Novel *in situ* normal streaming potential device for characterizing electrostatic properties of confluent cells

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(Received 26 February 2012; accepted 24 June 2012; published online 25 July 2012)

The characteristics of transport across confluent cell monolayers may often be attributed to its electrostatic properties. While tangential streaming potential is often used to quantify these electrostatic properties, this method is not effective for transport normal to the apical cell surface where the charge properties along the basolateral sides may be important (i.e., confluent cells with leaky tight junctions). In addition, even when cells have a uniform charge distribution, the shear stress generated by the conventional tangential flow device may dislodge cells from their confluent state. Here we introduce a novel streaming potential measurement device to characterize the normal electrostatic properties of confluent cells. The streaming potential device encompasses a 24 mm cell-seeded Transwell® with two AgCl electrodes on either side of the cell-seeded Transwell. Phosphate buffered saline is pressurized transversal to the Transwell and the resultant pressure gradient induces a potential difference. Confluent monolayers of HEK and EA926 cells are used as examples. The corresponding zeta potential of the cell-membrane configuration is calculated using the Helmholtz–Smoluchowski equation and the zeta potential of the confluent cell layer is deconvolved from the overall measurements. For these test models, the zeta potential is consistent with that determined using a commercial dispersed-cell device. This novel streaming potential device provides a simple, easy, and cost-effective methodology to determine the normal zeta potential of confluent cells cultured on Transwell systems while keeping the cells intact. Furthermore, its versatility allows periodic measurements of properties of the same cell culture during transient studies. © 2012 American Institute of Physics. [<http://dx.doi.org/10.1063/1.4737140>]

I. INTRODUCTION

Transport across and through cell layers, such as endothelial cells in the vascular system or mucosal cells (M-cells) at the mucosal-systemic barrier, is important for overall system homeostasis. In particular, the transport of nanoparticles for mucosal vaccines has been recently coupled to the electrostatic properties of the solution.¹ It is speculated that the local normal electrostatic potential across cellular barriers, such as intranasal M-cells, is a critical factor for vaccine delivery and cellular regulation in general. Thus, a critical evaluation of cellular normal electrostatic potential could prove valuable in determining appropriate mucosal vaccine delivery vehicles and other relevant transport processes.

One common approach for determining cellular electrostatic properties is through a zeta potential analysis. Bangham *et al.* invented the first zeta potential (ζ) measuring device.² Subsequently, ζ using laser Doppler spectroscopy, light-addressable potentiometric sensors, Doppler electrophoretic light scattering techniques, ion selective field emission transistors, microchip analytical devices, and dynamic light scattering techniques have been employed.^{3–10} A number of devices such as ZetaCad® and Malvern Zetasizer® have been commercially designed to measure the electrostatic properties of particulates. The limitations of the current techniques

for measuring ζ are: somewhat device-dependent values, the size of the cells, the concentration of cell suspension in surrounding media, the use of tangential flow devices, and the inability to measure normal ζ for a confluent layer of cells.

But while the streaming potential (SP) is typically used to determine the electrostatic properties of confluent cell layers, these methods are often measured tangential to the cellular surface.^{11–16} Haydon provided an excellent overview of how the surface charge of living cells can be quantified using ζ .¹⁷ Innumerable studies demonstrated that the electrostatic charges of cellular bilipid membrane layers can be correlated to the absorption and permeability of cations, particles, and preferential adhesion to bacterium, leukocytes, nanoparticles, etc.^{18–28}

Even though the above mentioned studies capture the electrokinetic characteristics of cellular layer alone, various intercellular and subcellular layer electrostatic properties (viz., pore dimensions, leaky junctions, and cellular surface conductivities) cannot be captured using tangential flow alone as shown in Figure 1(a). Hence, to substantially understand the cell layer electrokinetic characteristics, employing normal or permeate flow (streams across the apical-basolateral side) as shown in Figure 1(b) is necessary. To summarize, tangential flow streams across the apical side of the cell and normal flow streams across the apical-basolateral side.

In this study, we introduce a novel streaming potential device to capture the intrinsic properties of living cells by obtaining the normal ζ of a confluent monolayer of cells. In our

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FIG. 1. Illustration of a cell with leaky junction. The figure shows (a) tangential flow and (b) normal flow configurations of measuring streaming potential.

work, cells cultured on commercially available Transwell® inserts are subjected to normal flow with varying pressure drop. The resulting membrane streaming potential is measured and the zeta potential of the cultured cell layer alone is mathematically determined.

