An In Vitro Model of Erythroid Egress in Bone Marrow

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An in vitro system has been developed that mimics the passage of erythrocytes from the bone marrow to the circulation. Bone marrow egress and its proper regulation are vital physiologic processes. However, because of the inaccessibility of the marrow, it is difficult to evaluate the various factors important in controlling these processes or even to define the precise mechanism by which egress occurs. The in vitro system has been designed to evaluate the importance of different physical parameters in regulating egress. It consists of a thin silicon wafer (thickness = 1.0 μm) cemented over the tip of a large (15.0 μm ID) micropipette. The wafer contains a single circular pore. Cells were observed under the microscope as they passed through the pore under controlled pressures. The rate and duration of passage were obtained from videorecordings of the experiment. The measured passage times agreed well with the predictions of a simple analytical model of a cell passing through a thin aperture. The experimental results confirm the conclusion reached from the analysis that the pressures needed to drive a cell through the pore are well within the physiologic range, and the time needed to complete egress is typically less than 1.0 seconds. These results support the hypothesis that erythrocyte egress may be driven by a hydrostatic pressure difference across the pore.

A median adult man has approximately 2.5 × 10^12 red blood cells in circulation at any given time, and the average lifespan of each cell is approximately 120 days. To maintain homeostasis, the bone marrow must supply about 2.5 × 10^6 new erythrocytes to the circulation every second. To get from the hemopoietic space, where the cells grow and differentiate, to the marrow sinuses, which are contiguous with the circulation, the cells must pass through small apertures in the endothelial barrier separating the two compartments. These apertures are generally less than 1.0 μm in thickness and range in diameter from 1.0 μm to 3.0 μm. The mechanism by which the cells pass through these apertures and the factors that regulate the flux of cells across the endothelial boundary are not well understood. We have developed an in vitro model of the egress process to evaluate the importance of different physical parameters in controlling this process.

The motivation for these investigations came from examining electron micrographs of reticulocytes in passage through endothelial pores in the bone marrow of the mouse (Fig 1). The spherical contour of the portion of the cell in the sinus suggested that the reticulocyte egress may be driven by a hydrostatic pressure difference across the pore. An approximate analysis was undertaken to obtain estimates for the time it would take a cell to complete egress at different pressures and to evaluate the importance of pore diameter and membrane viscoelastic properties on the rate of egress. Based on this analysis, it was concluded that there is a threshold pressure on the order of 1.0 to 3.0 mmHg below which the cell would not pass through the pore because of the elastic resistance of the membrane to deformation. It was also concluded that for pressures appreciably greater than the threshold pressure, the time required for the cell to complete its passage was short, typically less than 0.5 seconds.

In the present paper an experimental system has been developed to test the analytical predictions. A thin (∼1.0 μm) silicon wafer with a single pore in it was cemented with epoxy over the tip of a large (15.0 μm ID) micropipette, and single erythrocytes were observed as they passed through the pore. The time course of the passage was observed as a function of the driving pressure. The experimental results agreed well with the analytic predictions. These observations support the hypothesis that erythrocyte egress may occur through preexisting endothelial pores and be driven by hydrostatic pressure.

MATERIALS AND METHODS

Cells were obtained by finger prick and suspended in a buffered saline solution containing 125 mmol/L NaCl, 24.4 mmol/L Na2HPO4, 6.1 mmol/L KH2PO4, 1.0 μmol/L sodium azide, and 3.0 mg/mL bovine serum albumin. The cell suspension was placed on the stage of an inverted microscope (Nikon M) in a chamber (1.0 × 1.0 × 0.1 cm) that allowed access for micropipettes from opposite sides. Experiments were performed at room temperature (−25 °C). The pipette-pore was connected via continuous water connection to an enclosed reservoir. Zero pressure across the pore was set by positioning the water-filled reservoir such that particles in or near the opening remained stationary. Changes in pressure relative to zero were produced by pressurizing the enclosed reservoir with an air-filled syringe. The pressure was monitored with a differential pressure transducer. The pipette-pore was introduced into the chamber on the microscope stage and an ordinary micropipette was introduced from the other side. The second micropipette was used to manipulate cells from the bottom coverglass to a place near the pore opening. The cell was sucked into the pipette-pore, then a constant outward pressure was set using the air-filled syringe. The passage of the cell out of the pore under constant pressure was observed, then the cell was reaspirated into the pore and the expulsion was repeated at a different pressure. Several passages at different pressures were observed for each cell. Experiments were recorded on videotape along with a digital display of the trans-pore pressure and the time.

