

A High-Throughput Bioreactor System for Simulating Physiological Environments

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Abstract—The optimization of *in vitro* cell culture for tissue engineering, pharmacological, or metabolic studies requires a large number of experiments to be performed under varying conditions. In this paper, we describe a high-throughput bioreactor system that allows the conduction of parallel experiments in a simulated *in vivo*-like environment. Our bioreactors consist of tissue-, organ-, or system-specific culture chambers and a mixing device controlled by an embedded system that regulates the insertion of gas in the culture medium in order to control pH and pressure. Each culture chamber and mixing device possesses an autonomous control system that is able to ensure an optimal environment for cells. A computer communicates with the embedded system to acquire data and set up experimental variables. With this apparatus, we can perform a high-throughput experiment controlling several bioreactors working in parallel. In this paper, we discuss the architecture and design of the system, and the results of some experiments which simulate physiological and pathological conditions are presented.

Index Terms—Bioreactor, cell culture, high throughput, mimic physiological environments.

I. INTRODUCTION

CELL CULTURE is an essential tool in biological science, clinical science, and biomedical studies. This approach is a fundamental step in preclinical drug testing, and for this reason, it is of great interest to the pharmaceutical industry to employ cheaper and more ethical systems which can supply accurate and predictive information on the effects of chemicals on the human body.

Because drug testing involves a large number of tests on identical cell cultures, a single well culture is inadequate and costly both in time and money. The “high-throughput screen-

ing” (HTS) is a methodology for scientific experimentation widely used in drug discovery, based on a brute-force approach to collect a large amount of experimental data in less time and using less animals.

HTS is achieved nowadays using multiwell equipment to contain the cell cultures subject to treatment [1]. An automatic machine collects data, usually with an optical system, during the treatment. Collected data can vary widely in nature, for instance, concentrations of physiological metabolites or proteins.

The parallel nature of HTS makes it possible to collect a large amount of data from a small number of experiments and in a very short time. The multiwell system, however, suffers from a significant problem that may affect the relevance of tests: the environment discrepancy problem [2]. The environment discrepancy problem lies in the fact that the tissue grown in wells is only a brutal approximation of biological reality. There are several relevant factors that are missing in this environment; for instance, the cells in the well are not subject to the convective flow of nutrients present in the physiological environment. Another meaningful example is the lack of the typical pressure peaks and the presence of constant solute concentrations, unlike in biological systems where gradients of concentration are the basis of most important processes. In [3], it is discussed how the multiwell approach does not scale fully as expected by an HTS system because the collected data are not directly usable in drug testing. This seems to be a paradox because the multiwell has been the core element of the HTS methodology.

In this paper, we propose to use bioreactors to obtain an HTS system that overcomes the limits due to the environment discrepancy problem and obtain valid data from experiments which simulate a physiological environment.

A bioreactor is a system able to maintain a cell culture in a controlled environment aimed at the simulation of a living organism. Two principal elements in the system combine to provide a biomimetic habitat: the environmental control system and the structural, chemical, and microfluidic dynamic framework of the bioreactor.

Although several different types of bioreactors [4], [5] have been reported, particularly for tissue engineering and pharmaceutical applications, none has been specifically designed for HTS. Pulsatile flow systems [6], rotating wall low shear reactors [7], [8], and compression bioreactors [9], for example, are all very large and bulky devices which do not lend themselves well to HTS or miniaturization. On the other hand, microfluidic systems, such as those described by Kane *et al.* [10], and the animal on-a-chip device in [11] are more amenable to parallel processing.

Here, we present a new bioreactor, called multicompartment bioreactor (MCB), through which we are able to perform

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high-throughput experiments in an *in vivo*-like simulated environment for a long time (more than a week). Two different compartmental cell culture chambers which simulate a blood vessel and the metabolic system, respectively, are described. Each chamber uses the same basic environmental control system and can be used for HTS experiments.

The first is a laminar flow chamber, in which known shear stresses and hydrostatic pressures can be applied in order to mimic physiological or pathological conditions in the cardiovascular system. The second unit is a multicompartamental device in which different cell types are cultured in series and in parallel to simulate a metabolic system [12]. Together with the environmental control system, the chambers provide a biomimetic habitat for cells and enable different *in vivo* scenarios to be more closely approximated.

The data extracted from this kind of culture is more predictive of the *in vivo* response with respect to the multiwell approach particularly for drug related studies because the bioreactor minimizes the environment discrepancy problem.

