DEFINED FLOW REGIMES: A NOVEL PERFUSION BIOREACTOR FOR OPTIMUM CELL GROWTH

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INTRODUCTION

Vascular cells are subjected to hemodynamic forces [1]. Shear stress acts tangentially to the long axis of the vessel and is caused by the blood flowing, while circumferential stress originates by changes in the vessel diameter and the compressive stress due to the hydrostatic pressure [2]. Laminar shear stress induces endothelial cells to flatten, elongate and align in the direction of the flow [3]. On the other hand, circumferential cyclic strain induces these cells to elongate and align transversally to the direction of stretch [4,5]. It is also known that these physical forces modify the biological response of vascular cells [6]. Recently it has been found that pulsatility is another factor that influences cellular proliferation, morphological rearrangements and biochemical production [7-9].

MATERIALS AND METHODS

Cell culture on tubular structures

Bovine aortic endothelial cells (BAEC) were isolated from bovine aortas according to the collagenase dispersion method [10] and cultured to passage 4 in Dulbecco's modified Eagle medium (DMEM, Gibco BRL Products, NY) containing 10% fetal bovine serum (FBS, HyClone, UT), 1% of penicillin-streptomycin (PS), and 1% L-glutamine (G) (Gibco BRL Products) in a humidified incubator at 37^oC and 10% CO_2.

BAEC were seeded on the inner surface of Silastic® Laboratory tubing (Dow Corning, NJ). Prior to cell seeding 14cm of Silastic® Laboratory tubing was washed for 20min with a 0.2% sodium dodecyl sulfate solution (Fluka, Switzerland) and then twice with distilled water. Subsequently tubes were plugged with metal luers and 1-way stopcocks (Popper&Sons, NY) and autoclaved for 20min. Once sterilized, the inner surface of the tubes was coated for 2h with a solution of 100μg/ml of fibronectin (Fn, Sigma-Aldrich, MO) in PBS (pH 7.4, Gibco BRL Products). Then, the Fn solution was replaced by complete medium for 1h incubation to remove loosely attached Fn. At this point, the tubes were filled with 2.5ml of cell suspension (109534±16192 cell/ml) and incubated at 10rph for 24h using a rotisserie apparatus to guarantee homogeneous cell attachment.

Cell number determination

After 24h seeding time (day 0), the tube content was collected, the luers removed and the tubes washed with PBS. Subsequently, to detach the cells, the tubes were immersed in 5ml of trypsin (Gibco BRL Products) for 5min at 37^oC. The enzymatic digestion was stopped by addition of 15ml of complete medium. Silastic® tubes were shaken several times to assure complete cell dispersion. Cell number was determined using a Coulter counter (Beckman, FL).

Physical Description of the Perfusion System

For every channel of the bioreactor, perfusate flows from a custom-made glass reservoir through a 40cm-inlet length rubber tubing (L_{in}, Tygon® laboratory tubing, 4.8mm ID, Saint Globain performance plastics, OH), and thereafter circulates through a 9cm-length segment (Silastic®, laboratory tubing, 4.78mm, Dow Corning, MI) containing the cultured cells (test segment). After the test segment, the medium flows through a 40cm-outlet length tubing (L_{out}), and returns to the reservoir via 35cm of the same Tygon® rubber tubing. The entire apparatus is placed outside the incubator. To maintain the media at 37^oC, the reservoirs are placed in a water bath (Napco, VA). A 10% mixture of CO_2 and air (Boc Gases, MA) is continuously bubbled in the media stored in the reservoirs. The amount of gas in the system is controlled using a CO_2 controller (CO_2 controller, Cole- Parmer, IL). Flow and pressure are controlled by an 8-channel programmable peristaltic Ismatec® pump (Cole-Parmer, IL), which propels media through peroxide cured silicone tubing (Cole-Parmer, IL). An analog circuit that generates a 0 to 5 VDC wave with a 60% of duty cycle gives the signal to the pump. Instantaneous and average volumetric flow are monitored using an ultrasound flowmeter (Transonic Animal Research, NY). These data were processed through an A/D converter, and stored for off-line analysis on a digital computer.