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The pipette-pore assembly. The assembly process is depicted diagrammatically in Figs 2(A–D). The silicon wafer pore was manufactured at the National Resource and Research Facility for Submicron Structures at Cornell University. Wafers were thinned to a thickness of approximately 1.0 μm, and a pattern was etched through the wafer (Fig 2A). Pipettes were formed from microneedles pulled on a David Kopf Instruments pipette puller. The needles were ground to a flat tip against an aluminum oxide surface in an ultrasonic bath. The intensity of the bath was adjusted using a Variac transformer. After a flat tip of the desired diameter was formed, the pipette was bent at a 90° angle using a microforge (Stoelting) mounted on the stage of the microscope (see Fig 2B).

To attach the pore to the pipette, the wafer was placed on a glass slide on the microscope stage, and a ring of ultraviolet-sensitive adhesive (Crystal Clear Adhesive, Loctite Corporation, Cleveland, Ohio) was painted around the pore to match the pipette circumference. The pipette was positioned such that the opening faced toward the objective and was centered over the pore (Fig 2C). The pipette was lowered onto the wafer with a micromanipulator until it contacted the glue and formed a seal. The assembly was exposed to ultraviolet light to harden the glue. Then using the micromanipulator the pipette was pressed down onto the wafer until the pore was punched out of the wafer along the perforated ring (Fig 2D).

Prior to the experiment, the pipette-pore assembly was filled with filtered, phosphate-buffered saline. The pipette-pore was inserted into a sealed, saline-filled chamber and the chamber was pressurized by depressing the plunger of a syringe, forcing saline through the pore into the tip of the micropipette. When the tip was filled, the pipette-pore was removed from the chamber and the shaft of the pipette was filled from the back using a 31 gauge needle.

Theoretical background. An approximate analysis of an erythrocyte passing through a thin, circular aperture has appeared elsewhere, and only a brief description of the analysis will be given here. The geometry used to approximate the cell contour is shown in Fig 3. The undeformed membrane consists of two flat disks of radius R, connected at the edge. To simulate egress, the upper disk is deformed into a spherical section. The area of the surface is conserved, but the cell volume is allowed to change freely. (Volume conserving models proved to be analytically intractable.) As the
spherical projection grows, material is drawn toward the aperture. In the planar region, the balance of forces in the radial direction is given by:

\[
\frac{dT_m}{dr} = -\frac{2T_r}{r}
\]

where \(T_m\) is the principal membrane tension in the radial (meridional) direction and \(r\) is the radial coordinate. A differential equation is obtained by combining Equations 1 and 2 to eliminate \(T_r\). Solution of the equation requires two boundary conditions. At the edge of the pore (\(r = R_p\)) we take \(T_m = -\Delta P R_p/2\), where \(R_p\) is the radius of the spherical section that has passed through the pore and \(\Delta P\) is the pressure difference across the pore. At the edge of the cell (\(r = R_c\)) we take \(T_m = 0\). The solution to the integration results in a first order differential equation relating the height of the spherical projection, \(h\), to time. In terms of dimensionless quantities this takes the form:

\[
\frac{dh}{dt} = \frac{\Delta P}{2} \left( h + 1 \right) - \frac{h^2}{2} - \ln \left[ 1 - \frac{h^2}{R_c} \right] - \frac{h^2}{2} + \frac{1}{2} \ln \left[ 2R_c - h^2 - 1 \right]
\]

and:

\[
\frac{dh}{dt} = \frac{\Delta P}{2} \left( h + 1 \right) + 1 - \frac{R_c}{2} - \frac{h^2}{2} + \frac{1}{2} \ln \left[ 2R_c - h^2 - 1 \right] - \frac{h^2}{2} + \frac{1}{2} \ln \left[ 2R_c - h^2 - 1 \right]
\]

where \(A_s\) is the area of the spherical section and \(A_{1/2}\) is half of the total cell area.