II. MATERIALS AND METHODS

The MCB bioreactor is a complex system. In this section, we discuss the three main elements of the system: the bioreactor hardware, the embedded system and the control software, and the algorithm used to regulate pH. Two HTS experimental protocols using two different culture chambers are also described.

A. Bioreactor Hardware

The bioreactor system consists of the following parts:

- 1) cell culture chamber;
- 2) mixing chamber;
- 3) electronic circuit and electrovalve box;
- 4) peristaltic pump;
- 5) PC;
- 6) heating system.

1) *Cell Culture Chamber*: This part of the system is the core of the bioreactor: It contains the cell monolayer or scaffold where cells are seeded. The chamber is made entirely of *polydimethylsiloxane* (PDMS), a biocompatible silicone polymer (Sylgard 184, Dow Corning).

We have developed several types of cell culture chambers [13], [14], and each unit can be plugged into the system and used for different kinds of experiments. A laminar flow chamber was specifically designed to enable cells to be subject to a large range of shear stresses. Several cardiovascular pathologies are associated with altered patterns of flow and shear stress. The study of endothelial cell response to different flow conditions is therefore important for understanding and curing cardiovascular diseases.

Using finite-element methods and imposing a number of design criteria such as maximum volume and minimum area for cell culture, as well as the range of shear stresses desired, we converged to a final design for the chamber. Fig. 1 shows the chamber design and the constant and controlled velocity vector in the cell culture zone.

By changing the flow rate of the fluid, constant and controlled shear stresses ranging from 40×10^{-3} to 900×10^{-3} Pa can be applied to simulate aortic as well as capillary shears in physiological or pathological states.

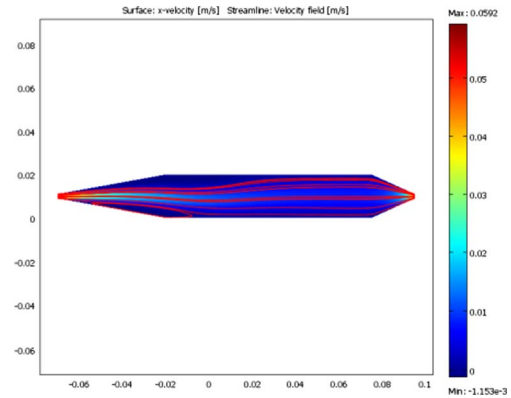


Fig. 1. Finite-element method simulation of the laminar flow bioreactor chamber with a medium flow speed of 0.05 m/s (12 mL/min).

Equation (1) allows the shear stress (τ) on the cell layer to be calculated from the applied volume flow rate (Q) with a medium viscosity (μ), cell chamber cross section (A), and a distance between the center of the chamber and the cell monolayer (z)

$$\tau = \mu \frac{Q}{A \cdot z}. \quad (1)$$

A completely different chamber configuration was adopted to simulate the metabolic system. Here, we used allometric principles to design a series of interconnected chambers in order to represent the principal components of the human metabolic system, focusing on the metabolism of glucose, which is the most important energy source for all organisms. Central to glucose metabolism are the liver and the pancreas, followed by insulin- and glucose-dependent target tissues. To design the system, the relationship between different parameters such as metabolism, time, volume distribution, and organ dimensions was considered in order to scale the physiological interactions present in the human body [15].

Thus, volume, flow rates, and cell numbers were downscaled using relationships based on allometry [16]. The simplest connected culture chamber device refers to a system with four cell types considered relevant to glucose metabolism (hepatocytes, pancreatic islets, visceral adipose tissue, and endothelial cells).

The general equation for allometric scaling is

$$Y = Y_o M^b \quad (2)$$

where Y is the parameter in question (flow rate, metabolism, etc.), Y_o is the proportionality factor, M is body mass, and b is the allometric scaling exponent. The mass of the standard human body is 68.5 kg, and our system is approximately 20 g.

In the case of volume and of the cell ratios, the exponent $b = 1$. Consequently, we first considered the ratios of cells in the system and scaled these down linearly in accordance with the allometric equation. The flow rate was scaled using $b = 3/4$ with respect to the portal flow rate. This flow rate also corresponds to small blood vessel and capillary flow velocities and to the range of shear stress typically found in blood vessels [17]. Because our main focus of interest is the study of glucose metabolism, the reference physiological data were referred to data on blood glucose levels in normal humans [18]. Through these data, the distance between the liver compartment and the other three compartments were estimated (liver–pancreas from

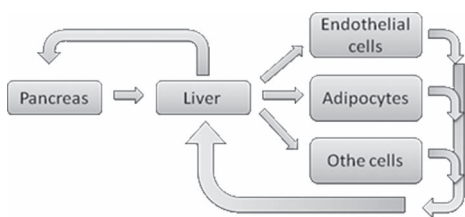


Fig. 2. MCB chamber topological scheme.

