Mercury free operation of the Coulter counter MultiSizer II sampling stand ⋆

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Abstract

Electronic particle counters have gained widespread acceptance as a means to measure osmotic properties of cell membranes. Because most current instruments do not allow for the collection of true volume as a function of time data, investigators use older models such as the MultiSizer II sampling stand. A significant drawback to this and other older models is that they rely on mercury to maintain a constant pressure and to connect electrodes. The presence of mercury is a human health hazard that is exacerbated by the sometimes irregular vacuum pressures that cause mercury spills inside of the machine. To eliminate this hazard, we have determined that the MultiSizer II model can be simply and easily modified to function and collect temporal volume data without the use of mercury.

Key words: cryobiology, Coulter counter, mercury, particle sizing, osmotic characteristics

Optimization of cryobiological procedures depends on a complete and accurate understanding of membrane biophysical characteristics [1]. Before the late 1980s, determining the membrane permeability characteristics such as hydraulic conductivity or solute permeability was tedious and time consuming. Typical methods for the determination of permeability characteristics included time to lysis[2], stopped flow [3] and microscopy [4]. With the introduction of the electronic particle counter (EPC) as an instrument to produce volume as a function of time data, this data collection became notably simpler for many cell types [5]. The EPC takes advantage of the "Coulter-principle," in which the resistance across a small aperture is proportional to the cross sectional area of the particle passing through it. The subsequent volume output of the Coulter counter is a measurement of the area under the voltage curve generated as a particle passes through the aperture, allowing the measurement of cell volume as a function of time as individual cells pass through. The method has been applied to a variety of cell types such as Islet cells[6,7], spermatozoa[8], and bioengineered corneal cells[9].

Traditionally, the EPC sampling stand of choice for cryobiologists has been the Beckman Coulter, Inc (Fullerton, CA) MultiSizer II. As opposed to more recent models, the MultiSizer II, attached to a control unit, allows the direct output and collection of volume as a function of time data. Unfortunately, the MultiSizer II model, along with other older models, uses mercury to regulate

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pressure while conducting electricity. Mercury is a known toxin and as such is unwanted in modern laboratories [10]. The Coulter counter user risks exposure to mercury due to common handling accidents such as shattering of the manometer. Users also risk exposure to mercury during regular use due to its current design—vacuum regulator malfunctions can cause a backflow of mercury into the aperture tube, which not only increases exposure to mercury vapor and liquid, but is difficult and time consuming to clean, requiring the assistance of trained hazardous waste personnel. With this problem in mind, we re-engineered the Coulter counter MultiSizer II to function without mercury.

In clinical applications, the Coulter counter has two primary functions: sizing particles and calculating particle density. For biophysical experiments that measure cell permeability to both solute and solvent, typically only the cell sizing function is necessary. In this case the Coulter counter maintains a negative pressure at the aperture by pulling a vacuum against the aperture and manometer (see Fig. 1). When the Coulter counter is in volume collection mode (i.e. the Reset/Count knob is in the Reset position and the Close/Fill knob is in the Close position), this negative pressure draws cells through the aperture and allows the measurement of the change in electrical impedance. When the Coulter counter is in count mode (e.g. the Reset/Count knob is at Count and the Close/Fill knob is at Close), the vacuum pump acts against a closed valve, and the weight of the mercury draws a specific amount of solution through the aperture until the mercury equilibrates. We deduced that during volume collection mode, none of the electrodes attached to the manometer were connected to the ground wire. It was determined that the only function of the mercury during volume collection mode is pressure regulation. It is therefore possible to replace the manometer with an appropriately sized

saline filled tube attached to the manometer inlet and clamped at the far end (see Fig. 1). For example we use a 1/4" inner diameter tube approximately 40 cm in length (sufficiently long to allow for convenient placement of the syringe) connected at the manometer port and a 60 cc syringe which functions as a tube clamp in the following manner: (1) the electrodes were disconnected from the manometer and were placed so that they would not make an electrical connection [1]; (2) the mercury filled manometer was removed and the mercury was disposed of properly; (3) the 60 cc syringe was filled with 20 ml of 0.9% saline; (4) the tube was then fitted and clamped over the luer tip of the syringe; (5) the open end of the tube was connected to the port where the manometer was attached (some of the original tubing may need to be removed); (6) the plunger of the syringe was depressed to fill the tube completely with saline while expelling air from the system. The pressure can be subsequently monitored and controlled by the internal vacuum regulator or the vacuum control unit (VCU). On our model (the MultiSizer II), pressure can be held constant reliably and efficiently using the regulator attached to the VCU, and can be monitored by connecting a pressure guage to an outlet from the waste jar (some VCU models (e.g. Model VCU VW II) have a pressure release port on the top of the waste jar which can be easily adapted to this purpose (see Fig. 1-B), other models in use (e.g. Gilford Instrument Vacuum Receiver 3021) have a two-holed rubber stopper on the waste jar which can be drilled to form an appropriate outlet (see Fig. 1-C); we have performed our tests with the former).