The variables in these expressions are:

\[
\begin{align*}
\bar{h} &= h/R_p \\
\Delta \bar{P} &= \Delta P R_p/4\mu \\
R_c &= R_{\text{cell}}/R_p \\
\bar{t} &= t - \mu/h
\end{align*}
\]

where \(\Delta \bar{P}\) is the pressure across the pore, \(R_p\) is the pore radius, \(R_{\text{cell}}\) is the radius of the undeformed disks, and \(h\) is the height of the spherical section (see Fig 3). Two expressions are required because of the different relationships between \(h\) and \(r\) for material that was originally on the upper or lower disk. Before half of the cell passes the pore edge, the integral encompasses regions from both the upper and lower disks (Equation 3). After half of the cell passes the pore edge (Equation 4), only material originally from the lower disk remains in the planar region.

Equations 3 and 4 were integrated numerically using Simpson’s Rule to obtain the height of the spherical projection as a function of time, as well as the total time for egress for a given set of parameters, \(\Delta \bar{P}\), \(R_c\), and \(R_p\). It is important to recognize that there is a threshold pressure, \(\Delta \bar{P}_{\text{min}}\), below which egress does not occur. Mathematically this occurs because for \(\Delta \bar{P} < \Delta \bar{P}_{\text{min}}\), \(dh/dt\) becomes zero before egress is completed; hence the integration of Equations 3 and 4 cannot be completed. Physically this occurs because the pressure must be sufficient to overcome the elastic resistance of the mem-

Fig 3. A schematic drawing of the geometric model used in the analysis. The initial cell radius is \(R_{\text{cell}}\). The pore diameter is \(D_p\). The radius of the spherical projection is \(R\), and its height is \(h\). The instantaneous cell diameter is \(R_{D0}\). The pore diameter is \(D_p\). When the radius of the lower disk equals \(R_{\text{cell}}\), egress is complete.

Fig 4. Results of the Analysis. (A) The dependence of the dimensionless threshold pressure (\(\Delta \bar{P}_{\text{min}}\) /4\(\mu\)) on the dimensionless cell radius (\(R_{\text{cell}}/R_p\)). Note that for a cell radius of 4.0 \(\mu\)m and a pore radius of 0.5 \(\mu\)m (\(R_{\text{cell}} = 8.0\)) the minimum pressure for egress is less than 2.0 mm Hg (2.7 \(\times 10^4\) dyn/cm\(^2\)) (\(\Delta \bar{P} = 4.75\)). (B) The effect of changing \(R_{\text{cell}}\) on the time course of egress. \(\Delta \bar{P}\) is held constant (\(\Delta \bar{P} = 10.0\)). For larger values of \(R_{\text{cell}}\) the final value of \(h\) is larger because the membrane has more surface area.
The pore used in the present experiments was approximately 1.6 m in diameter and 1.2 m in thickness, as measured in the light microscope. A cell in the process of passing through the pore is shown in Fig 5. A total of 28 cells were tested. Six of these were discarded because they tended to adhere to the silicon pore and their passage times were artifactually long. The data from the remaining cells agreed well with the analytic predictions, both for the height of the projection as a function of time and for the total time as a function of the pressure difference across the pore. The sequence in which the different pressures were applied was not ordered. When a pressure was repeated for a given cell, the egress times were similar, i.e., there appeared to be no history dependence of the pressure-time relationship.

Three examples of the height of the projection as a function of time are shown in Fig 6. The data were obtained from recordings of a single cell passing through the pore three different times, each time at a different pressure (0.62 mm Hg, 0.72 mm Hg, and 0.90 mm Hg). The solid lines show the theoretical prediction (Equations 3 and 4) for the projection height as a function of time for a cell with an initial radius of 4.0 μm passing through a pore with a radius of 0.8 m at pressures of 0.62 mm Hg, 0.72 mm Hg, and 0.84 mm Hg. Because of the finite thickness of the wafer and uncertainty about the exact location of the point, h = 0, each of the data sets was shifted vertically to minimize the average vertical distance between the data points and the corresponding curve.

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ing curve. No other adjustments in the data or the theoretical parameters were made.

It should be noted that not all of the measured time courses of egress agreed as well with the analytic prediction. At pressures near the threshold pressure (the minimum pressure needed to push the cell through the pore) small errors in the values chosen for \( R_p \) or \( \Delta P \) resulted in large differences in the predicted time course of egress. At high pressures, the accuracy of the measurements made from the videorecordings was limited by the framing speed of the camera, and it was not possible to evaluate the accuracy of the analytic prediction. At intermediate pressures, however, the agreement between theory and observation shown in Fig 6 was typical, although in some cases the values for \( R_p \) or \( \Delta P \) had to be adjusted to obtain the best fit. Considering the simplicity of the analytic model, the agreement between theory and experiment was remarkably good.