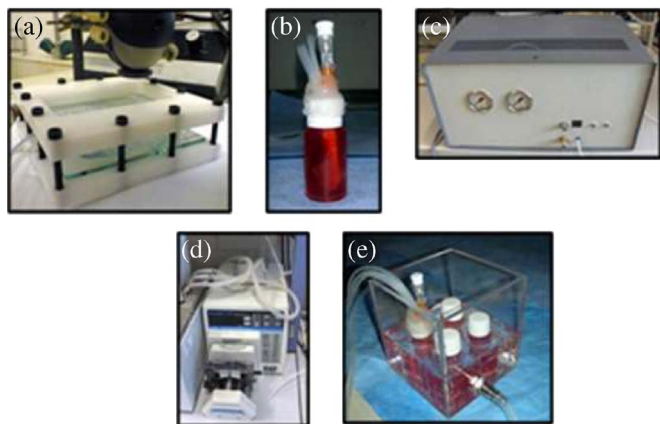


Fig. 3. (a) Cell culture chamber. (b) Mixing chamber. (c) Electronic and electrovalve box. (d) Peristaltic pump. (e) Heating box with four mixing chambers ready for the experiment.

5 to 7 cm and liver–adipocyte from 9 to 17 cm, with times of about 2 and 15 min, respectively) [19]–[21]. A schematic of the connected culture MCB chamber is shown in Fig. 2.

Each MCB unit is realized using an appropriate mould, in which silicone tubing is inserted during the fabrication process such that it is integrated into the chamber. PDMS is cast and cured in the mould by heating at 65 °C and then carefully removed, washed, and sterilized with H₂O₂ gas plasma. Following the sterilization phase, cells are seeded onto the chambers as necessary, and the bioreactor is assembled. The PDMS base is then covered with a gas plasma sterile glass plate, and the two are clamped together by a Teflon frame which can be tightened with screws [Fig. 3(a)] [22].

Medium flow in the chambers is generated by a peristaltic pump (P720, Instech, Plymouth, PA, USA) connected to the tubing and controlled by the electronic control unit for the regulation of flow rate. The electronic unit also controls a heating system below the culture chamber, which maintains the cell culture at constant temperature.

2) *Mixing Chamber*: The mixing chamber [Fig. 3(b)] is connected in series with each cell culture chamber and serves for pH and oxygen regulation as well as to remove air bubbles; essentially, the medium is perfused with gas according to the measured pH. The medium is inserted in this chamber through a needle with the flow imposed by the peristaltic pump. pH regulation is performed by inserting two different gases in the mixing chamber: CO₂ and air (not O₂ because of flammability risks). The culture medium contains bicarbonate buffer, and its pH can be closely regulated through diffusion of gases; in particular, the diffusion of O₂ (Air) tends to raise the pH, whereas CO₂ tends to lower it [23].

3) *Electronic Circuit and Electrovalve Unit*: This part of the system is the heart of the bioreactor control unit; there

are the electronic circuits for the sensors, for communication with the PC, and for the actuation of the electrovalves. The electrovalves are used to select the gas that is injected in the mixing chambers and are connected to a pressure regulator used to control the pressure inside the cell culture chamber.

4) *Computer*: The computer is plugged to the electronic circuit and electrovalve unit through a network cable; there is an embedded system board responsible for controlling the system. We use a *Wildfire 5282* [24], a microcontroller unit based on a Motorola Coldfire microcontroller. The *Wildfire* board is used to control the pressure regulator and the electrovalves and for reading the sensor data; the data are stored on a secure digital card and are sent to the computer using the user datagram protocol (UDP) over the Ethernet connection.

The system is equipped with a liquid crystal display to allow an immediate control of the experiment parameters without a PC.

5) *Heating System*: The bioreactor heating system consists of a Plexiglas box where the mixing chambers¹ are inserted [see Fig. 3(d)]. Water heated by a thermostatic bath is inserted in the heating box, or it is also possible to equip the box with a resistor heater placed under the culture unit.

Culture chambers are also heated independently using a dedicated resistive heater [25]. Both the resistive heaters are controlled by the electronic unit through a pulsewidth-modulation (PWM) power regulator. The PWM signal is generated by one of the digital I/O wildfire channels and is amplified by a dedicated FET-based electronic circuit.

Two thermistors are used to monitor the temperature of the mixing and culture chambers, respectively, and feedback control ensures that the temperature is constant [26], [27].