II. METHODS

A. Normal zeta potential measurements using streaming potential device

Streaming potential experiments are performed using an *in situ* streaming potential device shown in Figure 2. The device measures the potential across the cells cultured on the hosted 24 mm Transwells (Corning, NY). Electrolytic solution of phosphate buffered saline (PBS) (1.87 mS/cm^2) flows through the feed side of the device, normal to the Transwell (with a known pressure drop), and exits out through the permeate side of the device. The developed normal streaming potential across the membrane is measured by the Ag/AgCl electrodes on the feed and permeate sides of the Transwell. PBS solution is used as an electrolyte in order to maintain fa-

FIG. 2. Schematic of the normal streaming potential device. Ag/AgCl electrodes are used to measure the streaming potential with a voltmeter (110 True RMS Multimeter, Fluke, Inc.). PBS (1.87 mS/cm^2) was used as the electrolyte solution and the pressure at the inlet was monitored using a pressure gauge. Polycarbonate Transwells seeded with confluent EA926 cells or HEK cells or with no cells are hosted in the chamber.

vorable physiological conditions. Since the valence of ions in electrolyte solution and its concentration highly affects the accuracy of streaming potential measurements, the PBS solution is kept at low ionic strength in this study to prevent masking of the streaming potential of the cellular monolayer. Zeta potential (ζ) is calculated using the Helmholtz–Smoluchowski equation.^{29,30}

B. Cell culture

EA926 cells and HEK cells (obtained from ATCC, (Manassas, VA)) were carefully chosen to represent diverse cell lines. Cells were cultured using Dulbecco's modified Eagle's medium (DMEM, Lonza, Inc.) and were supplemented with 10% FBS (Gibco, Inc.) and antibiotics (Primocin, Invivogen, Inc.). Freshly passaged cells were grown on Transwell filters (24 mm, $3.0 \mu\text{m}$ pore sized polycarbonate Transwells, Corning, 3414; Lot #02011017, Corning, NY) to facilitate their insertion into the *in situ* streaming potential device. The polycarbonate Transwells were presoaked in DMEM for 2 h before being seeded with cells (with a density of 1×10^6 cells/cm²). The cells were cultured overnight in triplicates. The polycarbonate Transwells presoaked in complete DMEM was used as the control for the experiments. The cell-cultured Transwell filters were maintained in humidified 95% air-5% CO₂ incubator at 37 °C. The integrity of the cellular layer was measured using a transcellular resistance measuring device. Cells were determined to be confluent if the transcellular resistance was greater than $1 \text{ M}\Omega$.

C. Zeta potential measurements using Zetasizer

The zeta potential of the HEK293 cells and EA926 cells was measured by dynamic light scattering using Zetasizer Nano ZS90 (Malvern Instruments, UK). Samples of HEK293 cells and EA926 cells were pelleted down and dispersed in PBS buffer (pH 7.4) with 0.01% of ethylene glycol tetraacetic acid and placed in a disposable capillary cell (DTS1061, Malvern Instruments, UK). ζ of each sample was measured at 25 °C for five times and was reported as mean $\pm$ standard deviation.

D. Calculation of normal zeta potential of confluent cells

Teychene *et al.* proposed a mathematical equation to decouple the streaming potential of a chemical particulate cake layer from that of the measured cake-membrane support potential.³¹ We apply a similar approach to decouple the streaming potential of the confluent cells from the cell-seeded membrane streaming potential.

Streaming potential of confluent cellular layers is considerably influenced by the number and size of their tight junctions. The proposed mathematical derivation assumes that the confluent cellular layer has low surface conductivity compared to that of the media and that the radii of tight junctions of the cellular monolayer are much larger than the Debye length of the media. This will ensure non-overlapping electrical double layers and the resulting non-linear potential which would not represent the ζ of the cell surface being considered.

The proposed method also takes into consideration the deviation in the isoelectric point of the membrane when a confluent cell layer is cultured on it. The obtained ζ assumes that the flow is laminar within the tight junctions of the confluent cell layers and the pores of the membrane; that the electric double layer is very thin compared to the thickness of the pores; and that the junctions and pores are coaxial.

At a given steady state, electric charge flows through the membrane and the cellular layer seeded on the membrane

$$I = I_c + I_m, \text{ i.e.,} \\ I = \iint_{S_c} \rho_c \vec{V}_c dS_c + \iint_{S_m} \rho_m \vec{V}_m dS_m, \quad (1)$$

where I_c and I_m denote the cell layer and membrane layer current, respectively. Here also ρ_i is the electric charge density of the cellular layer or the membrane, ds_i is the cellular surface element and membrane surface element, V_i is the velocity field of the media across the cellular layer and the membrane layer,^{32–34} where $i = c$ or m with c denotes the cell layer and m denotes the membrane layer. The resulting streaming potential is expressed as the ratio of the measured electrical potential and the pressure gradient³³

$$SP = \frac{\Delta\psi}{\Delta P}, \quad (2)$$

where $\Delta\psi$ is the measured electrical potential (V) and ΔP is the transmembrane pressure drop (Pa). The streaming potential is used to calculate ζ of the cell-seeded membrane using the Helmholtz–Smoluchowsky equation.^{29,30} Thus, for a given pressure drop, ζ can be determined based on the variations of streaming potential across the charged membrane layer as

