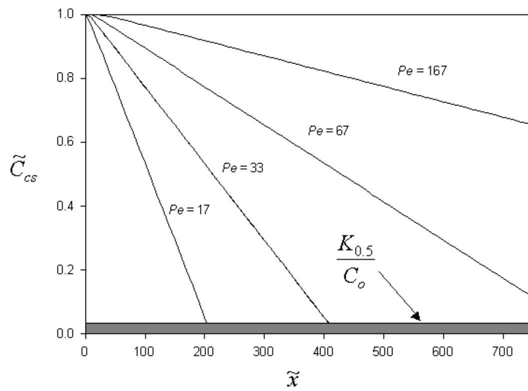

Figure 2: Non-dimensional cell surface oxygen concentration $(\tilde{C}_{cs} = C_{cs} / C_o)$ as a function of axial direction ($\tilde{x}$) for the bioreactor without internal membrane oxygenation at various *Pe*.

$$\tilde{C}(\tilde{x},\tilde{y}) = \frac{C}{C_o} = 1 - \left[\frac{Da}{Pe}\tilde{x} + Da\left[\frac{3\tilde{y}^2 - 1}{6} - \frac{2}{\pi^2} \sum_{n=1}^{\infty}\frac{(-1)^n}{n^2}\cos(n\pi\tilde{y})e^{-(n\pi)^2\frac{\tilde{x}}{Pe}}\right]\right] \quad (2)$$

where n is an integer. The non-dimensional average concentration can be obtained as

$$\tilde{C}_{ave}(\tilde{x}) = 1 - \frac{Da}{Pe}\tilde{x} \quad (3)$$

Flat-Plate Bioreactor With Internal Membrane oxygenation

For very small channel heights (~100 μm) and low flow rates (< ~1 mL/min), typical conditions used in small-scale bioreactors [19], the time constant for convection, $t_{transit} = L/U$, is much larger than the time constant for diffusion, $t_{diffusion} = h^2/D$. Thus, delivery of oxygen to the bioreactor surface is primarily dominated by the diffusion characteristics of the system. Under these conditions, for a Michaelis-Menten-behaving system, as already reported by Peng and Palsson [20] and Yarmush [21], the dimensionless cell surface oxygen concentration, $\hat{C}_{cs}$, is given by the following equation:

$$\hat{C}_{cs} = \frac{-(Da+\beta-1) \pm \sqrt{(Da+\beta-1)^2 + 4\beta}}{2} \quad (4)$$

where $\hat{C}_{cs} = \frac{C_{cs}}{C*}$, $\beta = \frac{K_{0.5}}{C*}$, $C*$ is the oxygen concentration at the aqueous membrane surface, and $K_{0.5}$ is a constant value of oxygen concentration at the cell surface for an OUR of $V_{max}/2$. The average oxygen concentration in the channel can be expressed as

$$\tilde{C}_{ave} = \frac{1+\hat{C}_{cs}}{2} \quad (5)$$

4. RESULT

4.1 OUTLET OXYGEN MEASUREMENTS IN BIOREACTORS WITH AND WITHOUT INTERNAL MEMBRANE OXYGENATION

Studies using porcine hepatocytes compared outlet oxygen concentration in bioreactors with and without the internal membrane oxygenator [1]. Figure 3 summarizes the effects of medium flow rates on outlet oxygen partial pressure in bioreactors, with and without the internal membrane oxygenator. In the bioreactor without the internal membrane oxygenator, the outlet oxygen tension decreased gradually from 154 mmHg at a flow rate of 3.5 mL/min to 114 mmHg at a flow rate of 0.5 mL/min, with an approximate overall decrease of 26%. As the flow rate was further decreased to 0.1 mL/min, output oxygen tension precipitously decreased to 2.0 mmHg, which corresponded to a decrease of about 99% from the concentration measured at the highest medium flow rate. This suggested significant oxygen limitations were occurring at volumetric flow rates of 0.1 mL/min, and lower, in this bioreactor configuration. Figure 2 also contains the model fit (Equation (3)) to the experimental data.