B. Control System

The microcontrolled board runs the μ TNetOS operating system (OS), a general purpose OS for microcontrolled systems [28] developed for the purpose.

μ TNetOS is a generated OS: The system generator takes as input the description of an eXtensible Markup Language (XML)-based protocol and generates an instance of the OS with the entire communication system tailored for the particular protocol. It uses cooperative multitasking to run concurrent activities.

We have developed an application targeting the instance of μ TNetOS tailored for bioreactors. The goal of the application is to make the bioreactor autonomous by monitoring the environmental variables and taking appropriate actions according to a policy defined through a networked computer and stored within the controller.

Because Ethernet is a communication bus, the graphical application used to control an experiment can receive UDP packets from several units running in parallel. This is very important in the context of HTS methodology [29]. The network also allows connections through the Internet, allowing remote monitoring of experiments, an important feature because experiments run for several days [30].

A high-throughput bioreactor experiment employs many bioreactors with different cell culture chambers. A pathology

¹At least four of them are allowed.

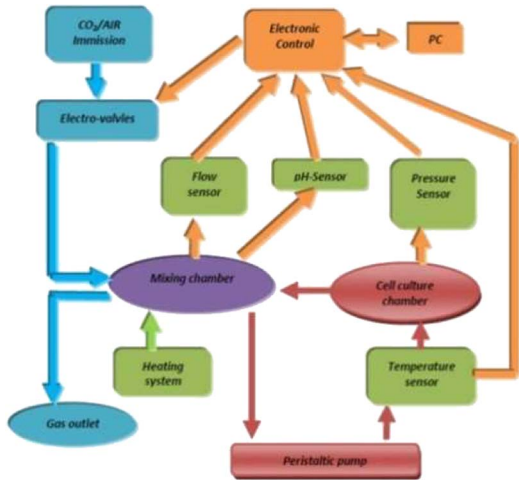


Fig. 4. Schematic flowchart of the bioreactor system. In orange are the electronic control unit and electronic connections; in green are the sensors, and in red are the culture chamber and the medium circuit. The gas connection and apparatus are represented in blue.

can be simulated in one of the Bioreactors, and the others used a control reference; we can setup the environmental variables of the bioreactors in order to simulate one or more pathologies and observe the influence of this different environment on tissue function during an experiment.

μ TNetOS messaging complies with an XML-based standard defined by a robotics programming framework called Robotics4.NET [31], [32]. The framework proposes a software architecture inspired by the architecture of the human nervous system. In this context, the software running on the autonomous embedded system of the bioreactor is perceived as a roblet, a sort of peripheral organ abstraction provided by the framework.

A program based on Robotics4.Net is composed of three ingredients.

- 1) The brain: It is the core of the control system.
- 2) The bodymap: It is a sort of black board used to send and receive messages.
- 3) The roblets: They are the appendix of the system, like the parts of our nervous system. They read data from the environment and convert the brain signal into an action.

In our case, each of the bioreactor systems is perceived as a roblet that communicates with the program on the computer hosting the bodymap.

Because the connections among the roblets and the bodymap are based on a data gram-oriented protocol, the brain can be powered off and restarted afterward without affecting the activity of roblets.

In a bioreactor system, it is necessary to control many parameters: the amount of nutrients flowing in the cell culture chamber, the hydrostatic pressure inside the system, the shear stress of the flow on the cell culture, the flow of gas, the temperature flux generated by the heating system, and the pH of the medium (Fig. 4). These parameters are established using a graphical interface and sent to the roblets running on the bioreactors.

To control the nutrient flow and the shear stress, it is necessary to regulate the speed of the peristaltic pump used to perfuse the nutrients inside the bioreactor, this control is operated by a serial connection between the pump and the controller board and is monitored in a feedback mode through an appropriate control pin of the pump. The hydrostatic pressure inside the

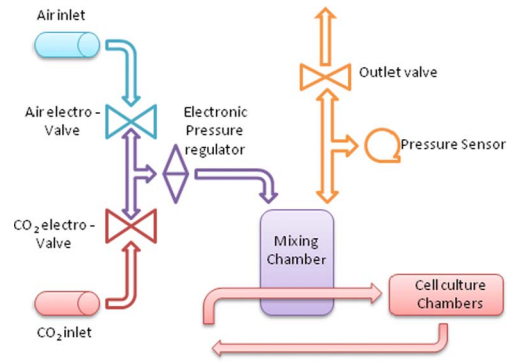


Fig. 5. Schematic connection of the pressure regulation system.