$$SP = \frac{\Delta\psi}{\Delta P} = \frac{\varepsilon_0 \varepsilon_r \zeta}{\mu \Lambda_0}, \quad (3)$$

where ε_0 is the permittivity of free space (8.854×10^{-12} F/m), ε_r is the relative dielectric constant of PBS (approximately that of water, 78.35 at 25 °C), μ is the viscosity of PBS (0.0098 g/cm/s), and Λ_0 is the conductivity of the PBS ($1.69 \mu\text{S/m}$).

Darcy's law is used to relate the permeability of the virgin membrane J ($\text{m}^3 \text{m}^{-2} \text{s}^{-2}$) to pressure drop as

$$J_m = L_{p_m} \Delta P = \frac{1}{\mu R_m} \Delta P, \quad (4)$$

where R_m as the hydraulic resistance (m^{-1}). The measured electrical potential is directly linked to the ζ and the hydraulic resistance (i.e., the permeability) of the membrane. Equation (4) when combined with Eq. (3), the electrical potential measured across the virgin membrane can be written as a function of the permeate flow (Eq. (5))

$$\Delta\psi = \frac{\varepsilon_0 \varepsilon_r \zeta_m}{\mu \Lambda_0} \Delta P = \varepsilon_0 \varepsilon_r \zeta_m \frac{R_m}{\Lambda_0} J_m. \quad (5)$$

As cells are cultured on the membrane ($c+m$), they increase the overall hydraulic resistance of the cell-seeded membrane. Assuming that there is no significant cell-membrane interac-

tion, the permeate flux can then be written as

$$J_{c+m} = L_{p_{c+m}} \Delta P = \frac{1}{\mu (R_m + R_c)} \Delta P, \quad (6)$$

where $L_{p_{c+m}}$ is the cell-seeded membrane permeability and R_c is the hydraulic resistance of the cellular layer. From Eqs. (5) and (6)

$$\Delta\psi = \frac{\varepsilon_0 \varepsilon_r \zeta_{c+m}}{\Lambda_0} (R_m + R_c) J_{c+m}. \quad (7)$$

Note that ζ_{c+m} is the zeta potential of the cell-seeded membrane. In addition, if the cultured layer of cells is considered as a hydraulic resistance with no interaction with the membrane, ζ_{c+m} can be written as a function of the zeta potential of the two layers (membrane and cultured cell layer). Applying the theoretical streaming potential of a porous medium composed of two different porous layers proposed by Szymczyk *et al.*,³³ the streaming potential of a confluent layer of cells on the membrane is

$$SP_{m+c} = SP_c \left(1 - \frac{L_{p_{m+c}}}{L_{p_m}}\right) + SP_m \left(\frac{L_{p_{m+c}}}{L_{p_m}}\right). \quad (8)$$

Finally, the streaming potential of the confluent cell layer is given as

$$SP_c = \frac{SP_{m+c} - SP_m \frac{L_{p_{m+c}}}{L_{p_m}}}{1 - \frac{L_{p_{m+c}}}{L_{p_m}}}. \quad (9)$$

III. RESULTS AND DISCUSSION

A. Measuring streaming potential

From Eq. (2), the streaming potential was determined using the slope of the streaming potential-pressure curves using least squares linear regression (SigmaPlot®, San Jose, CA). The measured streaming potentials across three membrane cases: the DMEM soaked virgin membranes, HEK confluent cell-seeded membranes, and EA926 confluent cell-seeded membranes, were determined to be -40.5 mV, -51.4 mV, and -52.1 mV, respectively. All the graphs obtained through our streaming potential device as shown in Figure 3 provided linear relationships with R^2 values between 0.986 and 0.9972.

FIG. 3. Representative graphs relating the measured potential ($\Delta\psi$) with variation in pressure (ΔP) for polycarbonate membranes with pore size of $3.0 \mu\text{m}$.

TABLE I. Results from normal streaming potential device. Phosphate buffered solution (PBS) with a conductivity of 1.81 mS/cm and 7.4 pH was used as the initial feed solution.

Case	Streaming potential SP (mV kPa ⁻¹)	Zeta potential ζ (mV)	Range of pressures P (kPa)	Hydraulic permeability L_p (cm ² s g ⁻¹) $\times 10^9$
DMEM soaked (membrane only)	-36.1 ± 2.0	-7.9 ± 0.44	23–127	28.9 ± 3.72
EA926 seeded with membrane	-52.1 ± 4.6	-11.4 ± 1.00	31–66	9.0 ± 8.24
HEK seeded with membrane	-51.4 ± 1.6	-11.3 ± 0.34	17–66	2.2 ± 1.99
EA926 cultured cells alone (deconvolved)	N/A	-7.8 ± 1.04	N/A	N/A
HEK cultured cells alone (deconvolved)	N/A	-7.4 ± 3.21	N/A	N/A

The slope of these curves is the streaming potential and the difference in the slopes correlate to variations in cellular characteristics.

