A Novel Multi-Coaxial Hollow Fiber Bioreactor for Adherent Cell Types. Part 1: Hydrodynamic Studies

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Abstract: A novel multi-coaxial bioreactor for three-dimensional cultures of adherent cell types, such as liver, is described. It is composed of four tubes of increasing diameter placed one inside the other, creating four spatially isolated compartments. Liver acinar structure and physiological parameters are mimicked by sandwiching cells in the space between the two innermost semi-permeable tubes, or hollows fibers, and creating a radial flow of media from an outer compartment (ECC), through the cell mass compartment, and to an inner compartment (ICC). The outermost compartment is created by gas-permeable tubing, and the housing is used to oxygenate the perfusion media to peritubal levels in the ECC. Experiments were performed using distilled water to correlate the radial flow rate (Qr) with (1) the pressure drop (∆P) between the media compartments that sandwich the cell compartment and (2) the pressure in the cell compartment (Pc). These results were compared with the theoretical profile calculated based on the hydraulic permeability of the two innermost fibers. Phase-contrast velocity-encoded magnetic resonance imaging was used to visualize directly the axial velocities inside the bioreactor and confirm the assumptions of laminar flow and zero axial velocity at the boundaries of each compartment in the bioreactor. Axial flow rates were calculated from the magnetic resonance imaging results and were similar to the measured axial flow rates for the previously described experiments. © 2002 John Wiley & Sons, Inc. Biotechnol Bioeng 77: 85-90, 2002.

Keywords: multi-coaxial bioreactor; phase-contrast velocity-encoded MRI; hydrodynamics; bioartificial liver

INTRODUCTION

Bioartificial organs are needed to support patients during organ failure (Gerlach, 1994). In 1990, more than 27,000 deaths in the United States were caused by liver failure (Nyberg et al., 1992). Even more deaths are caused by multi-organ system failure, where liver failure is the proximal cause of death (Baue et al., 1998). Therefore, a bioartificial liver is a logical means to bridge liver function until transplantation or until the organs regain function. Oxygen is a limiting nutrient in bioartificial livers (Catapano, 1996; Macdonald et al., 1999). In general, if the bioreactor relies solely on diffusion for the mass transfer of oxygen, hepatocytes must be within 150 to 200 μm of an oxygen source to survive and proliferate (McClelland and Coger, 2000).

Conventional hollow fiber bioreactors consist of a bundle of hollow fibers forming two compartments, a cell compartment and a media or plasma compartment. Coaxial hollow fiber bioreactors contain a fiber within a fiber to form a third media or plasma compartment to enhance the mass transfer of nutrients to the cell compartment. The coaxial hollow fiber bioreactor has performed four-fold better than conventional hollow fiber bioreactors in antibody production per unit of glucose (Custer, 1988). Initial studies of isolated rat hepatocytes maintained in a coaxial bioreactor found that if the distance between concentric fibers is greater than 200 μm, then radial convection, or flow of media across the coaxial fibers and through the cell mass, is necessary to maintain cell viability (Macdonald et al., 1998). Previous theoretical mass transfer models of the coaxial bioreactor predicted similar results (Cima et al., 1990; Fowler and Robertson, 1990), given the oxygen consumption rates of hepatocytes (Suleiman and Stevens, 1987; Smith et al., 1996). Figure 1A illustrates the orientation of the two innermost coaxial fibers. An interfiber distance of 500 μm matches the average distance from the portal triad to the central vein in a liver acinus as shown in Figure 1B (McCuskey, 1994; Saxema et al., 1999). Mimicking this dimension will permit normal sinusoidal structures to form (inset, Fig. 1B) while greatly increasing the cell volume of the bioreactor. The optimal...
choice of cells is hypothesized to be progenitors given their expansion and differentiation potential (Xu et al., 2000). Clonal expansion of rat progenitors under defined conditions has demonstrated that a single liver progenitor can yield thousands of liver cells (Kubota and Reid, 2000). At this expansion rate, the cells could fill volumes equally as large in the cell compartment. The interfiber volume limits the three-dimensional clonal expansion. The surface area of the inner fiber, which is the surface onto which cells are seeded (Macdonald et al., 1998), determines the number of progenitor cells required for inoculation. The coaxial bioreactor embodiment used by Macdonald and others (1998) with a 500-μm as compared to 200-μm interfiber distance creates a three-fold increase in cell compartment volume, resulting in three-fold increase in cell mass for the same number of inoculated progenitor cells (see Equation 1 in Materials and Methods). However, a culture of eucaryotic cells in a coaxial bioreactor with a 500-μm interfiber distance was unsuccessful (Williams et al., 1997). The goal of this study is to define the optimum permeabilities of the two innermost coaxial fibers (Fig. 1A) to attain acceptable radial flow rates (Qr) and pressure profiles (ΔP) using commercially available hollow fibers for the construction of bioreactors. These findings will lead to the successful three-dimensional culture of hepatocytes in a bioreactor with a 500-μm interfiber distance.

