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TRANSTUBULAR BIOREACTOR: A PERFUSION DEVICE FOR MAMMALIAN CELL CULTIVATION

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SUMMARY

A transtubular mammalian cell perfusion chamber has been developed that allows separate delivery and control of medium and gas flow to the cells. The hydrodynamic and mass transfer characteristics of the reactor are studied experimentally for both gas and liquid components. Feasibility and potential of the transtubular reactor are demonstrated by the growth of a hybridoma cell line.

INTRODUCTION

Large scale cultivation of mammalian cells *in vitro* to produce vaccines, growth factors, monoclonal antibodies and other pharmaceutical products has become a growing area of interest in biochemical engineering (Boraston, 1982; Thilly, 1985; Hu and Wang, 1986). The fastidious character of animal cells combined with their fragility places unique demands on the design and operation of bioreactors used for their cultivation. The main challenges are to provide sufficient transfer of nutrients to the cells, especially oxygen; to create a uniform environment for the cells; to facilitate easy product removal; and to provide controllability of the system (Glacken et al., 1983).

Perfusion approaches are gaining favor for cost effective production of MAb's (van Wezel et al., 1985; Hu and Sinskey, 1988). In a perfusion reactor one can grow cells to high densities for high volumetric productivities. This culturing method involves continuous addition and removal of medium with retention of the cells and provides opportunities to replace missing components selectively, to remove inhibitors and toxins, to exert a high degree of control, and to keep the cells viable for long periods of time.

In an attempt to meet these requirements, a new perfusion bioreactor has been developed (Halberstadt and Midgley, 1988). This reactor, described here, will be shown to have the advantages of a gradient free, uniform environment without significant shear forces, and good oxygen mass transfer characteristics without the aid of gas sparging. The hydrodynamics and mixing in the reactor have been studied, and the mass transfer characteristics have been modeled. The potential of the transtubular reactor is illustrated by the growth of a hybridoma cell line.

THE TRANSTUBULAR REACTOR

The transtubular bioreactor consists of one or more separate tubings for medium and gas exchange (Fig. 1A). Oxygen is provided through a silicone tube (Silastic^{TM} from Dow Chemical, Midland, MI), which eliminates the need for gas sparging. For medium delivery and removal, microporous Teflon^{TM} tubing (Gore-Tex, Elkton, MD) is used. This Teflon^{TM} tubing has a pore size of 3.5 μm with an overall porosity of 70%, and has a burst pressure greater than 140 psi. Thus, mammalian cells cannot enter or degrade the tubing and high flow rates can be achieved. The large pore size in the medium tubes allows for significant flow of medium components across the tube wall while retaining the cells in the reactor.

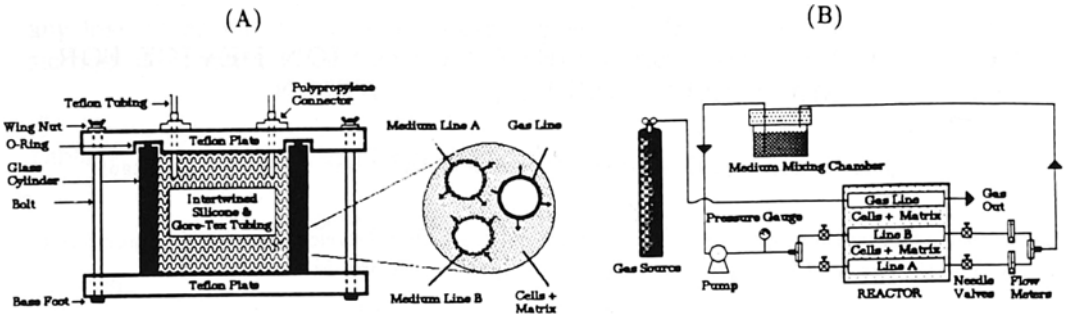

Figure 1: (A) Transtubular reactor. (B) Bioreactor operation.

The bioreactor is comprised of these tubes bundled inside a glass cylinder (Fig. 1A). The resulting reactor has been autoclaved repeatedly without detectable physical changes and all materials that contact medium (glass, Teflon^{TM}, polypropylene, Norprene, silicone rubber, etc.) have been shown to be non-toxic as estimated by growth rates of a hybridoma cell line. The silicon tubing is known to form pinholes after repeated autoclaving. Although this problem was not encountered in our studies, it may limit the number of autoclaving.