RESULTS AND DISCUSSION

Instantaneous flow rates and pressure gradients were directly recorded for several flow frequencies. As an example, graphs a and b (cf. Fig. 1) show the flow wave form obtained at 0.12Hz and 1Hz, respectively.

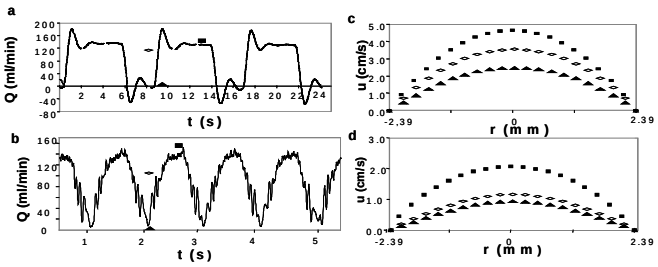

Figure 1. Flow profiles and waveforms

Only at low frequencies a square wave is obtained. At high frequencies due to physical limitations the form of the wave becomes more sinusoidal. The measures of the gradients of pressure and flow were used to calculate the velocity profiles (cf. Fig. 1c, 1d). These profiles were then applied to compute the shear rate and shear stress (viscosity being measured separately). Shear stress was calculated, assuming laminar flow of a Newtonian fluid through a straight rigid tube with a periodic axial pulsatile pressure gradient and following a Womersley-type solution [11]. The instantaneous axial velocity profile can be obtained using the following expression:

$$u(r,t)=\frac{-G_0}{4\nu}(R^2-r^2)+\sum_{n=1}^{\infty}\frac{G_n}{in\omega}\left[1-\frac{J_0(i^{3/2}\alpha_n r/R)}{J_0(i^{3/2}\alpha_n)}\right]e^{in\omega t} \qquad (1)$$

Where R is the radius of the tube, ω is the fundamental angular frequency, ν is the kinematic viscosity, r is a radial coordinate is the time, J_0 is the Vessel function, G_n is the pulsatile pressure gradient amplitude, α_n is the Womersley parameter that is calculated as:

$$\alpha_n = R\sqrt{\frac{n\omega}{\nu}} \qquad (2)$$

The constants G_n are determined by the Fourier coefficients of the pressure gradient waveform using the equation:

$$\Delta P = \sum_{n=0}^{\infty} G_n e^{in\omega t} \qquad (3)$$

The shear stress is the calculated using the waveform and the expression:

$$\tau(t) = \mu \frac{\partial u(r,t)}{\partial r}_{r=R} \qquad (4)$$

The computed average low shear stress throughout one cycle remained between 0.94 and 1.02 dyn/cm^2 for all frequencies. The values for high shear stress vary between 3.76 and 4.08 dyn/cm^2. The number of attached cells was measured after 24 hours of incubation in the perfusion system (day 1). The experiments were performed at low and high shear stress values and in a range of frequencies from 0 Hz (steady flow) to 1.5Hz. The results obtained are summarized in table 1.

Frequency/Hz	Low shear stress	High shear stress
0	1.65±0.07	1.68±0.10
0.25	1.44±0.07	1.43±0.06
0.5	1.71±0.21	1.10±0.09
1.0	2.88±0.28	3.00±0.10
1.5	1.51±0.18	1.68±0.23

Table 1. Quotient between cell number measured at day 1 and cell number at day 0

Interestingly, cell number at day 1 was 3-fold the cell number at day 0 only when 1Hz flow frequency was applied to the system independent of the mean shear stress.

CONCLUSIONS

A new profusion system have been developed and fully characterized to study cell growth under defined flow conditions. Cells cultured using this system are viable throughout all the experiments; and more importantly, cells experienced maximum growth at 1Hz flow frequency at both levels of shear stress applied. Culture conditions are a key point in the development of artificial grafts. In order to optimize the growth of cells cultured *in vitro* is essential to reproduce the environmental conditions that cells usually confront *in vivo* and this is the main goal of this work.

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