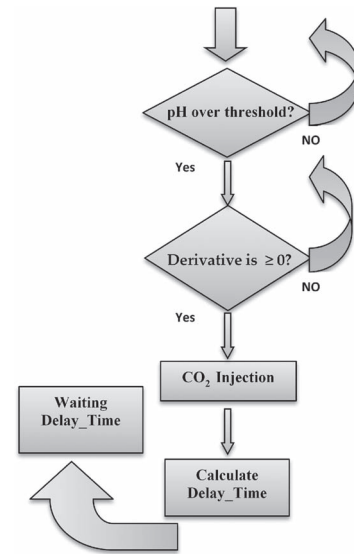


Fig. 6. Flow diagram of the control algorithm for pH regulation.

circuit and the flow of gas through the mixing chamber are regulated by fine control of the pressure regulator and of the outlet; the outlet can be set manually in order to obtain the desired pressure in the mixing chamber. The system reads the pressure inside the mixing chamber with a pressure sensor plugged in parallel with the outlet; in this way, we can control the system in a classic feedback mode (Fig. 5).

The heat flux and the temperature of the cell culture chamber is controlled by a feedback system, which reads the temperature of the cell culture chamber through a negative temperature coefficient sensor placed near the cell monolayer and imposes a voltage across a dedicated resistive heater placed under the cell culture chamber.

As mentioned in [33], the pH is more complex to control than the other parameters because of delays between gas infusion and ionic dissociation; for this reason, we studied a dedicated control strategy based on a variable delay time, which is reported in the following section.

C. pH Control Algorithm

To control the pH in the medium, we developed a control adaptive algorithm based on a step strategy. This control algorithm is a high priority service running on μ TNetOS.

A formal model of the algorithm has been also defined using the abstract state machines (ASM) formalism [34].

The pH response to the diffusion of CO₂ and air is very difficult to predict, because it depends strongly on the environmental variables of the particular experiment such as temperature, volume of medium, type of medium, hydrostatic pressure, number of cells, and their metabolic state. For this reason, we do not use the mathematical model of the CO₂ and air diffusion in water medium but instead use a function defined by an algorithm.

Our system continuously inserts air in the mixing chamber through an appropriate needle; when the pH goes over the safety threshold (0.05 units of pH over a user-defined threshold), the control inserts a known CO₂ impulse, in the mixing chamber, and waits a *delay time*. In this way, we can insert a known amount of CO₂ inside the mixing chamber and wait to evaluate the effect of this operation. If the pH returns under the safety threshold, we can stop the pH control phase; otherwise, after the *delay time* has passed, the control inserts a new impulse of CO₂ and waits for the *delay time*. The *delay time* is adjusted at every step in a manner dependent on the value of pH and the derivative of pH with time.

The pH control strategy includes one safety logic test used to evaluate whether the pH is decreasing or not; in the case of a negative derivative, if the pH value is over the safety threshold, the control does not insert CO₂ because we can assume that the last CO₂ injection was sufficient for the pH control and the pH can return under the threshold in a short time; this test prevents an excessive fall in pH, because the CO₂ injection causes a large drop in pH but with a substantial delay (Fig. 6)

$$\text{TDelay}(n) = \text{TDelay}(n-1) \times [A(\text{pH} - \text{pH}^*) + B(\text{pH}(n) - \text{pH}(n-1))]. \quad (3)$$

Equation (3) describes how the time delay is calculated by the roblets running on the Wildfire 5282 board. The delay time to use at the current step is proportional to the delay time of the last step but with an additional value that is directly proportional to the difference of the current and expected pH values and a part that is proportional to the first derivative of the pH signal coming from the pH meter.

The multiplicative constants A and B are between zero and one; in this way, we added only a part of the $(n-1)$ *delay time* for every control step.

These constants can be predicted by the formal model based on ASM [35] for a water environment but are very sensitive to the physical condition of the experiment (volume and type of medium, pressure, and temperature). Consequently, we have to calibrate the system through the user interface before starting a new session, in order to match the pH control parameter with the experimental setup.

D. GUI Software

Our *graphical user interface* (GUI) is developed in C#.Net and is based on a multitab structure; we use the GUI to read data from the bioreactors and to set up the experimental variables of each one. The user interface also features a tool for sensor calibration, in order to perform the sensor calibration with the same software that we used for bioreactor control.

The user has control over the experiments, including a manual overdrive, although the autonomous control software

running on the embedded systems avoids commands that could damage the system or the experiment.

When the user interface is open, it seeks out connected bioreactors; when one is found, the GUI switches in the view mode. In this section, we can see data of the bioreactor selected by the menu, and experimental settings can be changed through the configuration tab.