The current system can be operated in several modes. Gas is provided by an air pump, filtered, passed through a flow meter, saturated with water and then passed through the silicone tubing inside the reactor (Fig. 1B). Medium, provided from a container, is taken *via* a peristaltic pump, measured by the pressure gauge and then pumped into one or more medium tubes, here illustrated as tubes A and B (Fig. 1B). Due to the porosity of the Teflon^{TM} tubing, media entering into line A, can flow out into the extratubular space of the chamber and then either enter into line A, or enter line B. In addition to these simple flow patterns, other flow regimes can be acheived via the use of external controls. For example, all medium flowing into line A, can be forced to flow into line B by blocking the outlet of line A. By placing a pinch valve external to the reactor, this net convective flow can be alternated between the two lines, causing an oscillatory flow regime to occur inside the reactor. After passing through the chamber, the medium passes through the flow meter and is either recycled back into the medium reservoir (batch-recycle operation) or taken into another container (net perfusion operation).

MATERIALS AND METHODS

Cell Line, Medium and Culture Maintenance

The murine hybridoma used was 167.4G5.3, kindly provided by Dr. J. Latham Claflin from the University of Michigan (Department of Microbiology and Immunology). The antibody produced by this cell line is an IgG_1, directed against phosphorylcholine (Claflin and Davie, 1975). The cells were initially grown in T-flasks using Iscove's Modified Dulbecco's Medium (Gibco Laboratories, Grand Island, New York) containing 10% Fetal Bovine Serum (Gibco Laboratories, Grand Island, New York) and containing antibiotic/antimycotic solution (for 1 ml, 1000 U penicillin, 0.1 mg streptomycin, and 0.25 μg amphotericin B) (Sigma Chemical Company, St. Louis, MO).

Reactor Characterization: Hydrodynamics, Mixing and Mass Transfer

In order to study the fluid dynamics in the reactor, tube A (Fig. 1B) was used both as an inlet and outlet line, while tube B was used exclusively as an outlet line. For these studies, the term intratubular flow will be used to describe the flow in line A and the term transtubular flow will be used to describe the flow in line B.

The hydrodynamics of the reactor was performed by changing the total flow rate at the inlet and measuring the pressures at the outlets of the tubes A and B.

Mixing patterns in the reactor were characterized by Residence Time Distribution (RTD) functions (Levenspiel, 1972). A pulse input was applied to the inlet of the reactor and the response was recorded at three locations: line A, line B, and the extratubular space. Acid (0.1 N HCl) was used as a tracer and was measured using a flow-through pH electrode (Cole-Parmer, Chicago, IL) with the output connected to a chart recorder. The pH values were then converted to hydrogen ion concentrations and these concentrations were used to determine the RTD.

The mass transfer coefficients for oxygen were measured by a dynamic dissolved oxygen (DO) technique modified for liquid flow. Nitrogen gas was initially passed through the silicone tubing. When a constant concentration of oxygen was observed in the liquid, the gas supply was changed from nitrogen to air and the response to this change was recorded continuously. The mass transfer coefficient was extracted from these response curves by using a mathematical model of the system..

Analytical Methods

Samples were taken daily and kept frozen for later analysis. The glucose and lactate concentrations were measured using a Model 2000 Glucose/L-Lactate analyzer (Yellow Springs Instruments, Yellow Springs, Ohio). IgG_1 antibody was quantitated using an enzyme linked immunosorbent assay (ELISA). Ammonia was measured with an ion selective electrode (Orion, Boston, MA). Viable cells in suspension were counted, using a hemocytometer, by the trypan blue exclusion method after diluting the sample 10 fold in serum-free medium.

RESULTS AND DISCUSSION

Hydrodynamics. Experimental data presented in Fig. 2A show that the pressure gradients in the transtubular reactor result in tolerable pressures even at reasonably high flow rates. After entering the chamber through line A, medium splits between lines A and B by convective flow. This convective flow occurs due to a pressure difference between these two lines.

Fig. 2B shows the relationship between transtubular flow, the flow in line A, and % transtubular flow. Transtubular flow is the secondary flow created by the bleeding of line A in the chamber. Percent transtubular is defined as the percent ratio of flow in line B (transtubular) to the flow applied to line A. As the intratubular flow increases, transtubular flow increases in a linear fashion. Percent transtubular flow remains constant at about 70%. Hence, the medium components are transported to the cells not only by diffusion, but also by bulk convective flow. As discussed above, this flow is due to a large pore size of 3.5 μm and porosity of 70%.

Mixing. The results of mixing experiments are outlined in Fig. 3A. This figure shows that the mixing patterns in the three regions are similar to a perfectly mixed case. However, by-passing occurs (corresponding to the peaks in the figure where E is greater than unity), which is expected due to the close proximity of the ends of the inlet and outlet tubes.

