

# Real-time monitoring of lactate extrusion and glucose consumption of cultured cells using a lab-on-valve system

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Microsequential injection ( $\mu$ SI) provides microfluidic operations that are ideally suited for cellular function studies and as a means of validating targets for drug discovery.  $\mu$ SI carried out within the lab-on-valve (LOV) manifold, is an ideal platform for spectroscopic studies on living cells that are grown on microcarrier beads and kept thermostated while their metabolism is probed in real-time. In this paper a microbioreactor is integrated into the LOV manifold allowing measurement of cellular lactate extrusion and glucose consumption rates of a cell culture that is automatically renewed prior to each measurement. Glucose consumption and lactate extrusion are monitored using NAD-linked enzymatic assays. The  $\mu$ SI-LOV setup has demonstrated a linear analysis range of 0.05–1.00 mM for lactate and 0.1–5.6 mM for glucose. These assays were conducted in a serial fashion requiring 3  $\mu$ L of cellular perfusate and 10 s for glucose determination and 30 s for the lactate assay. Overall waste generated per lactate/glucose assay is < 200  $\mu$ L. This work was performed using two different transfected hepatocyte cell lines, which adhere to Cytopore<sup>®</sup> microcarrier beads. This novel approach to metabolic screening allows for the rapid evaluation of the effects of dosing cells with chemical agents.

## Introduction

The rate of cellular lactate extrusion, combined with the rate of cellular glucose consumption, provides a means to assess the metabolic regimen of a cell culture. An estimate can be made as to the portion of the cells' energy that is being generated by anaerobic glucose metabolism by comparing these values.<sup>1</sup> The percentage of glucose that is metabolized to lactate can be determined using the stoichiometry of anaerobic metabolism, (2:1 lactate to glucose) and the rates of lactate extrusion and glucose consumption. Furthermore, the percentage of the cells' energy that is derived through aerobic metabolism can be estimated by subtracting the anaerobic percentage from 100. In this way, the portion of the cells' energy that is generated by anaerobic and aerobic metabolism can be approximated.<sup>1</sup>

The metabolic regimen of a cell line can be used to rank the metabolism of that cell line against others. For example, cell lines that have undergone stable transfection can be screened in this manner. Subsequent to transfection each cell line can be screened individually and their metabolic regimens compared. In this manner the effects of the transfection, if any, on cellular glucose metabolism can be examined. In a similar fashion lactate and glucose monitoring can be used to rank primary and secondary cell cultures. Primary cell cultures tend to be much more aerobic than are secondary ones.<sup>2</sup>

Metabolic screening can be used to elucidate the effects of chemical compounds on live cells. By comparing the basal (unperturbed) rates of lactate extrusion and glucose consumption to those in the presence of a chemical agent, the relative effects of the compound on cellular metabolism can be determined.

## Transfected hepatocyte cell lines

The cell lines selected for this work are two stably transfected versions (TABX2S and TABX1A) of a hepatocyte cell line. The cell lines are transfected to either overexpress or not express an outer mitochondrial membrane protein known as Bcl-x<sub>L</sub>, which

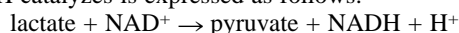
regulates the permeability of important metabolites going into and out of the mitochondria. It is hypothesized that the expression of Bcl-x<sub>L</sub> may have ramifications for the sensitivity of the cell lines to metabolic inhibitors such as antimycin A.<sup>3</sup> The TABX2S cell line is stably transfected to overexpress Bcl-x<sub>L</sub>. The TABX1A cell line is transfected with an antisense construct such that it effectively reduces Bcl-x<sub>L</sub> expression to undetectable levels. The current project is to rank the basal parameters of glucose consumption and lactate extrusion for the two hepatocyte cell lines (TABX2S and TABX1A). In addition, the technique is used to monitor events that perturb metabolism, such as exposure to electron transport inhibitors like sodium azide. Further studies with this system and these cell lines will be used to elucidate the effects of the graded expression of Bcl-x<sub>L</sub>. When highly expressed, as found in many cancer cell types, these anti-apoptotic proteins (Bcl-x<sub>L</sub>) confer cellular resistance to a wide variety of cytotoxic chemotherapeutics.<sup>3</sup> The exact molecular mechanism provided by these proteins that accounts for their anti-apoptotic activity is yet to be clearly defined.