E. Cell Culture Experiments

The MCB bioreactor system was tested in a “*virtual laboratory*,” in which we simulated a typical parallel experiment with four “animals.” For each experiment, we installed four bioreactors, and we performed laminar flow and connected culture HTS trails, respectively, with cells in the bioreactor under a constant flow of nutrients and gas.

These experiments demonstrate how our bioreactors can be used to conduct experiments in a high-throughput manner with four parallel chambers and how we can control and store data with a single computer placed in any location.

1) *Laminar Flow Chamber*: The cells used for the laminar flow experiments were human umbilical vein endothelial cells (HUVEC) [36]. They were placed in the cell culture chambers and in an incubator for 24 h in order to ensure their adhesion on the gelatin-treated PDMS base. After incubation, the cell culture chambers were plugged into the bioreactor system, and we ran four sets of experiments at four different flow rates (including zero) for 24 h. At the end of each experiment, we analyzed the culture medium for nitric oxide (NO), a vasodilator, and endothelin (EN-2) [37], a powerful vasoconstrictive molecule which counteracts the effects of vasodilation. Cell morphology and orientation were also assessed by calculating cell eccentricity.

2) *Connected Culture Chamber*: To demonstrate the application of HTS in the MCB connected culture chamber, hepatocytes and endothelial cells (HUVEC) were used in a ratio similar to that in the liver. Hepatocytes were extracted directly from freshly explanted murine (black mice, strain C57BL/6) liver and cultured according to standard procedures [38]. The endothelial cells were prepared according to the method described by Jaffe *et al.* [36].

Glass slides of 12-mm diameters with 80 000 hepatocytes and 8000 HUVEC were inserted into the connected culture bioreactors and then connected to the mixing device and pump and filled with 30-mL William’s culture medium. Controls using only hepatocytes in the absence of HUVEC were also conducted. For these experiments, a flow rate of 175 $\mu\text{L}/\text{min}$ was applied to four systems running in parallel. Experiments were carried out for 24 h, during which 2 mL of the medium was withdrawn for analysis at 2 h 40 min, 4 h 20 min, 6 h, and 24 h and was replaced with 2 mL of fresh medium. During each withdrawal, albumin, an important biomarker for hepatocyte synthetic function, was quantified using a commercial enzyme-linked immunosorbent assay kit and the data compared with standard static multiwell cultures.

III. RESULTS

The experiments showed how the high-throughput bioreactors system can run for up to 24 h in order to simulate the classic parallel “animal” experiments; in this way, we can greatly

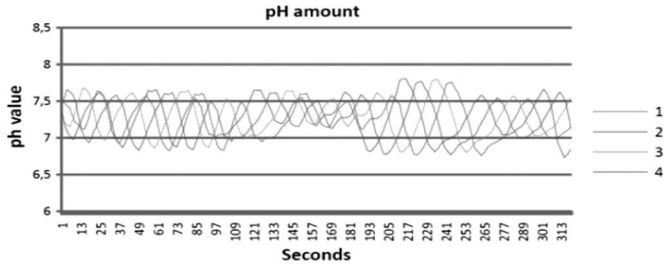


Fig. 7. The pH in the four bioreactor systems during the laminar flow experiment; the numbers in the legend are the four bioreactors ID labels. The acquisition was carried out over 24 h but a 300-s window is shown here.

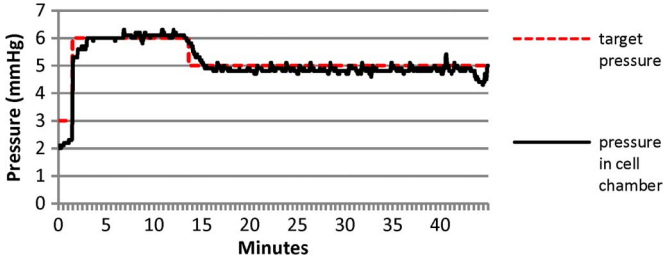


Fig. 8. Pressure graph shows how the control system is able to maintain the desired value of pressure inside the bioreactors.

reduce the number of “animals” that are necessary for the experiments. Another important aspect of the new bioreactor system is that, in this mode, it is possible to perform experiments in an *in vivo*-like biomimetic habitat instead of in a multiwell, which is a brutal approximation of the physiological environment.

The data extracted from the bioreactors during the experiments show how the new system is able to correctly control the environmental variables.