An advantage of this pressure control is that the rate of flow through the

[1] An elegant but less reversible solution is to clip off the electrode ends and cap the exposed wires with electrical tape.

aperture can be varied. This gives investigators the option to use less solution during long experiments (such as those carried out at low temperatures). A negative aspect to this pressure control is that we have found that the aperture becomes "clogged" more readily at lower pressures. We recommend an operating pressure of approximately 18 kPa to emulate the pressures generated by the Coulter counter with mercury. However, we investigated the sample uptake of our MultiSizer II at three pressures (measured at the waste chamber as described above). Coulter counter cuvettes (Beckman Coulter, Fullerton, CA) were filled with ∼20 ml 0.9% saline, weighed, placed into the a sampling stand adjusted to either 10, 15, or 20 kPa pressure measured with a 0.1 MPa Yamamoto Keiki Instruments vacuum gauge (VWR Scientific, West Chester, PA), and held for 15 minutes. Cuvettes were then removed from the sample stand and weighed. The difference in mass was divided by the exposure time to yield values in g/min. Assuming 1 mL 0.9% saline ≈ 1 g 0.9% saline, this yielded flow rates of 0.524 ± 0.025 mL/min (mean ± SD, $n = 3$), 0.435 ± 0.008 mL/min, 0.328 ± 0.011 mL/min at pressures of 20 kPa, 15 kPa, and 10 kPa, respectively (see Fig. 2). A linear regression yields the apparent relationship over this pressure range between flow rate f in mL/min, and pressure P in kPa: $f(P) = 0.02P + 0.14$, with a correlation coefficient $r = 0.9828$.

Finally, to demonstrate that the measured volume of particles is a function of both the flow rate and the voltage, we measured 20 μm and 15 μm spherical latex calibration beads (Beckman Coulter, Fullerton, CA) while varying the pressure from 10 kPa to 20 kPa. The resulting measurement of volume as a function of time can be seen in Fig. 3. Note that as the flow rate increases, measured volume decreases, as expected. This also demonstrates that strict control of pressure must be maintained to ensure that the volume measure-

ments are accurate.

In conclusion, we have demonstrated a simple modification to a common experimental cryobiological tool that removes the necessity of mercury in the instrument. Moreover, although the volume output is pressure dependant, the volume measuring function of the instrument is independent of the amount of pressure. Thus, we have shown a method to precisely control the rate of flow through the aperture—a significant advantage to investigators working at lowered temperatures or with reduced cell concentrations.

Figure Legends

Figure 1. Panel A: Diagram of the modified system. A saline filled 1/4" inner diameter tube is attached to the manometer port and clamped distally with a 60cc syringe. Panel B: Top view of a VCU VW II waste jar. The valve in the pressure release port is removed and replaced with tubing connected to a pressure gauge. Panel C: Side view of a Gilford Instrument Vacuum Receiver 3021 waste jar. The rubber stopper is drilled to form a third hole. (a) and (b) are connected to the sampling stand as usual, and (c) is connected to a pressure gauge.

Figure 2. Plot of pressure as a function of sample uptake.

Figure 3. Calibration bead volumes as a function of pressure.