**MATERIALS AND METHODS**

**Design Considerations of the Novel Bioreactor**

Acceptable $Q_r$ and $ΔP$ can be obtained from physiological measurements of liver sinusoid blood flow and pressure, respectively, (McCuskey, 1994) and theoretical predictions (Cima et al., 1990; Fowler and Robertson, 1990). Figure 1B shows a schematic of the structure of a liver acinus and physical parameters found in the hepatic sinusoid. Blood flow velocity in the sinusoid ranges from 0.003–0.03 cm/s; the average hydrostatic pressure is typically 7 to 10 mm Hg (McCuskey, 1994); and oxygen tension normally ranges from 70 mm Hg in the periportal region to 20 mm Hg in the pericentral region (Warless, 1999). A novel multicoaxial hollow fiber bioreactor (Fig. 1A) is described that mimics the geometry of the liver acinus (Fig. 1B). The multicoaxial bioreactor differs from the previous coaxial designs (Robertson and Kim, 1985; Custer, 1988; Cima et al., 1990; Fowler and Robertson, 1990; Williams et al., 1997; Macdonald et al., 1998) by the incorporation of a fourth compartment for integral oxygenation, or oxygenation within the bioreactor proper. Integral oxygenation eliminates axial gradients (Macdonald et al., 1999) and can oxygenate media in the extracapillary compartment (ECC) to levels similar to those (70 mm Hg, Fig. 1B) found in the periportal region of the liver just prior to entering the cell compartment (Fig. 1A). To replicate blood flow in the liver, flow in the coaxial bioreactor is directed radially from the ECC through the cell mass to the inner media compartment or intracapillary compartment (ICC) (Fig. 1A). The hydraulic permeability is the critical physical characteristic of semipermeable hollow fiber membranes that determines the relationship between $Q_r$ and $ΔP$ and $Q_r$ and $P_r$. The predicted relationships were compared with flow experiments and to phase–contrast magnetic resonance imaging (MRI) measurements of flow (Moran, 1982) for validation.

**Sources of Equipment and Supplies**

Bioreactors were constructed from polysulfone hollow fibers (AG/Technology, Needham, MA), Silastic™ tubing and silicone glue (Dow Corning, Midland, MI), 10-mm [outer diameter (O.D.)]; 9 mm inner diameter (I.D.)] NMR tubes (Wilmark/Lab Glass, Buena, NJ), 13-mm O.D. (10 mm I.D.) polycarbonate tube and 1-inch diameter polypropylene rod (Piedmont Plastics Inc., Raleigh, NC), 0.394 inch by 0.079 inch viton 0-rings (Apple Rubber Products, Lancaster, NY), and medical-grade polyurethane (Caschem Inc., Bayonne, NJ). The 1–60 rpm variable pump, Pharmed™ tubing, polypropylene barbed fittings, plastic tubing clamps, and Teflon™ needle valves were purchased from Cole Parmer (Chicago, IL). The pressure-monitoring apparatus was constructed using 0 to 260 mm Hg pressure sensors (SensyM Inc., Milpitas, CA), a pressure cali-
brator (Crystal Engineering Inc., San Luis Obispo, CA), and TDS210 oscilloscope (Tektronix Beaverton, OR). The MRI studies and the flow experiments used a model M361 Sage™ syringe pump from Orion Research Inc. (Beverly, MA).