The egress times for 22 different cells over a range of pressures are shown in Fig 7. The theoretical curves are calculated for a pore radius of 0.8 \( \mu \)m and initial cell radii (from left to right) of 3.2 \( \mu \)m, 4.0 \( \mu \)m, and 4.8 \( \mu \)m. For this curve, 14 dimensionless time units correspond to one second, and one dimensionless pressure unit is 350 dyn/cm\(^2\) (0.26 mm Hg). No parameters were used to adjust the data to fit the theoretical prediction. The agreement is again quite good, especially at the larger pressures. At very low pressures, the cells tended to pass through the pore more quickly than expected, and the actual threshold pressure was slightly lower than was predicted.

**DISCUSSION**

The experimental results confirm the major theoretical predictions. First, there is a threshold pressure on the order of 0.5 mm Hg required to push the cell through the pore, and second, for pressures greater than 1.0 mm Hg, passage times are typically less than one second. The accurate prediction of the change in the height of the spherical projection with time also supports the validity of the analysis and its underlying assumptions.

One slight, but potentially important, difference between the analytic prediction and the experimental results is the lower-than-expected threshold pressure that was observed in the experiments. This difference is most probably due to the cell folding as it passes through the aperture. Such folding would reduce the amount of surface deformation required for passage compared to the deformation of the analytic model. It is important to recognize that because of their ability to fold, real cells can negotiate an aperture at pressures lower than that predicted by the theoretical model.

The slightly higher-than-expected passage times that were observed for pressures between 1.0 and 2.0 mm Hg are probably due to adhesive or frictional interactions between the cells and pore. Cells that took longer than expected to complete their passage generally did not follow the predicted dependence of the projection height on time. The differences between the predicted and the observed time courses were consistent with small frictional or adhesive interactions between the cell and the pore.

**Constraints of surface area and volume.** The present analysis does not take into account restriction on passage resulting from the conditions of constant cell surface area and volume. Although the present analysis is sufficiently accurate to predict cell passage times when the surface-to-volume ratio is sufficient for cell passage, the constraints of constant area and volume limit the size of an aperture that a cell can negotiate. The strength of this limitation is illustrated by the experimental results shown in Fig 8. A single cell was transferred with a micropipette into a series of chambers with successively decreasing salt concentrations. The pressure needed to aspirate the cell into the pipette \( (P_t) \) was measured in each chamber. Over the range of concentrations between 260 mOsm and 230 mOsm only a slight increase in \( P_t \) occurred. This slight increase was probably due to a decrease in the ability of the cell to fold, because more and more membrane area was being taken up to encompass the cell volume. The abrupt change in \( P_t \) at around 225 mOsm occurred because at this salt concentration the surface area of the cell was not sufficient to enclose the cell volume within the constraint of the pipette geometry.

Whether or not a cell is restricted from passage through a particular pore depends on the surface and volume of the cell and the size and shape of the aperture. In the Appendix a relationship is derived to calculate the minimum surface area a cell with a given volume must have to negotiate a cylindrical pore of given dimensions. In Fig 9, this minimum area is shown as a function of cell volume for three different-sized pores. The solid curve corresponds to a pore of radius \( (R_p) \) 0.8 \( \mu \)m and thickness \((L)\) 1.2 \( \mu \)m. These are the measured dimensions of the pore used in the present study. The short-dashed curve corresponds to \( R_p = 0.8 \mu \)m and \( L = 0.0 \mu \)m. This relationship would be appropriate for the present study if the pore was hour-glass shaped, such that the thickness of the wafer at the aperture was less than the
The pressure ($P_r$) required to pull a cell into a micropipette (3.5 μm ID) is shown as a function of the osmolarity of the suspending solution. Points represent measured aspiration pressures for the same cell into the same pipette in solutions of different salt concentrations. The solid curve is "hand-drawn" and has no theoretical significance. The abrupt change in $P_r$ at around 225 mOsm occurs because the volume of the cell at this concentration is too large to be contained by the cell membrane within the constraint of the pipette geometry. The volumes shown in parenthesis were calculated based on a volume of 100 μm$^3$ at 230 mOsm assuming that the cell behaved as an osmometer with a nonideality coefficient of 0.8: $[V - V_i/V_i = 0.6 (C_i - C)/C]$, where $V_i$ is the isotonic volume (88.5 μm$^3$) and $C_i = 290$ mOsm.