Fig. 3B shows the experimentally determined residence times plotted against the theoretical residence time for a perfectly mixed vessel. Since the flow rate to the well mixed zone is less than the

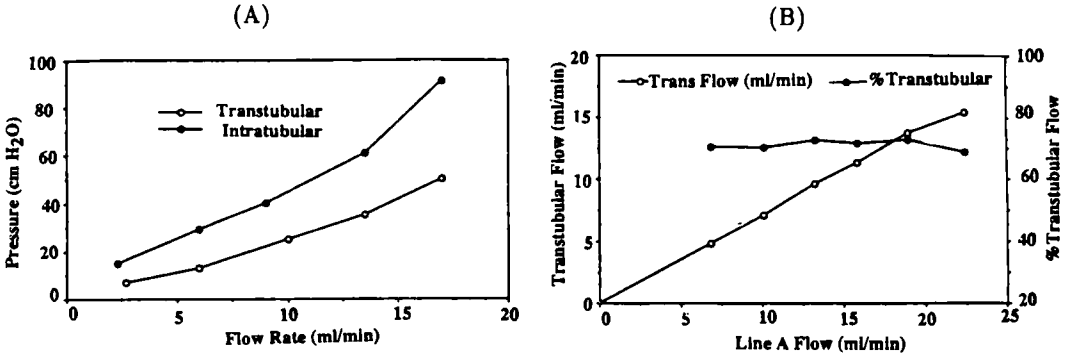

Figure 2: Hydrodynamics in Transtubular reactor. (A) Total flow rate and pressures in the system. (B) Transtubular flow (% transtubular=flow at outlet of line B as % of flow at outlet of line A).

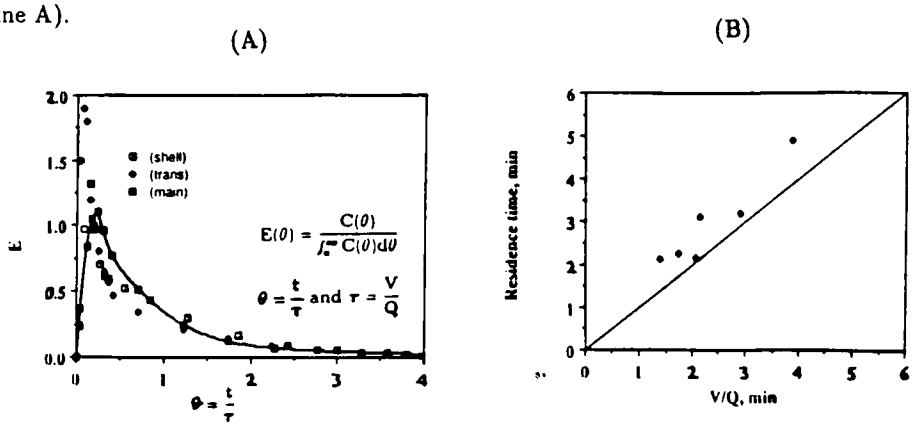

Figure 3: Mixing in Transtubular reactor. (A) Residence Time Distributions in different regions of the reactor. (B) Effect of by-passing on the residence time.

total flow rate, we obtain slightly higher residence times than for a perfectly mixed reactor. These results indicate about 12% of the incoming stream flows almost directly through the chamber while the remaining 88% flows through a "well mixed" region. These experiments were performed without cells or a matrix in the chamber, which would likely perturb this mixing pattern. However, our preliminary studies with Sephadex G-200 beads indicated that the mixing patterns in the reactor is not influenced by the solid matrix.

As expected, lines A and B are found to be well-mixed. In Fig. 3A the same value (total liquid volume) is used as a reference volume (V) and the residence time distributions for these tubings fall into the same curve. This means that any particle entering the tube can see the whole volume in the reactor. Hence, a single tube allows liquid to pass to the extratubular space of the reactor and return into the same tube.