Somewhat paradoxically, the compound antimycin A, and also a derivative, 2-methoxy antimycin A, induce cell death selectively in cells that overexpress either Bcl-2 or Bcl-x<sub>L</sub>. There exists a potential to develop novel cancer therapies using antimycin A to selectively induce cellular apoptosis in cells expressing high amounts of Bcl-2 or Bcl-x<sub>L</sub>. Furthermore, cell lines can be used to rank the relative effects of antimycin A between cells expressing varying amounts of Bcl-x<sub>L</sub>.

## $\mu$ SI-LOV for monitoring lactate and glucose

For lactate detection using the microsequential injection ( $\mu$ SI) lab-on-valve (LOV) system, the well characterized enzymatic assay using lactate dehydrogenase (LDH) was selected.<sup>4–6</sup> The reaction for the determination of lactate is ideally suited to the  $\mu$ SI-LOV method developed for glucose assay, as both of these assays utilize the same type of enzyme chemistries (NAD-linked). In addition, the same detection parameters, wavelength

and integration time, can be used for both assays. The reaction that LDH catalyzes is expressed as follows:



where  $\text{NAD}^+$  and  $\text{NADH}$  are the oxidized and reduced forms, respectively, of nicotinamide adenine dinucleotide. Similar to the  $\mu\text{SI-LOV}$  assay for glucose using glucose reagent<sup>7</sup> the progress of the enzymatic reaction can be monitored by the accumulation of  $\text{NADH}$ . In turn, the increasing concentration of  $\text{NADH}$  with time can be monitored by absorbance at 340 nm. Finally, it follows that the methodology described below is versatile, as it will accommodate other  $\text{NAD}$ -linked enzymatic assays.

Currently, in order to determine cellular lactate and glucose simultaneously in real-time dual immobilized enzyme electrodes are used.<sup>8</sup> However, these electrodes cannot be reconditioned in a simple automated fashion, and are susceptible to protein fouling.<sup>9,10</sup> Once the electrodes are fouled they require manual reconditioning. Also, this method does not allow for the automated renewal of adherent cells. In contrast, the  $\mu\text{SI-LOV}$  technique provides spectrophotometric monitoring of glucose consumption and lactate extrusion in a simple automated fashion, allowing the cells and reagents to be rapidly renewed before each assay. Thus, if the cell column becomes fouled or destroyed it can simply be replaced by microfluidic manipulation in a matter of a few seconds.

## Experimental

### Instrumentation

A FIALab 3000 (FIALab Instruments, Medina, WA) was used for this study. Included in the instrument is a high precision bi-directional syringe pump with a 1.0 mL syringe and a 6-position lab-on-valve (LOV). An auxiliary Cavro XL 3000 syringe pump with a 250  $\mu\text{L}$  syringe (Cavro Scientific Instruments Inc., Sunnyvale, CA) and a 6 port multiposition valve (MPV) (Valco Instruments Co. Inc., Houston, TX) were added to the system (Fig. 1). The system was plumbed with 1.59 mm outer diameter (OD) PEEK and stainless steel tubing (Upchurch Scientific, USA). The bead retention plug/nozzle (Fig. 1B) was made from a 2 mm length of 0.178 mm inner diameter PEEK tubing. The plug/nozzle was inserted into the channel of the LOV and kept from entering the flow cell by the end of a fiber optic (Fig. 1B). Holding coil #1 was fashioned from stainless steel tubing and has a volume of 1.0 mL, holding coil #2 was also fashioned from stainless steel tubing and has a volume of 250  $\mu\text{L}$ . FIALab software version 5.0 (FIALab Instruments, Medina, WA) was used to control all of the system components and was also used for data collection and analysis. The flow cell was illuminated by a long wave UVA pencil light (Spectronics Corp., Westbury, NY) coupled to a 600  $\mu\text{m}$  UV fiber optic. Absorbance measurements were accomplished *via* a 600  $\mu\text{m}$  UV fiber optic connection to an Ocean Optics SD-2000 CCD spectrophotometer (Ocean Optics, Dunedin, FL) containing a #1 (UV sensitive) grating. Absorbance was monitored at 340 nm at a frequency of 1.5 Hz with 500 ms of integration. The fiber optics were spaced 10 mm apart, resulting in a flow cell volume of 22.7  $\mu\text{L}$ . The entire apparatus was placed inside an Imperial III incubator (Lab-Line, Barnstead Int., Dubuque, IA) to provide precise temperature control over all of the instrument components, as well as, to maintain a physiological temperature (37 °C) for all studies.