B. Calculating normal zeta potential

The zeta potential for three membrane cases: (1) virgin membrane, (2) EA926 cells seeded membrane, and (3) HEK cells seeded membrane were determined using the corresponding streaming potentials calculated using the Helmholtz–Smoluchowsky equation (Eq. (3)). Table I summarizes the results of this work for the three membrane cases. The hydraulic permeability (L_p) for each case was calculated by measuring the flux flowing across the membrane cases for the pressure drop.

C. Deconvolving zeta potential of cells from composite zeta potential of cell-cultured membrane

The values for the zeta potential were deconvolved to be -7.8 ± 1.04 mV and -7.4 ± 3.2 mV for the EA926 and the HEK confluent cells, respectively. Figure 4 compares the ζ of the virgin membrane, the composite membranes (EA926 confluent cell-seeded membrane or HEK confluent cell-seeded membrane), and the ζ of the EA926 cells and HEK cells alone deconvolved from the composite ζ of cell-cultured membranes using Eq. (9).

FIG. 4. Representative graphs comparing the measured zeta potential (ζ) for three membrane cases: virgin polycarbonate membranes, HEK confluent cell-seeded membrane, and EA926 confluent cell-seeded membrane and the deconvolved confluent cells. Using Eq. (9), the zeta potential (ζ_c) of the HEK cells and EA926 cells were deconvolved from the cell-membrane configurations.

D. Sensitivity of the calculated zeta potential of cells

The sensitivity of determining the zeta potential of the cells directly corresponds to the sensitivity of the corresponding streaming potential measurements. Using Eq. (9), the sensitivity of the overall streaming potential to changes in the streaming potential of the confluent cells only is approximated

$$\frac{\partial SP_{m+c}}{\partial SP_c} = \left(1 - \frac{L_{P_{m+c}}}{L_{P_m}}\right). \quad (10)$$

The equation clearly shows that the potential in capturing zeta potential of confluent cells from the proposed device is dependent on significant reductions in hydraulic permeability of the confluent cell-seeded membrane from the virgin membrane. As the L_p of confluent cell-seeded membrane increases for the same membrane, the error propagated through the system decreases.

It should also be noted that care should be taken in ensuring that the system has reached steady state when recording measurements associated with streaming potential or hydraulic permeability.

E. Zeta potential measurements using Zetasizer

Although the normal zeta potential for the confluent cells does not have to correspond to the zeta potential of single cells, their comparison is instructive. Figure 5 compares the zeta potential measured using a Zetasizer (for fluid dispersed cells) to that of the normal zeta potential of the

FIG. 5. Comparing the zeta potential of EA926 and HEK cells deduced from the streaming potential measured using our streaming potential device and that measured using commercially available Malvern Zetasizer.

corresponding confluent cell layer. The measured zeta potential for both HEK and EA926 cells, in this study, are not statistically different.

IV. CONCLUSION

Here we develop a simple and novel device to determine the normal ζ of confluent cells that are cultured on membranes. The device has the potential to be used to study cell surface interactions to identify and monitor the dynamic responses of the cells in response to administration of chemical or biological stimuli. Current ζ devices employ methodologies incorporating electrokinetic, sesimokinetic, or electroosmotic concepts; however, they are limited by the inability to measure normal zeta potential of cells and cellular monolayers.

In summary, we investigated the ζ of two different cellular (HEK cell line and EA926 cell line) monolayers on polycarbonate membranes. Using Helmholtz–Smoluchowski's equation and Darcy's law, we developed a methodology to deduce the zeta potential of confluent cellular layers that is derived from the work of Teychene *et al.*³¹ The proposed method assumes minimum conductivity inside the tight junctions and uses the conductivity of the bulk media flowing perpendicular to the confluent cell layer. The method is applicable when the mean size of the tight junctions is not much smaller than the Debye length of the confluent cellular layer. The results are sensitive to the ratio of the hydraulic permeability of the virgin membrane and that of the confluent cell-seeded membrane. Moreover, for isotropically charged cells that may be susceptible to removal by shear using a tangential flow device, the proposed normal flow device offers an ideal alternative that will maintain cell adherence to the membrane. We demonstrate that this ζ measurement device is a convenient laboratory approach and show that this method can be used to quickly calculate electrostatic properties of confluent cellular surfaces.

ACKNOWLEDGMENTS

We also thank Dr. Bahman Anvari (University of California, Riverside) for his support with the Malvern Zetasizer.

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