**Construction of Bioreactors and Quality Assurance**

The embodiment of the multi-coaxial hollow fiber bioreactor is shown in Figure 2A. Construction consisted of four procedures to attach both ends of the various takes to their respective manifolds. First, the housing, a 6 cm long polycarbonate tube (13 mm O.D., 10 mm I.D.) is attached to the inner side of manifold 3 with epoxy. Prototypes for the flow experiments used a glass NMR tube for the housing (10 mm O.D., 9 mm I.D.) with endcaps and sealed with o-rings and silicone glue. The outer Silastic™ tube (aeration fiber) with a 7.92 mm O.D. is inserted into the housing. The ends of the aeration fiber are fitted into two polypropylene manifold end pieces (manifold 3) extending the total bioreactor length to 9 cm, and the aeration fiber ends are epoxied to the manifolds with medical-grade polyurethane. This forms the gas compartment (Fig. 2A). Next, the middle polysulfone hollow fiber (0.1-μm pore size) with a 3-mm outer diameter is inserted through the lumen of the aeration fiber, and the ends are inserted into the second set of polypropylene manifold end pieces (manifold 2). Each fiber end is subsequently potted with epoxy and cured in an oven at a temperature of 50°C. This second construction procedure extends the bioreactor length to 11 cm creating the ECC. Finally, the inner polysulfone hollow fiber (0.65-μm pore size) with a 1.2-mm outer diameter is inserted through the lumen of the middle polysulfone fiber and potted to the next set of manifolds (manifold 1) by the same aforementioned procedure. This creates the cell compartment and ICC. The final length of the two-sided bioreactor is 15 cm, resulting in a cell compartment volume of 0.33 ml, calculated from the following equation:

\[
\text{Cell compartment volume} = L_{MF} \pi (r_{idmf}^2 - r_1^2) \tag{1}
\]

where \(L_{MF}\) is the length of the middle fiber, \(r_{idmf}\) is the inner radius of the middle fiber, and \(r_1\) is the outer radius of the inner fiber. This equation is used to calculate the increase in the cell compartment volume as a function of \(r_{idmf}\) as described in the Introduction.

Quality assurance (QA) was performed at each of the three stages of the bioreactor construction. The first QA procedure was the visible inspection of leaks in the outer gas compartment: the outer compartment is filled with double distilled water and pressurized to 200 mmHg, and an inspection is made to test for water leakage at the housing/manifold 1 and aeration fiber/manifold 1 interfaces. At the second and third stages of construction, QA protocols are similar to those previously described in which the cell compartment and ICC are pressurized to 200 mmHg, and if leaks are visible around the potted fiber, they are sealed with epoxy or discarded. This rarely occurs. Rather, more frequently, the fibers are clogged with epoxy, in which case these bioreactors are discarded.

**Radial Flow**

Radial flow through the hollow fibers (\(Q_r\)) was predicted based on the following general equation for flow-through hollow fibers (Cussler, 1998):

\[
Q_r = A L_p [\Delta P - \Delta \Pi] \tag{2}
\]

where \(L_p\) is the hydraulic permeability, \(A\) is the surface area of the hollow fiber, \(\Delta P\) is the pressure drop across the hollow fiber, and \(\Delta \Pi\) is the osmotic pressure drop across the hollow fiber. Because distilled water was used in these studies, the value for \(\Delta \Pi\) is zero. The hydraulic permeability values, \(L_{p1}\) and \(L_{p2}\), of the inner and middle polysulfone hollow fibers, respectively, were determined empirically using similar methods as described by Lewinska et al. (1997). Figure 2B depicts the various parameters measured in the flow experiment and in
Equation 2 (see figure legend for definition of additional variables). Single fibers were sealed at one end with epoxy connected to Pharmad™ tubing using a silicon sealant. The tubing was connected in series with a pressure transducer and syringe pump, and the syringe pump was set to several different flow rates, and the pressure was recorded at each. This method was repeated to collect data for three inner fibers and for three middle fibers.