Fig. 7 shows how the new bioreactor system is able to maintain a pH value in the medium around the physiological value of 7.4. It has a maximum error deviation of -1.2 and $+0.3$; the asymmetry of the pH error deviation is a consequence of the pH control strategy. In fact, when CO_2 is inserted inside the chamber, we have a rapid drop of pH; otherwise, when air is inserted, the pH increases slowly; for this reason, it is very difficult to prevent the excessive undershoot of pH because an opening time of the CO_2 electrovalve of only 0.5 s is necessary to control the pH but at the same time gives rise to a slow but large drop in pH. The pressure graph (Fig. 8) shows a pressure step experiment; in this experiment, we choose to increase the pressure in the cell culture chamber with a step in order to simulate hypertensive stress.

A. HTS Laminar Flow

The vascular endothelium is a dynamic organ that responds to various physical and humoral conditions by producing several biologically active substances, both vasoconstrictors and vasodilators, which control these processes. Therefore, to assess the ability of the laminar flow system in maintaining functional properties that cells possess in physiological conditions, we examined the production of these important vasoactive factors (NO and EN-2) by endothelial cells in different shear stress conditions. The results are shown in Figs. 9 and 10.

Endothelin production decreases even at very low shear stresses, indicating that the cells respond to static conditions by overexpressing vasoconstrictive functions in an effort to com-

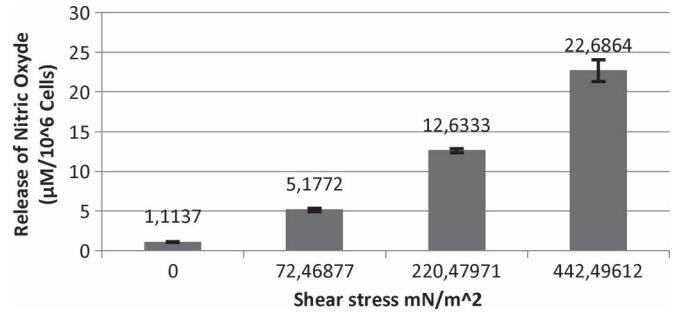


Fig. 9. NO production (micromole per 10^6 cells) after 24 h in the laminar flow bioreactor chambers with different shear stresses.

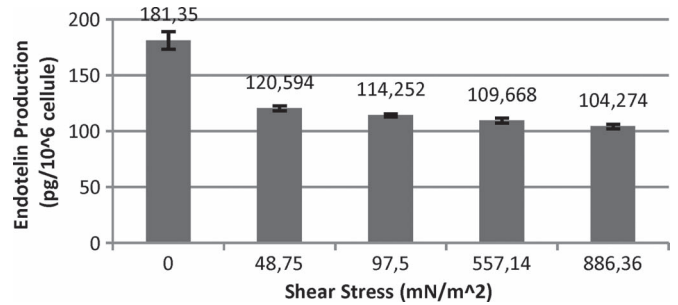


Fig. 10. Endothelin production (picogram per 10^6 cells) after 24 h in the laminar flow chamber with different shear stresses.

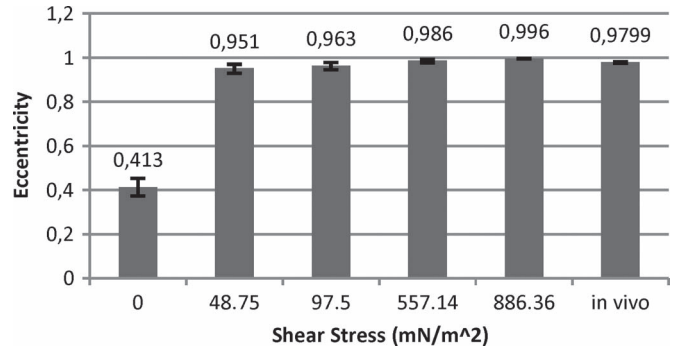


Fig. 11. Change in the cell eccentricity E during a 24-h experiment in a laminar flow chamber with different shear stresses compared with the capillary physiological eccentricity ($E = a/(2b)$) with a as the major radius and b as the minor).

pensate for the lack of mechanical stimulus normally produced by flow. This is also reflected in NO production, which increases steadily with shear stress. Fig. 11 shows the change in eccentricity of endothelial cells, which respond to shear stress by orienting and elongating along the direction of flow as observed *in vivo*.

In Fig. 12, a cell culture after 24 h of laminar flow treatment with the MCB laminar bioreactors is shown. We can observe the vitality of the cells, that do not show any type of cell membrane damage or formation of picnotic nuclei.

The cells can be used as sensors of the environment, to evaluate the consequence of flow treatment. In the micrograph, the endothelial cells are oriented with the medium flow and have elliptical shapes similar to their *in vivo* morphology.