This observation is at odds with expectations based on the data of Tsang,$^7$ who used high-resolution interference holograms to measure the surface area and volume of over 1,500 cells from a variety of donors (see also Fung et al$^8$). Tsang's data are shown as the shaded region in Fig 9. A significant fraction of the cells in Tsang's study lie below the minimum area curves for the pore size used in our study. Cells falling below the curve for a particular aperture should be incapable of passing through, except under very large pressures or after lysis. If the surface areas and volumes of the cells used in our study had the same surface area and volume distribution as the cells in Tsang's study, we would have expected a significant fraction of them to be unable to pass through the pore under the pressures used in our experiments. The fact that all of the cells in our study passed through was surprising.

The reason for this discrepancy is not clear. It is unlikely that it is due to an error in measuring the pore diameter, because the pore would have to have a diameter twice as large as was measured to accommodate most of the cells in Tsang's study (note the long-dashed line in Fig 9). Other explanations seem more plausible. Differences in the suspending buffers could account for at least part of the difference. (Tsang used a TRIS buffer whereas we used phosphate.) Additionally, there may have been unconscious bias in cell selection for our measurements. An earlier, less extensive study by Evans$^9$ using the same technique as Tsang found volumes 4.0% smaller and surface areas 4.0% larger than those reported by Tsang. This difference was thought to be due to bias in cell selection. The discrepancy between our observations and Tsang's results could be accounted for by such small differences.

Clearly, when the area of the cell is insufficient to encompass the cell volume during passage, the present analysis is inapplicable. However, as the data in Fig 8 show, when the area is sufficient, the cell volume has little effect on the dynamics of cell passage.

Implications about bone marrow egress. The results of these experiments support the feasibility of pressure-driven egress of reticulocytes from the bone marrow. Although leukocytes clearly have the capability to move themselves across an endothelial boundary, the mechanism by which reticulocytes pass into the marrow sinuses has remained obscure. Circulating reticulocytes show no evidence of intrinsic motility. If reticulocyte egress is pressure-driven, a major question remains as to the source of the pressure gradient across the pore. Two features of the present results bear on this question. The first is that the pressure required to force a cell through an aperture is small compared to systemic pressures. Michelsen$^{10}$ has measured pressure differences of 20.0 mm Hg between the marrow tissue and the emissary vein just outside the cortical bone in rabbits. It is reasonable to hypothesize that a part of that pressure drop sufficient to
drive reticulocyte egress (2.0 to 4.0 mm Hg) might exist across the pore between the hematopoietic space and the marrow sinus, which is contiguous with the emissary vein.

The second feature of the present results that bears on the question of the origin of the driving pressure is the short time required to complete egress (~0.3 s) when the threshold pressure is exceeded. The pressures that Michelsen recorded were pulsatile, and it is likely that pressures across the pore sufficient to cause egress, occur only transiently. Dabrowski et al. have shown a correlation between transient increases in marrow pressure and an increase in the release of reticulocytes into the circulation in rabbits. The fact that the times for cell passage that we have measured are less than, or on the order of, the heart rate is an important observation because it shows that if sufficient pressures for egress occur transiently with the pulse, there would still be sufficient time for egress to be completed.

It is important to recognize that in the present study we have used mature erythrocytes to develop and test our theories. If the membrane elastic modulus or the membrane viscosity of early reticulocytes were significantly different from mature cells, our conclusions might be affected. However, preliminary experiments in our laboratory on blood cells of the rabbit indicate that differences in membrane properties between mature erythrocytes and reticulocytes are slight. The average value measured for reticulocyte shear modulus of 5.5 × 10−2 dyn/cm was similar to the control value, 4.5 × 10−2 dyn/cm, and the viscoelastic recovery time constants (the ratios of the membrane viscosity to the membrane shear modulus) were also similar: 0.074 seconds for reticulocytes and 0.081 seconds for controls. The reticulocyte samples in those experiments were prepared by density gradient centrifugation. More than 90% of the cells in those samples were reticulocytes as determined by New Methylene Blue staining. However, in our experiments we selected against oddly shaped cells in favor of those that were biconcave and our data sample probably included more late than early reticulocytes. Nevertheless, it is unlikely that the properties of even very young reticulocytes are so different from mature cells that the conclusions of the present work would be affected substantially.