To experimentally determine the relationship between $Q_t$ and $\Delta P$, double distilled water was channeled into the ICC and ICC at a constant flow rate using two syringe pumps. The experimental design for these flow studies is represented schematically in Figure 3. A pressure drop between the ICC and ECC directed radial flow inward from the ECC to the ICC. Pressures at the ICC and ECC inlets and outlets were measured with four pressure sensors (with a range of 0 to 260 mm Hg) attached to an amplifying electronic circuit (Sensym, 1998). The Tektronics oscilloscope measured the amplified voltage from the sensors, and this voltage was used to calculate the pressures. A crystal engineering pressure calibrator traceable to National Institute of Standards and Technology (NIST) standards was used to determine the pressure voltage relationship for each sensor under controlled conditions. Pressure in the cell compartment was measured using the same pressure calibrator. A Teflon™ valve was attached to the ECC exit to control the radial flow rate. Radial flow rate into the ICC was measured by collecting the ICC effluent and then subtracting the known ICC inlet flow. Similarly, radial flow out of the ECC was measured by subtracting the collected ECC effluent from the ECC inlet flow. The results shown are an average of these two measurements.

MRI Studies

To determine and visualize axial flow in the ECC, phase-contrast velocity-encoded MRI (Moran, 1982) experiments were conducted on a 7.1 T horizontal-bore instrument (Bruker Instruments, Fremont, CA). The same setup described above and shown in Figure 3 was used except that none of the pressure sensors were present. The bioreactor was placed such that its center axis was oriented along the bore axis (i.e., laboratory z-axis) of the MR magnet. In all, three sets of MRI flow measurements were performed, each with a different flow configuration. The first configuration was with the valve wide open; the second was with the valve partially closed to allow approximately half of the ECC influx to flow radially into the ICC; and the third configuration was with the valve completely closed so that all the ECC inlet flow was forced through the fibers radially into the ICC.

For each set of flow measurements, a three-dimensional MR image (2.0 cm FOV and 256 × 128 matrix size in the radial plane, 16 consecutive slices of 1.5-mm thickness in the axial direction) was obtained using a standard spin echo sequence (150 ms TR, 13.3 ms TE, and 2 averages) inserted with a pair of half-sine-velocity-encoding magnetic field gradient pulses (duration $\delta$ of 3 ms, and separation $\Delta$ 7.2 ms) with peak amplitudes $G$ at each of 1, 3, 8, and 9 G/cm. With this velocity-encoding scheme, the pixel intensity phase $\phi$ would be related to the flow velocity $v$ according to:

$$\phi = \phi_0 + \frac{2\gamma v\delta G}{\pi},$$

where $\gamma$ is the gyromagnetic ratio constant for protons (42.58 MHz/T), and $\phi_0$ is the phase-constant offset not associated with the velocity-encoding pulses.

Subsequent to data acquisition, flow velocity was calculated from phase-sensitive reconstructed MR images on a pixel-by-pixel basis using Matlab (v. 5.3, Math Works, Inc., Natick, MA). The middle plane was used to calculate the axial flow for each flow configuration. The velocities in the ECC region were integrated and multiplied by the area of the field of view. The MRI-derived axial flow was compared to the experimental measurement of axial flow to corroborate both methods. In principle, the radial flow can be assessed either directly by velocity encoding in the corresponding gradient directions (i.e., laboratory $x$- and $y$-axes), or indirectly from differences of axial flows between consecutive slices. However, the finite signal-to-noise ratio (SNR) available at the image resolution and the extremely low velocity values involved, precluded accurate radial flow measurements.