B. HTS Connected Cultures

The connected culture system was conceived using biomimetic principles to reproduce salient features of the

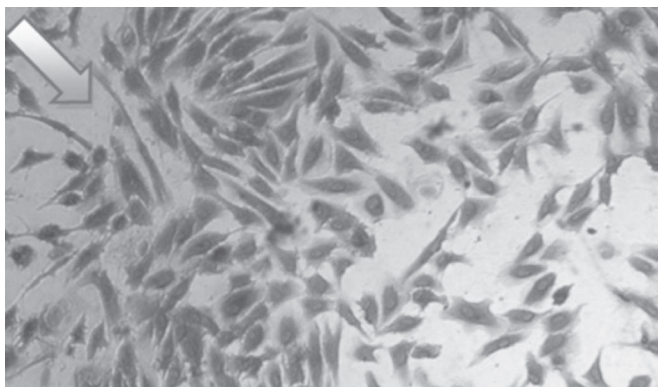


Fig. 12. Cell culture after 24 h of treatment; we can observe how the cells have an elliptical shape and are oriented with the medium flow (indicated by the arrow).

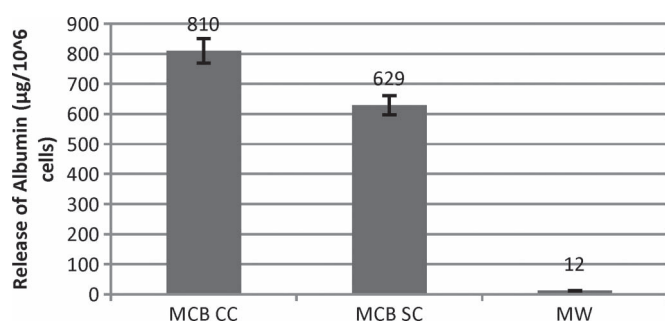


Fig. 13. Albumin production after 24 h of experiment in an HTS MCB connected culture; the MCB CC system is a dynamic (175 µl/min) connected culture of hepatocytes and HUVEC, MCB SC is a dynamic culture in MCB with only hepatocytes, and MW is a static hepatocyte multiwell culture.

metabolic system with particular regard to the metabolism of glucose, fat, and protein. The chamber was designed using allometric laws conserving proportions and ratios between different organs. It allows cell–cell crosstalk to be studied in a rigorous and controlled manner, by addressing and interrogating one cell type at a time, and could lead to a better understanding of the physiological and biochemical complexity. Obviously, a focal point of the system is the hepatocyte chamber, because the liver is the principal metabolic organ in the body. This initial study was therefore on a hepatocyte–endothelial connected culture model considering only proportions between the two cell populations in the liver. Overall, large differences in albumin production were observed between static multiwell and dynamic cultures, and smaller but nonetheless significant differences were observed between dynamic monocultures and connected cultures: evidence of crosstalk between two cell types. Strictly speaking, the static monoculture conditions cannot be directly compared with the connected culture as the medium volumes are quite different. The availability of oxygen and nutrients is increased, the fluid is well mixed, and nutrient depletion in the medium is negligible in the bioreactor system. This is shown in Fig. 13, which shows that albumin production is upregulated by about 1.5 times when compared with connected cultures with dynamic monocultures and over 50 times when comparing dynamic and static multiwell monocultures.

IV. CONCLUSION

In this paper, we have presented an alternative HTS approach for cell culture. Using a purposely designed and constructed

autonomous software and electronic control and acquisition system combined with specific cell culture chambers, it is possible to simulate a biomimetic habitat for cells. The control system and software enable the temperature, pH, pressure, and flow to be tightly regulated or modulated to simulate physiological or pathological environments. Special chamber designs can be used for approximating the geometry, fluid dynamics, and chemical environment of different organs or systems.

The experiments demonstrate how the high-throughput MCB bioreactor system is able to perform experiments in parallel, combining the advantages of *in vivo*-like simulated environments with an HTS control strategy. So as not to limit the applications of the systems, the OS μ TNetOS was generated and not written *ad hoc*; in this way, we can easily change the hardware platform without reengineering the OS. A further innovative aspect is the modular architecture of the hardware which is mirrored by the human nervous system-inspired software architecture.

Our efforts are now being directed to predictive drug toxicology, nanotoxicology, and the works of hypertension-related cardiovascular diseases.

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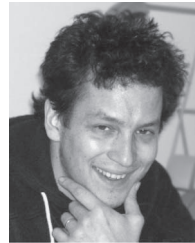
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