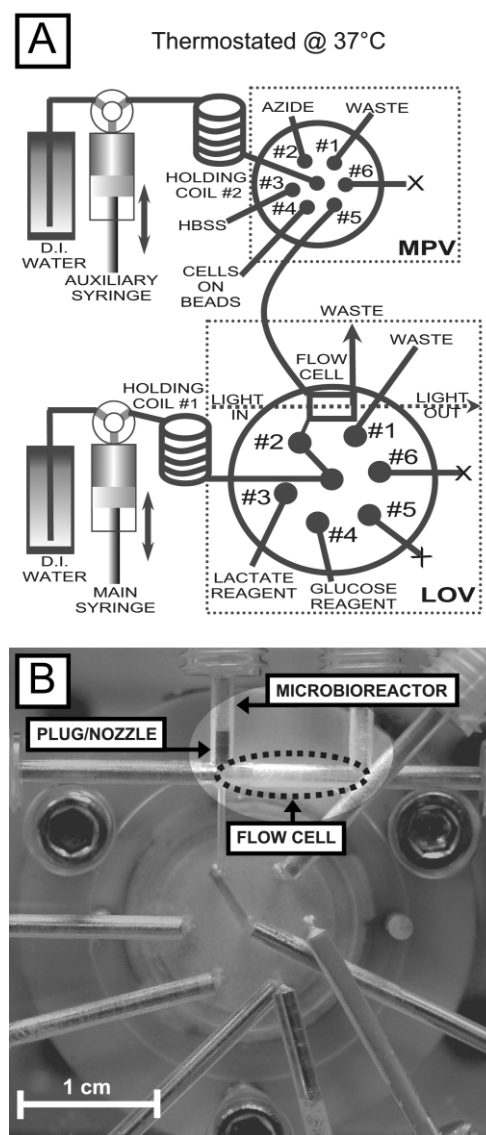
### Materials and reagents

Infinity™ glucose reagent, glucose standard, lactate standard, bovine heart lactate dehydrogenase (LDH), stock glycine buffer and sodium azide were obtained from Sigma Chemical (St.

Louis, MO). All standards and reagents were prepared fresh daily using ultrapure water made by a Nanopure® system (Barnstead Thermolyne Corp., Dubuque, IA). The glucose reagent was at a final concentration of  $>1500 \text{ U L}^{-1}$  hexokinase,  $>3200 \text{ U L}^{-1}$  glucose-6-phosphate dehydrogenase, 2.1 mmol  $\text{L}^{-1}$  adenosine triphosphate (ATP) and 2.5 mmol  $\text{L}^{-1}$  nicotinamide adenine dinucleotide ( $\text{NAD}^+$ ). The lactate reagent was at a final concentration of 2000  $\text{U mL}^{-1}$  LDH and 2.5 mmol  $\text{L}^{-1}$   $\text{NAD}^+$  in glycine buffer. Cytopore® beads were obtained from Amersham Pharmacia Biotech (Uppsala, Sweden). Hank's Balanced Salt Solution (HBSS) was obtained from Gibco (Gibco BRL, Grand Island, NY). The HBSS was fortified with calcium chloride (Sigma Chemical, St. Louis, MO) to a final concentration of 1.8 mmol  $\text{L}^{-1}$ .

### Cell culture

The mouse hepatocyte cell lines, TABX2S and TABX1A, were cultured by our colleagues at the Fred Hutchinson Cancer Research Center onto Cytopore® microcarrier beads in a manner identical to that previously described.<sup>11</sup> The final dilution was 1 mL of bead slurry in 10 mL of HBSS, resulting



**Fig. 1** (A) Diagram of the  $\mu\text{SI-LOV}$  system for monitoring glucose and lactate. The cells-on-beads, buffer, and inhibitor are located around the MPV, while all of the reagents for the assays are grouped about the LOV. Connections to the symbol (X) represent unused ports that are plugged. (B) Expanded view of the LOV flow cell.

in a bead slurry concentration of approximately 1600 beads mL<sup>-1</sup>.

The number of cells per microbead was determined each day using a hemacytometer. Prior to each day of experiments, cell number was determined by the following method: 100 µL samples of uniformly suspended beads were diluted 1:10 in a solution containing 0.1 M sodium citrate with 0.2% crystal violet for at least 1 h. The number of beads was counted in a defined volume to determine the exact bead concentration, and the cell number was determined by counting the released nuclei in a hemacytometer. The number of cells per bead could then be determined. Experiments were done with densities between 100 and 500 cells per bead.