RESULTS

A novel multicoaxial bioreactor was developed for adherent cell types such as liver, and liver cells are being used to test its efficacy. The bioreactor design mimics critical parameters of the liver acinus. The radial hydrodynamics in this multicoaxial bioreactor were
assessed to determine the hydraulic permeability of the membranes necessary to generate the radial flow rate desired \( Q_r \) at a given cell compartment pressure \( P_c \).

The hydraulic permeability values of the middle and inner fibers were determined experimentally from the slope of the least-squares linear fit of \( Q_r \) versus transmembrane pressure data. The hydraulic permeability, \( L_{p1} \) and \( L_{p2} \), with 95% confidence are \( 3.611 \pm 0.07 \times 10^{-3} \) mL/min/mm Hg/mm² \( (r^2 = 0.982) \) and \( 5.311 \pm 0.033 \times 10^{-6} \) mL/min/mmHg/mm² \( (r^2 = 0.974) \), respectively. These results show the large effect that pore diameter (0.65 μm for inner fiber and 0.1 μm for middle fiber) and fiber thickness (0.2 mm for inner fiber and 0.5 mm for middle fiber) has on the hydraulic permeability value.

The experimentally determined hydraulic permeability values of the two fibers, \( L_{p1} \) and \( L_{p2} \), were used to predict the relationship between \( \Delta P \) and \( Q_r \) and between \( P_c \) and \( Q_r \). These results were compared to radial flow experiments. Figure 4A shows the correlation between \( \Delta P \) and \( Q_r \) from the experimental data as well as the predicted relationship. Flow was directed toward the ICC where the gage pressure was zero throughout the experiment. Therefore, the ECC was the site of the variable pressure, and \( \Delta P \) can be considered the same as the ECC pressure. As expected, experimental data showed that the pressure drop increased linearly with increasing radial flow rate \( (r^2 = 0.989) \). The fiber with the smallest hydraulic permeability value has the greatest effect on \( \Delta P \). The middle fiber is relatively nonporous as compared to the inner fiber and, therefore, the middle fiber requires a large transmembrane pressure gradient to drive the radial flow. Figure 4B is a graph of \( P_c \) versus \( Q_r \) with experimental data from the same flow studies displayed in Figure 4A and the theoretical prediction. Using \( L_{p1} = 3.61 \times 10^{-4} \) to calculate the pressure drop across the inner fiber with Equation 3, there is a linear relationship between \( P_c \) and \( Q_r \) that compare reasonably well with the experimental data \( (r^2 = 0.884) \). The permeability of the middle fiber has a negligible effect on \( P_c \) using this flow configuration.

Figure 5 displays the transaxial phase-contrast velocity-encoded MRIs of the axial velocity for three different radial flow configurations. In all three cases, there is no axial flow in the cell compartment, whereas the parabolic velocity profile in the ECC suggests the flow to be laminar. The MRIs also show laminar flow in the ICC for the no radial flow condition (Fig. 5A), but no velocity is seen for the other two cases (Fig. 5B and C). The apparent lack of flow in the later two cases is due to large velocities in this region, which results in a phase shift that is too large to be “unwrapped,” resulting in the velocity being filtered out as noise. Table I shows that the axial flow values obtained from the MRIs were not significantly different than those obtained from the flow experiments, further supporting the model’s assumption that radial flow is constant along the length of the bioreactor. Also, the MRIs demonstrate that the fibers are not always precisely centered with respect to each other when in a coaxial configuration, as previously described (Macdonald et al., 1998), causing asymmetric flow and possibly non-uniform pressure profiles. The MRI data corroborate the model’s boundary condition that assumes that there is no axial flow at the walls of the fibers.

**DISCUSSION**

A novel multi-coaxial bioreactor is described with hydrodynamic properties appropriate for three-dimensional growth of adherent cell types such as liver. It is hoped that this bioreactor design can be used to expand human liver cells to generate a human bioartificial liver (Xu et al., 2000). A minimum number of cells can be inoculated per coaxial fiber pair and, therefore, a larger annular cell compartment volume will permit greater expansion of the cells resulting in greater biomass per cell inoculated. However, to accommodate the mass transfer requirements of this greater biomass, radial
convection is needed. Therefore, hydrodynamic studies were performed to predict the optimum hydraulic permeability values of the inner and middle coaxial fibers using physiological blood flow velocities and hydrostatic pressure as input parameters. Empirical studies of bioreactors constructed from commercially available hollow fibers confirmed predicted relationships between radial flow and pressure (Fig. 4). Using Equation 2, the radial pressure profiles were generated.