Since measurement of the local oxygen concentration near the hepatocyte surface is difficult, we used a mathematical model to predict the cell surface oxygen concentration, which can be estimated from Equation (2). Figure 2 shows non-dimensionalized cell surface oxygen concentration (C_{cs}/C_0) in the axial direction of the bioreactor without the internal membrane oxygenator at various *Pe*. The inlet PO_2 for the reactor was fixed at 159 mmHg, based upon the constraint that the medium was oxygenated with 21% oxygen prior to its entrance into the bioreactor. The model predictions were based on a *Da* of 0.08, which was obtained from the experimental data fit. The average oxygen concentration decreased in a linear fashion along the length of the bioreactor as more cells consumed oxygen. Assuming that porcine hepatocytes were oxygen-limited when the oxygen concentration at the cell surface was below the normalized $K_{0.5}/C_o$ (~ 0.05, [7]), one can predict the extent of oxygen metabolism in the bioreactor. For Pe = 33.3 (corresponding to Q = 0.1 mL/min), the average concentration fell below $K_{0.5}/C_0$ at $\tilde{x}$ ~ 400, which predicted that approximately 50% of the cells in the bioreactor of $\tilde{x}$ = 750 would be exposed to oxygen partial pressures of less than $K_{0.5}$ and thus would be oxygen limited. At Pe = 167 (corresponding to Q = 0.5 mL/min), no hepatocytes were exposed to oxygen rate-limiting conditions. Although higher *Pe* would theoretically result in less oxygen limitations due to increased medium velocity, there would be a practical upper limit due to the potential deleterious effects of increased shear stresses on hepatocyte viability and function. Additionally, in the clinical setting, the flow rate would be governed by the cardiac output of the patient.

4.2 EFFECTS OF INTERNAL MEMBRANE OXYGENATION

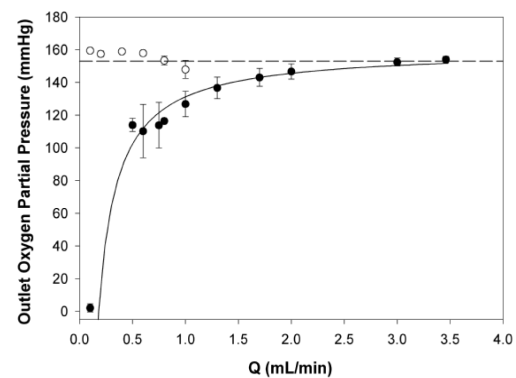

Figure 3: Outlet oxygen partial pressure (PO_2) as a function of flow rate (Q) for the bioreactor without (●) and with (○) internal membrane oxygenation. The solid line represents the mathematical model fit (Da = 0.08) to the experimental data for the bioreactor without the internal membrane oxygenator. The dashed line represents the model prediction (Da = 0.08, β = 0.052) for the bioreactor with internal membrane oxygenation (Equation 5) [1].

In the bioreactor with the internal membrane oxygenator (Figure 1), the variability of outlet PO_2 tension across all flow rates was minimal when compared to that exhibited by the bioreactor without the internal membrane oxygenator, with average values ranging from 148 mmHg at 1.0 mL/min to 160 mmHg at 0.1 mL/min (Figure 2). Flow rates between 0.1 and 1.0 mL/min were selected because of the low oxygen tensions encountered in the bioreactor without internal membrane oxygenation at these flow rates. At the lowest flow rate, the resulting mean outlet oxygen tension of the bioreactor with internal membrane oxygenation demonstrated a 75-fold increase over that seen in the

bioreactor without the internal membrane oxygenator ($p < 0.01$). Statistical significance was also seen between the performances of the two reactors for the 0.5 to 0.6 mL/min flow range (p=0.012), and for the 0.75 to 0.8 mL/min flow range (p = 0.015), as determined by one-way ANOVA.

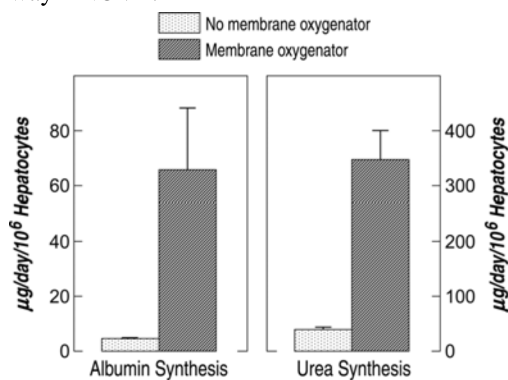

Figure 4: Albumin and urea synthesis on Day 3 of culture for rat hepatocytes co-cultured with 3T3-J3 fibroblasts in bioreactors with (130 μm channel height) and without (115 μm channel height) the internal membrane oxygenator. Volumetric flow rates set at 0.06 mL/min. Corresponding shear stresses were 0.14 dynes/cm^2 in the bioreactor without the internal membrane oxygenator and 0.18 dynes/cm^2 in the bioreactor with the internal membrane oxygenator. Results are expressed as mean of 3 experiments ± SD.