## Results and discussion

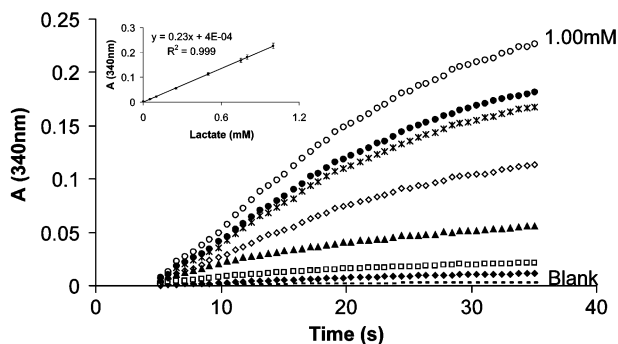
### Lactate assay calibration

The µSI-LOV system was calibrated for the lactate assay using lactate dehydrogenase. Using the previously determined<sup>11</sup> optimized sample volume (3 µL) and flow rate (30 µL s<sup>-1</sup>) the µSI-LOV system was calibrated with injections of 0.05, 0.10, 0.25, 0.50, 0.75, 0.80 and 1.00 mM lactate standard (Fig. 2). The absorbance signals at the end of the thirty second assay period were fit with their respective lactate concentrations to obtain a calibration equation:  $A_{340\text{ nm}} = 0.23 \times [\text{lactate (mM)}] + 4\text{E-}04$ ,  $R^2 = 0.999$  (Fig. 2 inset). The reproducibility of the lactate assay is ±4.5% over ten replicate injections, and the day-to-day reproducibility is ±7.3%.

### Experimental protocol

The protocols for the cell studies in the current µSI-LOV platform differ from those previously described<sup>11</sup> in the addition of the multiposition valve (MPV). The MPV was added so that all of the components for the cell studies, cells-on-beads, buffer, and inhibitors, can be grouped about the MPV, leaving the LOV ports available for the assay reagents (lactate reagent and glucose reagent) (Fig. 1).

An assay is initiated by packing a column of bead adherent cells in the microbio reactor, which is located upstream from the LOV flow cell. A column is packed by aspirating an aliquot of the bead slurry from port #4 of the MPV using the auxiliary syringe. The beads are then dispensed into the microbio reactor using the auxiliary syringe by switching the MPV to port #5. The beads are dispensed at a flow rate of 30 µL s<sup>-1</sup>, and are retained in the microbio reactor volume by the bead retention plug/nozzle. The cells-on-beads are then perfused with HBSS by the auxiliary syringe pump for 120 s at a flow rate of 2 µL



**Fig. 2** Calibration reactions for lactate dehydrogenase and lactate standard. The system was calibrated with injections of increasing concentration of lactate standard of 0.05, 0.10, 0.25, 0.50, 0.75, 0.80, and 1.00 mM. Inset shows the linear plot of the endpoint absorbance at each respective concentration of lactate standard.

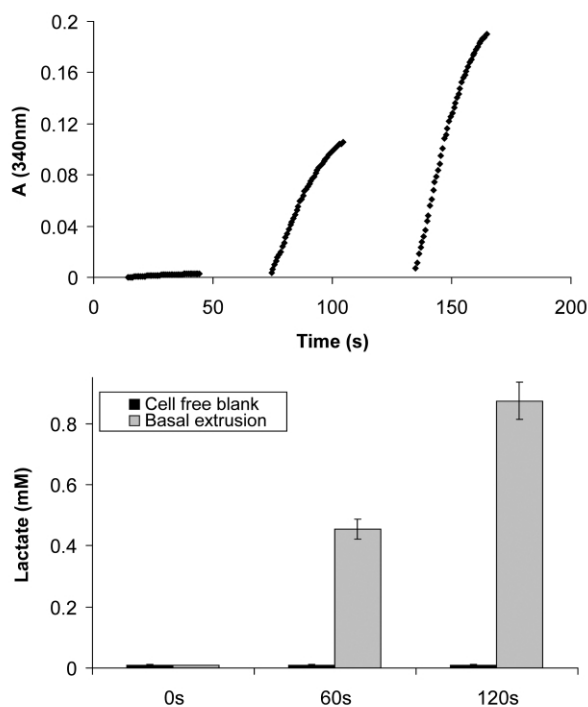
s<sup>-1</sup>. Subsequent to the perfusion period the cell column is incubated in the interstitial HBSS during a stop flow period, which allows extruded cellular lactate to accumulate, and allows for the cells to consume glucose from the HBSS (5.6 mM). During the stop flow period the flow cell is flushed with deionized water, using the main syringe pump, and is then loaded with enzyme reagent from either port #3 or port #4 of the LOV. After the stop flow period, 3 µL of the interstitial HBSS is injected through the plug/nozzle into the LOV flow cell and the concentration of either glucose or lactate was determined by spectrophotometric measurements at 340 nm. At the end of the assay period the cell column is removed by aspirating through the plug/nozzle using the auxiliary syringe. The MPV is then switched to port #1 and the beads are flushed to waste. The metabolic assay protocol is then repeated on a new column of fresh cells.