Table I. Mid-plane axial flow rate (mL/min).

<table>
<thead>
<tr>
<th>Flow configuration</th>
<th>Flow Experiment</th>
<th>MRI</th>
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<tbody>
<tr>
<td>No radial flow</td>
<td>1.0 ± 0.03</td>
<td>0.96 ± 0.04</td>
</tr>
<tr>
<td>Half radial flow</td>
<td>0.70 ± 0.02</td>
<td>0.68 ± 0.03</td>
</tr>
<tr>
<td>Full radial flow</td>
<td>0.50 ± 0.04</td>
<td>0.52 ± 0.03</td>
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Figure 5. Transaxial phase-contrast velocity-encoded MRIs of the bioreactor obtained at five different locations and at three different radial flow rates 0 mL/min (A), 0.2 mL/min (B), and 0.3 mL/min (C). The colored velocity scale is shown on the side.

Figure 6. A radial pressure profile across the bioreactor with the fibers used in the study (A) and the optimum fibers predicted from the model (B) for flow going in the direction of either the ECC to ICC or ICC to ECC.

Figure 6 shows the radial pressure profile for an inner fiber permeability value of $3.61 \times 10^{-4}$ mL/min/mm Hg/mm$^2$ and two different middle fiber permeability values, $5.31 \times 10^{-6}$ mL/min/mmHg/mm$^2$ (Fig. 6A—existing middle fiber permeability values) and $1.56 \times 10^{-4}$ mL/min/mmHg/mm$^2$ (Fig. 6B). The hydrostatic pressure in the cell compartment will depend on the permeability value of the inner fiber if the radial flow is directed inward from the ECC to the ICC, or on the permeability value of the middle fiber if the radial flow is directed outward, from the ICC to the ECC. For example, Figure 6A demonstrates that the cell compartment pressure is 23 or 678 mmHg depending on the direction of radial flow (0.024 cm/s). Flow directed from the ECC toward the ICC better mimics the liver acinar blood flow (Fig. 1B) and is required in the present multioxaxial bioreactor configuration due to oxygenation of media in the ECC before entering the cell compartment (Fig. 1A). Therefore, the optimum hydraulic permeability values for $L_{p1}$ and $L_{p2}$ are $3.61 \times 10^{-4}$ mL/min/mmHg/mm$^2$ and $1.56 \times 10^{-4}$ mL/min/mmHg/mm$^2$, respectively, using a radial velocity of $2.4 \times 10^{-2}$ cm/s and a cell compartment pressure of 23 mmHg. The pressure profile calculated for this configuration is shown in Figure 6B.

High pressure in the ECC may result in out-gassing, where gas comes out of solution and forms small bubbles in the compartment. These bubbles typically have a deleterious effect on most cell types. Oxygen is the least
soluble of the physiologically relevant gases, and would be most likely to out-gas particularly due to the direct oxygenation of a high pressurized ECC compartment (Fig. 1A), as depicted in Figure 6A. For example, the solubility of 20% oxygen in water at 0 mmHg and 760 mmHg (gauge reading) is 0.223 µmol/mL and 0.446 µmol/mL (Bailey and Ollis, 1986), respectively, and oxygen would be forced out of solution as it passes through the middle fiber, going from high pressure in the ECC to low pressure in the cell compartment (Fig. 6A). In addition, concentration polarization resulting from membrane fouling, or the clogging of membrane pores, increases the concentration of charge at the surface of the membrane (Belfort et al., 1994), thereby decreasing local oxygen solubility at the membrane surface. In fact, small bubbles form on highly fouled polypropylene membranes during radial flow of serum-containing media (Macdonald et al., 2001). Therefore, to avoid out-gassing, the pressure in the ECC should be minimized.