There was no statistical difference in the performances of the two reactors at 1.0 mL/min. In this bioreactor configuration, air entering countercurrent to the medium flow supplies bioreactor without internal membrane oxygenation. Utilizing the expression for $\tilde{C}_{ave}$ developed for the bioreactor with the internal membrane oxygenator, the average concentration of oxygen in the channel was found to be 153 mmHg and shows as a dashed line in Figure 2. This simple model is able to predict the oxygen concentration with reasonable accuracy.

It is important to predict the channel height at which hepatocytes become oxygen-limited in the bioreactor with internal membrane oxygenation. Computing the oxygen concentration at the cell surface (Equation (4)) as a function of both Da and β (*i.e.,* $K_{0.5}/C^*$) reveals as Da increases to 2, the outlet oxygen concentration decreases to the $K_{0.5}$ value. This corresponds to a channel height of 865 μm, above which the cellular oxygen metabolism is rate limited. Since the channel height in the bioreactor was maintained below this value, there were no oxygen limitations, thereby resulting in increased outlet oxygen concentration in the bioreactor with the internal membrane oxygenator under the flow conditions of 0.1 to 1.0 mL/min.

4.3 HEPATOCYTE FUNCTION IN BIOREACTORS WITH AND WITHOUT INTERNAL MEMBRANE OXYGENATOR

To assess the beneficial effect of internal membrane oxygenation on the function of hepatocytes within the two bioreactor configurations, rat hepatocytes co-cultured with 3T3-J2 murine fibroblasts were used in the bioreactor. The rat hepatocyte/3T3-J2 fibroblast co-culture combination was used because this culture system has shown long-term stability and has been extensively characterized and published in the literature [7]. Volumetric flow rates within the two bioreactors containing hepatocyte co-cultures were maintained at 0.06 mL/min. The channel height for the bioreactor without the internal membrane oxygenator was 130 μm with a corresponding wall shear stress of 0.14 dynes/cm^2 and the channel height of the bioreactor with the internal membrane oxygenator was 115 μm with a corresponding wall shear stress of 0.18 dynes/cm^2. Figure 4 shows the albumin and urea synthesis rates on day 3 of perfusion within the two bioreactors. The albumin synthesis rate for the bioreactor without the internal membrane oxygenator was 4.8 μg/day/10^6 hepatocytes and was 65.9 μg/day/10^6 for the bioreactor with the internal membrane oxygenator. The urea synthesis rate was 38.7 μg/day/10^6 in the bioreactor without the internal membrane oxygenator and was 347.2 μg/day/10^6 in the bioreactor with the internal membrane oxygenator. This corresponded to greater than a 1300% increase in the albumin synthesis rate and greater than a 500% increase in the urea synthesis rate within the bioreactor with the internal membrane oxygenator compared to the bioreactor without the internal membrane oxygenator, clearly indicating the significance of oxygenation in the bioreactor.

4.4 EFFECT OF SHEAR STRESS ON HEPATOCYTE FUNCTION

Since hepatocyte function was shown to be significantly decreased in the bioreactor without the internal membrane oxygenator, all experiments to determine the effects of various flow conditions were only conducted in the bioreactor with the internal

membrane oxygenator. The flow rate (mL/min) – channel height (μm) combinations used to asses the effect of flow conditions on the function were: 0.06 – 500; 0.06 – 180; 0.06 – 115; 0.06 – 85; 3.8 – 180; 3.8 – 115; and 3.8 – 85, which corresponded to wall shear stresses of 0.01, 0.07, 0.18, 0.33, 5, 10, and 21 dynes/cm^2, respectively. These combinations were sorted into a low shear stress group (0.01 – 0.33 dynes/cm^2) and a high shear stress group (5 – 21 dynes/cm^2). Figure 5 shows the results of daily albumin synthesis rates, presented as percentages of the corresponding daily static controls, at both the low (upper panel) and high (lower panel) shear stresses. For flow conditions that resulted in low shear stresses, the normalized daily albumin synthesis rates were not significantly different throughout the three days in the bioreactors across the four shear stresses tested (ANOVA, p=0.12). There also were no statistically significant differences between the normalized daily albumin synthesis rates for day 3 and day 0 (non-perfused, prior to placement into bioreactor) for any of the four shear stresses. For flow conditions that resulted in high wall shear stresses, the normalized daily albumin synthesis rates decreased throughout the three days in the bioreactor for the three wall shear stresses tested (ANOVA, p<0.01). Across the group, the day 3 normalized daily albumin synthesis rates were statistically lower than those on day 0 (Tukey's test, p<0.05).