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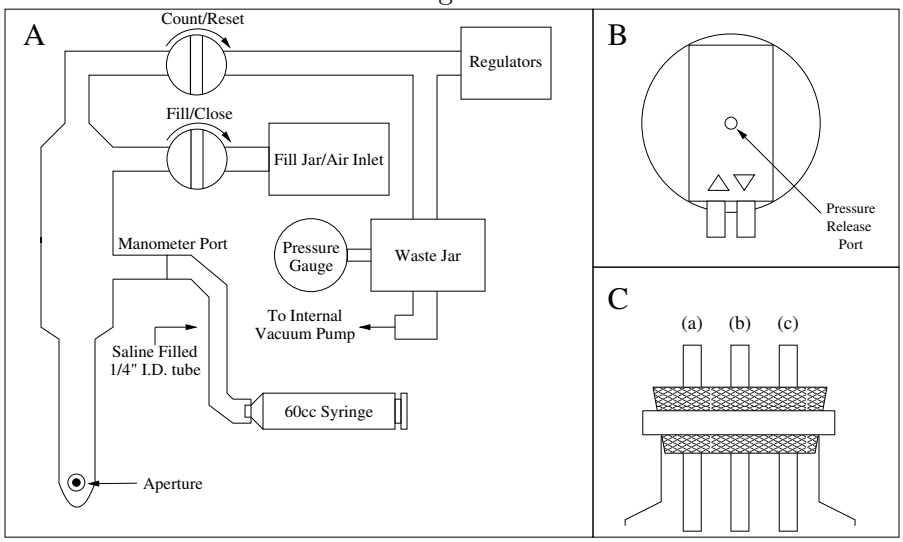
Fig. 1.


A
Count/Reset
Regulators
Fill/Close
Fill Jar/Air Inlet
Manometer Port
Pressure Gauge
Waste Jar
To Internal Vacuum Pump
Saline Filled 1/4" I.D. tube
60cc Syringe
Aperture
B
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C
(a)
(b)
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Fig. 2.

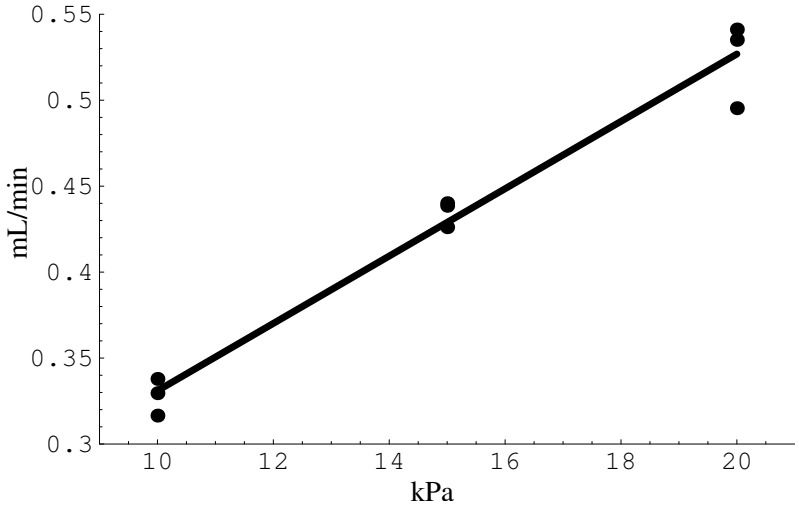
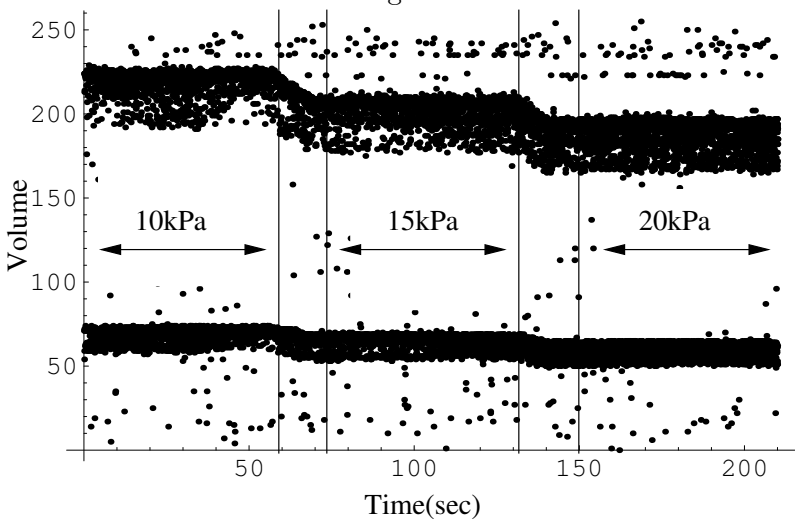


Fig. 3.


250
200
150
100
50
Volume
10kPa
15kPa
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Time(sec)