The fiber permeability will decrease with cell culture because of membrane fouling, and this is especially true for a radial-flow configuration whereby macromolecules in the media or derived from cells will clog the membrane pores (Tharakan and Chau, 1986a and 1986b). Fouling will decrease continually the hydraulic permeability values with time (Belfort et al., 1994). A cross flow configuration (i.e., flow parallel to the membrane surface) is far superior to dead-ended flow configuration (i.e., flow perpendicular to the membrane surface) in reducing membrane fouling (Macdonald et al., 2001), primarily due to enhanced shear force at the membrane surface (Belfort et al., 1994).

MRI studies of bioreactors have been reviewed recently (Fernandez, 1996; Brindle, 1998; Macdonald et al., 1999; Xu et al., 2000). T1 and T2-weighted MRI has been used to determine cell distribution in coaxial hollow fiber bioreactors (Custer, 1988; Macdonald et al., 1998), whereas Fourier velocity zyngmatographic imaging has been applied to observe Starling flow in conventional hollow fiber bioreactors (Hammer et al., 1990; Donoghue et al., 1992; Zhang et al., 1995) and axial flow in spirally-wound bioreactors (Fleidrig et al., 1997). However, this is the first report of quantifying axial flow rates by phase-contrast velocity-encoded MRI in a radial-flow hollow fiber bioreactor. The experimental measurements from velocity-encoded MRL corroborated those obtained from the radial flow experiments, and confirmed the assumptions and boundary condition of the hydrodynamic model.

The velocity-encoded MRI (Fig. 5) clearly shows that there is no axial flow at the walls of the middle fiber and that the axial fluid flow in the ECC and ICC is laminar. Also, flow experiments determined that there was a negligible pressure drop axially across the ICC and ECC and, therefore, the axial flow was considered to be constant across the axis of the bioreactor as found by others for similar radial-flow configurations (Tharakan and Chau, 1986b; Tharakan et al., 1988). Therefore, the data obtained from the flow experiments correlated well with the predictions of the radial flow (Fig. 4, A and B), and the MRI studies corroborated the assumed axial flow profiles in the ICC and ECC compartment. The combination of standard flow experiments of bioreactors as described by others (Tharakan and Chau, 1986b) and velocity encoded MRI are a powerful combination of empirical methods to establish mathematical models for bioreactors.

One unique and important finding resulting from the non-invasive aspect of MRI was that flow is not axissymmetric in all compartments; for example, (1) near the entry port of the ECC (data not shown), (2) the ECC when the middle fiber is not centered with respect to the aeration fiber (Fig. 5), and (3) the radial flow is asymmetric in the cell compartment when the inner fiber is not centered with respect to the middle fiber. The ECC inlets are located at the top outer radius of manifold 2 (Fig. 2A), resulting in higher velocities near the inlet point and lower velocities at the bottom of the ECC compartment. A flow distributor could be used to negate this effect. The middle fiber is now pulled taut before potting to center the middle fiber with respect to the aeration fiber. The asymmetric inner fiber orientation, as previously found by T2-weighted MRI of a coaxial bioreactor (Macdonald et al., 1998), has been shown to become centered by cell culture, as cells expand to fill the cell compartment (Custer, 1988). Similar results would be expected for the expansion of liver cells in the bioreactor. These results show the value of using MRI to determine the velocity distribution inside the bioreactor and will likely be of greater value after inoculation of cells to determine cell viability and distribution, and radial flow paths.

We found that commercially available hollow fibers that are geometrically large enough for the middle fiber typically have hydraulic permeability values that are approximately two orders of magnitude less than optimum. A more permeable middle fiber is required to prevent excessive pressures. MRI is a powerful noninvasive technique that can be used to monitor changes in hydrodynamics, cell distribution, and fiber orientation during proliferation of the liver cell culture, and also to empirically test flow and geometry assumptions.

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