Urea synthesis rates were noted to decrease throughout the three days of perfusion in both the low and high shear stress groups (ANOVA, p<0.05) (Figure 6). In the high shear stress group, there was also a statistically significant decrease in the normalized daily urea synthesis rates on day 3 compared to day 0 (lower panel), whereas in the low shear stress group, there were no statistically significant differences between day 3 and day 0 urea synthesis rates (upper panel) (Tukey's test, p<0.05). Comparison of day 3 results between low and high shear stress groups showed that albumin and urea production rates were 2.6 and 1.9 times greater, respectively, than that at high shear stress (t-Test, p<0.01).

These results are of great benefit in the selection of proper bioreactor operating conditions. For example, increasing medium flow rate is beneficial in delivering oxygen to the cell surface. However, our results indicate there is a critical flow range above which synthetic function of the hepatocytes can be greatly

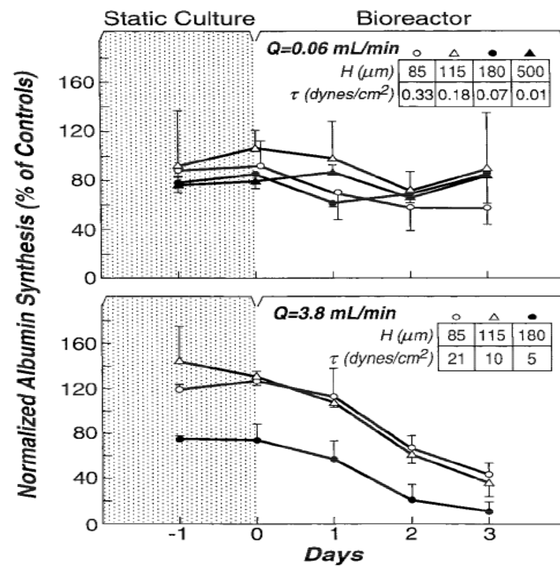

Figure 5: Normalized albumin synthesis at low (upper panel) and high (lower panel) shear stresses for rat hepatocytes co-cultured with 3T3-J2 fibroblasts for 3 days of continuous perfusion in the microchannel flat-plate bioreactor with internal membrane oxygenator. Results are expressed as the mean of three experiments ± SD. *H* = channel height (μm); τ = shear stress (dynes/cm^2).

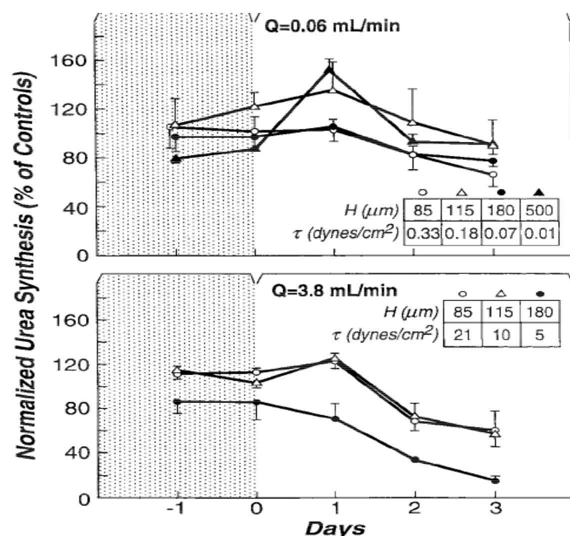

Figure 6: Normalized urea synthesis at low (upper panels) and high (lower panels) shear stresses for rat hepatocytes co-cultured with 3T3-J2 fibroblasts for 3 days of continuous perfusion in the microchannel flat-plate bioreactor with internal membrane oxygenator. Results are expressed as the mean of three experiments ± SD. *H* = channel height (μm); τ = shear stress (dynes/cm^2) [2].

diminished by the increased shear stress. Therefore, in a BAL device where recirculation is used, our results suggest that there is an upper limit on the recirculation rate for optimal functioning of the hepatocytes, unless the hepatocytes are protected from the detrimental effects of high shear stresses caused by the flowing medium [22]. Also, in the design of a BAL, minimizing the dead volume of the bioreactor by reducing the channel height, for the same medium flow rate, causes an increase in wall shear stress, thereby placing a lower limit on the dead volume within the bioreactor.

5. CONCLUSION

An extracorporeal BAL device is a promising technology for the treatment of liver failure. These studies using our microchannel flat-plate hepatocyte bioreactor provide information on the optimal design of hepatocyte bioreactors, and will hopefully lay a foundation for the eventual clinical application of this device.

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