### Cell studies with TABX2S

The raw data corresponding to a lactate assay on the TABX2S cell line after 0, 60 and 120 s of stop flow incubation is presented in (Fig. 3). The absorbance increases with time due to the accumulation of lactate in the interstitial volume of the microbio reactor.

The basal concentration of cellular lactate extrusion per minute was calculated in the following manner; the difference in absorbance between the blank run (0 s) and the 60 and 120 s stop flow runs was fit to the calibration equation (refer to previous section):  $(A_{340} - 4 \times 10^{-4})/0.23 = \text{mM lactate}$ . The average concentration of extruded lactate over a one minute period was determined to be  $0.46 \pm 0.04 \text{ mM}$  ( $n = 7$ ) (Fig. 3).

A similar set of experiments at 0, 60, and 120 s of stop flow incubation were conducted using the glucose assay (Fig. 4). The absorbance decreases with time because the cells are consuming glucose from the HBSS in the interstitial volume of the microbio reactor. The concentration of basal glucose consumed per minute was determined in a similar manner as was used for the lactate calculation. The data in Fig. 4 was fit to the glucose

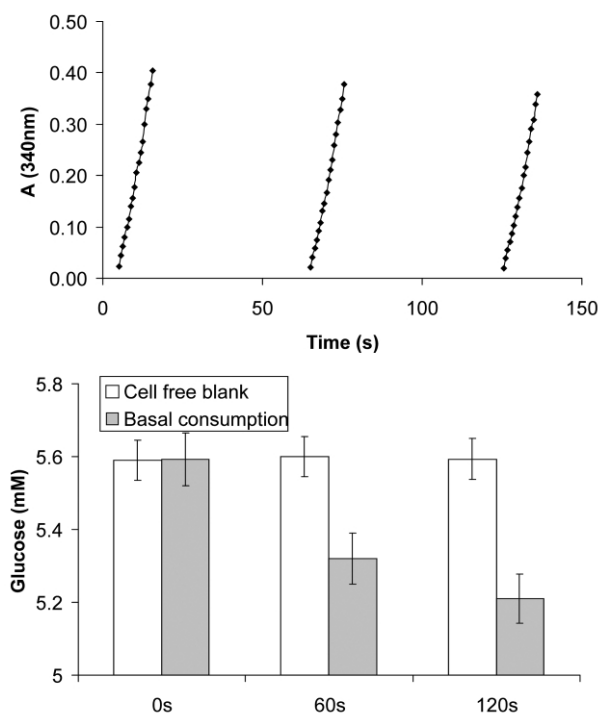


**Fig. 3** Lactate extrusion from the TABX2S cell line during 0, 60, and 120 s of stop flow incubation. Cell free blank corresponds to an identical set of experiments conducted over a column of blank Cytopore® beads.

calibration equation which was determined previously:<sup>11</sup>  $(A_{340} - 5 \times 10^{-3})/0.07 = \text{mM glucose}$ ,  $R^2 = 0.999$ . The average concentration of glucose consumed by the cells over a one minute period, was determined to be  $0.28 \pm 0.02 \text{ mM}$  ( $n = 7$ ) (Fig. 4).

An estimate can be made as to the portion of glucose metabolism that is anaerobic by comparing the concentrations, over the same time period, of extruded lactate to that of consumed glucose. The generation of lactate from glucose is a 2:1 stoichiometric ratio in cellular metabolism. Using the calculated concentrations above, the percentage of glucose that is converted to lactate by the cells can be calculated:  $[(0.46 \text{ mM lactate}) \times 0.5]/0.28 \text{ mM glucose} = 83 \pm 8\%$  anaerobic conversion under basal conditions.