Oxygen Mass Transfer. Mass transfer coefficient determinations are presented in Fig. 4 as a function of residence time. As shown, the mass transfer coefficient increases with the flow rate. Mixing occurs in the absence of agitation due to the convective current between the porous Teflon

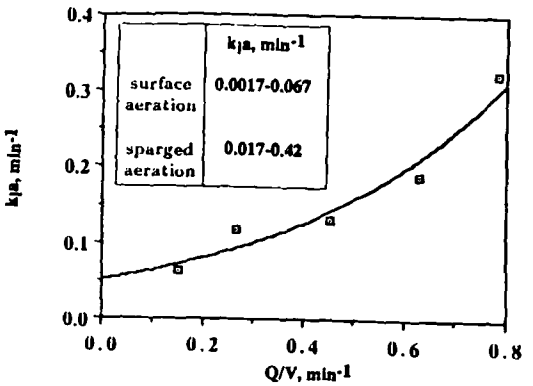

Figure 4: Mass transfer from silicone tubing

tubings. Since the silicone tubing in the reactor is exposed to a convective flow, higher mass transfer coefficients are observed. Hence, by increasing the flow rate, one can increase the mass transfer coefficient of oxygen in the reactor.

The dependency of the mass transfer coefficient on the convective currents in the reactor indicates that the mass transfer limitations are confined to the boundary layer on the outer surface of the tubing. This resistance can be decreased by external agitation. However, as can be seen in Fig. 4, even without agitation the mass transfer coefficient permits delivery of sufficient oxygen to support a high density of cells. This mass transfer is higher than those obtained in surface aeration ($k_l a = 0.0017 - 0.067\ min^{-1}$) and comparable to sparged systems ($k_l a = 0.017 - 0.47\ min^{-1}$)(Lavery and Nienow, 1987). The oxygen demand of hybridoma cells, is 6-11 $10^{-6} \mu g$/cells/hr (Hu and Wang, 1986). Using the solubility value for oxygen 7 mg/l at air saturation and the mass transfer coefficients in our reactor, we can calculate that the reactor could provide enough oxygen for viable cell concentrations up to $3 \cdot 10^7$ cells per ml.

Cell Growth. To determine feasibility of the bioreactor, a hybridoma cell line (167.4G5.3) was grown in the reactor for 24 days. The 60 ml reactor was initially seeded with a cell concentration of $1 \cdot 10^5$ cells per ml.

The reactor was run in two separate modes: batch recycle and perfusion. In the batch recycle mode the medium was recycled from the chamber back into the medium reservoir at a flow rate of 60 ml/hr. This mode was used to condition the medium. In the perfusion mode the medium was replaced continuously at a flow rate of 10 ml/hr. Fig. 5 shows the results of this run when the reactor was run sequentially in these modes. The increase in glucose concentrations and decrease in lactate shown in Fig. 5A are due to the additions of fresh medium. As can be seen in Fig. 5B, the mean MAb concentration differs greatly between the two modes. However as the cell count during the fermentation period is not known and the flow rate was varied in the perfusion mode, a quantitative conclusion cannot be drawn from this data. At the end of this particular run we examined the cells. The total cell concentration in the reactor was $3.5 \cdot 10^7$ cells per ml, with a viable cell concentration of $7.0 \cdot 10^6$ cells per ml. This corresponds to a cell viability of 20 %.

CONCLUSION

- Our goal was to design a chamber that: a) provides a shear-free, uniform environment, b) allows the constant removal of waste products without significant radial or axial gradients, and c) provides

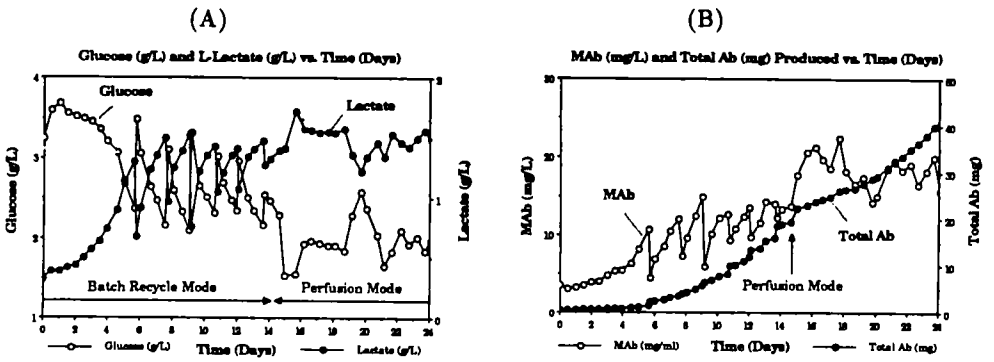

Figure 5: (A). Glucose/lactate data, (B) MAb data

high mass transfer coefficients for nutrients and oxygen. From the data presented in this paper, it has been shown that this system can fulfill all of these criteria. In addition, our preliminary results with hybridoma cells demonstrate that the transtubular bioreactor can be used for the growth and maintainance of mammalian cells *in vitro*.

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