Azide, a cytochrome C oxidase inhibitor, was used to validate the data derived from both the lactate extrusion and the glucose consumption experiments. In the presence of azide, cellular glucose metabolism is shunted to the anaerobic pathway, and consequently lactate extrusion should increase. Furthermore, the anaerobic metabolism of glucose is less efficient (less ATP per glucose) than aerobic metabolism, and therefore cellular glucose consumption should increase as well (Table 1). For these studies HBSS containing 5 mM azide was perfused over the cells for 120 s. In the presence of azide the concentration of lactate extruded from the cells, per minute, was determined to



**Fig. 4** Glucose consumption by the TABX2S cell line during 0, 60, and 120 s of stop flow incubation. Cell free blank corresponds to an identical set of experiments conducted over a column of blank Cytopore® beads.

**Table 1** Summary and comparison table of the metabolic parameters for the two cell lines TABX2S and TABX1A

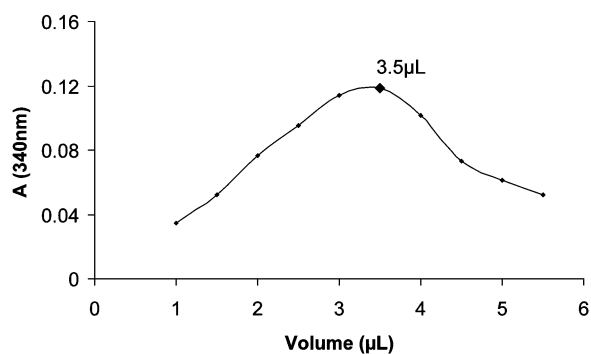
Metabolic parameters	TABX2S	TABX1A
Basal glucose consumption/nmol min <sup>-1</sup> per 10 <sup>6</sup> cells	4.3	3.4
Basal lactate extrusion/nmol min <sup>-1</sup> 10 <sup>6</sup> cells	7.1	5.2
Basal % anaerobic	83	77
Azide glucose consumption/nmol min <sup>-1</sup> per 10 <sup>6</sup> cells	4.8	3.7
Azide lactate extrusion/nmol min <sup>-1</sup> per 10 <sup>6</sup> cells	9.3	7.2
Azide % anaerobic	97	97

be  $0.59 \pm 0.05 \text{ mM}$  ( $n = 5$ ); an increase of 30% over basal. Likewise, the concentration of glucose consumed per minute in the presence of azide increased 11% over basal, to  $0.31 \pm 0.02 \text{ mM}$  ( $n = 5$ ). Comparing, as before, the concentrations of lactate and glucose to determine the percentage of anaerobic glucose conversion in the presence of azide, it was calculated that the TABX2S cells were  $97 \pm 5\%$  anaerobic.

### Cell number determination

In order to correlate cell numbers with the concentration of extruded lactate, and of consumed glucose, the number of cells contained in the microbio reactor must be determined. A packed microcolumn of bead adherent cells occupies approximately 10  $\mu\text{L}$  of volume in the microbio reactor. For each assay, 3  $\mu\text{L}$  of HBSS surrounding the cells is pushed through the microbio reactor into the flow cell. The 3  $\mu\text{L}$  used for the assays is derived from the *interstitial volume* of the microbio reactor. In order to determine the number of cells interacting with the 3  $\mu\text{L}$  interstitial volume, the following experiment was devised: using a microcolumn of bead adherent cells (TABX2S), corresponding to 10  $\mu\text{L}$  of packed beads, increasing volumes of HBSS were pushed off the microcolumn after a 1 min stop flow period. The lactate in the sample was monitored in the flow cell using LDH (Fig. 5). The absorbance due to the enzymatic conversion of lactate steadily increases with increasing sample volumes, up to a volume of approximately 3.25  $\mu\text{L}$ . Since there is no lactate in the stock HBSS, the decrease in signal at sample volumes larger than 3.5  $\mu\text{L}$  is due to dilution of the HBSS contained in the interstitial volume with that contained upstream from the microbio reactor. Therefore, the interstitial volume of a 10  $\mu\text{L}$  packed column can be approximated as 3.25  $\mu\text{L}$ . Using the ratio of column to interstitial volume, 3.08, the volume of microbeads interacting with the 3  $\mu\text{L}$  interstitial volume used for each assay can be calculated as follows:  $3 \mu\text{L} \times 3.08 = 9.23 \mu\text{L}$ . This means that the 3  $\mu\text{L}$  assay volume corresponds to the interstitial volume of 9.23  $\mu\text{L}$  of packed microbeads. The number of beads contained in 9.23  $\mu\text{L}$  can now be calculated using the volume of a microbead ( $\sim 8.2 \text{ nL}$ ). Assuming a 67% packing efficiency, there are approximately 760 microbeads in 9.23  $\mu\text{L}$  of packed column volume. Finally, the number of cells per microbead ( $250 \pm 10$ ) and the number of microbeads (760) can be used to determine the number of cells interacting with 3  $\mu\text{L}$  of interstitial volume, which was found to be  $1.9 \pm 0.1 \times 10^5$  cells.

The rate of lactate extrusion can be determined, using the sample volume (3  $\mu\text{L}$ ), the average concentration change per minute (0.45 mM) and the number of cells ( $0.19 \times 10^6$ ). The



**Fig. 5** Interstitial volume determination. The absorbance steadily increases up to approximately 3.25  $\mu\text{L}$  where it plateaus until approximately 3.5  $\mu\text{L}$  (highlighted on the chart). At volumes greater than 3.5  $\mu\text{L}$  the signal decreases as the interstitial volume is diluted with HBSS contained upstream from the cell column. 3.25  $\mu\text{L}$  was selected as the interstitial volume for a 10  $\mu\text{L}$  column as this is the point at which the signal ceases to steadily increase.

average calculated rate of lactate extrusion for the TABX2S cell line was  $7.1 \pm 0.6 \text{ nmol min}^{-1}$  per  $10^6$  cells ( $n = 7$ ). This value lies within the range ( $0.5\text{--}15 \text{ nmol min}^{-1}$  per  $10^6$  cells) of expected basal lactate extrusion rates for a cell line of this type.<sup>12–16</sup> In a similar manner, the average basal rate of glucose consumption for the TABX2S cell line was determined to be  $4.3 \pm 0.3 \text{ nmol min}^{-1}$  per  $10^6$  cells ( $n = 7$ ). This value lies within the range of expected basal glucose consumption rates ( $0.5\text{--}7 \text{ nmol min}^{-1}$  per  $10^6$  cells) for a cell line of this type.<sup>12–19</sup>

### Cell studies with TABX1A

In a manner identical to that described for the TABX2S cell studies the basal rates of glucose consumption and lactate extrusion for the TABX1A cell line were determined to be  $3.4 \pm 0.3 \text{ nmol (glucose) min}^{-1}$  per  $10^6$  cells ( $n = 6$ ) and  $5.2 \pm 0.5 \text{ nmol (lactate) min}^{-1}$  per  $10^6$  cells ( $n = 6$ ). Comparing these rates, as before, to determine the percent anaerobic conversion for this cell line, it was calculated that under basal conditions the TABX1A cell line is  $77 \pm 8\%$  anaerobic. In order to validate the basal rates of glucose consumption and lactate extrusion, the TABX1A cells were exposed to  $5 \text{ mM}$  sodium azide in HBSS (Table 1). In the presence of azide the rate of glucose consumption was calculated to be  $3.7 \pm 0.3 \text{ nmol min}^{-1}$  per  $10^6$  cells ( $n = 5$ ), an increase of  $8.8\%$  over basal. The rate of lactate extrusion for the TABX1A cells in the presence of azide was calculated to be  $7.2 \pm 0.8 \text{ nmol min}^{-1}$  per  $10^6$  cells ( $n = 5$ ), an increase of  $38\%$  over basal (Table 1). In the presence of  $5 \text{ mM}$  azide the TABX1A cell line was determined to be  $97 \pm 5\%$  anaerobic.

### Comparing the TABX2S and TABX1A cell lines

The results of the cell experiments are summarized in Table 1. Comparing the basal rates of glucose consumption and lactate extrusion for the two cell lines, the TABX2S cells are consuming glucose and extruding lactate at a faster rate than the TABX1A cells. This relationship is loosely supported by comparing the basal percentage of glucose metabolism that is anaerobic for each cell line. The TABX2S cell line were determined to be  $83 \pm 8\%$  anaerobic under basal conditions, and the TABX1A cell line was determined to be  $77 \pm 8\%$  anaerobic under identical conditions. Since anaerobic glucose metabolism is less efficient than aerobic metabolism, it stands to reason that the cell line that is more anaerobic would be consuming more glucose, as well as, extruding more lactate.

In the presence of azide, both cell lines behaved as expected, with concurrent increases in glucose consumption and lactate extrusion. After exposure to azide both cell lines converted nearly all of the glucose consumed into extruded lactate.

### Conclusion

The  $\mu$ SI-LOV system with an integrated microbioreactor has successfully been used to monitor cellular glucose consumption and lactate extrusion by NAD-linked enzymatic analysis. The system has demonstrated a dynamic linear range for glucose determination of  $0.1\text{--}5.6 \text{ mM}$ , and has also demonstrated a dynamic linear range for lactate determination of  $0.05\text{--}1.00 \text{ mM}$ . The design of the  $\mu$ SI-LOV system allows for rapid and reproducible mixing to be achieved through nozzle assisted mixing of cell perfusate and enzyme reagent directly in the flow cell.<sup>11</sup> The compact dimensions of the system allow the entire apparatus to be easily placed inside an incubator, providing precise physiological temperature control, which is ideal for cell studies. In addition, the design of the  $\mu$ SI-LOV system locates cells and reagents in close proximity allowing for rapid mixing of reagents and cellular perfusate. This facilitates the quantifica-

tion of glucose within a ten second time period and the quantification of lactate within thirty seconds. The assays for glucose and lactate were conducted in a serial fashion, with each assay generating  $< 200 \mu\text{L}$  of waste. The automation provided by the  $\mu$ SI-LOV system allows reagents and cells to be renewed in a rapid and reliable manner allowing for glucose and lactate assays to be conducted by the same apparatus.

The use of a high number of cells ( $> 10^5$ ) for each experiment allows the  $\mu$ SI technique to screen the responses indicative of tissues. Currently, there is extensive research devoted to techniques for the study of single cells.<sup>20</sup> However, the value of techniques for the study of many cells at once should not be underestimated, as these techniques provide a higher degree of statistical validity due to a greater sample population. In addition, examining many cells at once is more indicative of the response witnessed in tissues since experiments are conducted on a cell population that contains cells in all phases of cell cycle and each cell is in close proximity to many others allowing for cytokine signaling.

Two stably transfected hepatocyte cell lines were screened and their rates of basal glucose consumption and lactate extrusion were quantified and compared. In addition, the metabolic inhibitor sodium azide was used to validate the technique. Thus it has been shown that the  $\mu$ SI-LOV system for monitoring glucose consumption and lactate extrusion is a sensitive means to elucidate the effects of chemical compounds on cellular metabolism, as was demonstrated with azide, by comparing the basal parameters with those in the presence of azide. Furthering this concept, novel compounds can be screened rapidly at varying concentrations in order to form a dose response series for a compound. From the dose response, the efficacy of the compound can be determined. This  $\mu$ SI-LOV method is a versatile technique for the validation of targets for drug discovery and for the ranking and determination of their relative potency.

In addition, the new method can also be used to elucidate the effects of environmentally induced metabolic perturbations. An ischemic (no oxygen) event can be induced in the  $\mu$ SI-LOV system by using carrier buffer that has been thoroughly degassed. Comparing the cellular metabolic parameters prior to and subsequent to an ischemic event will allow investigation of how the cells are generating their energy before and after hypoxia. An ischemic induced metabolic change has been witnessed in cardiac myocytes. Subsequent to an ischemic event, these cells use glucose metabolism to generate a greater portion of their energy than under basal conditions.<sup>21,22</sup> Glucose metabolism consumes less oxygen than fatty acid metabolism, and hence glucose metabolism is more catered to a low oxygen environment. Experiments to examine environmentally induced metabolic perturbations will provide needed information into the mechanisms underlying events such as strokes or cardiac arrest.

The  $\mu$ SI-LOV system in its current configuration can be used for multiple NAD-linked enzymatic assays. Since there are well over 250 members of the NAD-linked family of enzymes the present methodology may become a tool for studying multiple metabolic pathways. For instance, fructose 5-dehydrogenase, isocitrate dehydrogenase and malate dehydrogenase can be used in the current  $\mu$ SI-LOV system to screen for cellular fructose, isocitrate and malate. Future applications of this technique to study cell physiology will include the use of many of these enzymes, in order to quantify a multitude of cellular compounds in real-time.

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