Our ability to critically evaluate these ideas is hampered by a lack of information about the local environment within the marrow. We have had to use pore dimensions measured in the mouse, pressure differences measured in the rabbit, and transit times and threshold pressures measured on human erythrocytes. Although differences in the architecture and hydrodynamics found within different animals may affect the details of the regulation process for each animal, the major conclusions of the present study should not be affected by anatomic differences between different species. The pressures required to move red cells across thin circular apertures are within the physiologic range, and the speed with which the passage is completed is sufficient for it to occur within the space of a single heartbeat.

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APPENDIX

Because erythrocytes maintain essentially constant surface area and volume, there are limits on the size and shape of an aperture that a cell can negotiate. In this appendix, a functional relationship is developed that makes it possible to calculate the maximum volume that a cell of a given surface area can have and still be able to pass through a cylindrical pore of given dimensions.

To begin the calculations, consider the shape shown in Fig 10. The surface area, A, and volume, V, of this surface of revolution are:

\[ f_A = A = 2\pi \left[ R_1 (R_1 + \sqrt{R_1^2 - R_2^2}) + R_2 (R_2 + \sqrt{R_2^2 - R_1^2}) + R_p L \right] \]  

\[ f_V = V = \frac{1}{2} \pi \left[ R_1^3 + (R_1^2 + R_p^2/2) \sqrt{R_1^2 - R_p^2} + R_2^3 + (R_2^2 + R_p^2/2) \sqrt{R_2^2 - R_p^2} + \frac{1}{6} R_p^3 L \right] \]

For a fixed volume, we wish to find the relationship between R_1 and R_2 that requires the largest surface area. This is the “critical geometry” for cell passage, i.e., if the cell can assume that geometry it will be able to negotiate the aperture. We use the method of Lagrange multipliers and define the function:

\[ H = f_A - \lambda (V - f_V) \]  

where \( \lambda \) is the Lagrange multiplier. At the extremum three conditions must be met:

\[ \frac{\partial H}{\partial R_1} = 0; \quad \frac{\partial H}{\partial R_2} = 0; \quad \frac{\partial H}{\partial \lambda} = 0. \]

The third condition simply gives the expression for the cell volume (Equation A2). The first two conditions require that:

\[ 4R_1 (R_1 + \sqrt{R_1^2 - R_p^2}) - 2R_p^2 \]

\[ + \lambda [2R_1^3 (R_1 + \sqrt{R_1^2 - R_p^2}) - R_1 R_p^2] = 0 \]

and,

\[ 4R_2 (R_2 + \sqrt{R_2^2 - R_p^2}) - 2R_p^2 \]

\[ + \lambda [2R_2^3 (R_2 + \sqrt{R_2^2 - R_p^2}) - R_2 R_p^2] = 0. \]

Fig 10. Schematic of a cell caught in a cylindrical aperture. The length of the aperture is L and its radius is R_p. The contours of the cell on either side of the aperture are spherical with radii R_1 and R_2.
After solving Equation A5 for \( \lambda \) and substituting that expression into Equation A6, it can be seen by inspection that both conditions will be met when \( R_1 = R_2 \). Let \( R_o \) be the outer radius when the two outer radii are equal. Then the expressions for area and volume at the critical geometry are:

\[
A = 4\pi R_o \left( R_o + \sqrt{R_o^2 - R_p^2} \right) + 2\pi R_p L \tag{A7}
\]

\[
V = \frac{\pi}{6} [R_o^3 + (R_o^3 + R_p^3/2) \sqrt{R_o^2 - R_p^2}] + \pi R_p^2 L. \tag{A8}
\]

Equation A7 can be solved for \( R_o \):

\[
R_o = C_1 \left( 2C_1 - R_p \right)^{-1/2} \tag{A9}
\]

where \( C_1 = A/4\pi - R_p L/2 \). Equation A9 can be substituted into Equation A8 to eliminate \( R_o \) and obtain a functional relationship among \( A, V, R_p, \) and \( L \):

\[
f(A, V, R_p, L) = 0 \tag{A10}
\]

To generate the curve in Fig 9, the pore dimensions \( R_p \) and \( L \) were specified, and an arbitrary value for \( A \) was chosen. \( R_o \) could then be calculated via Equation A9, and the volume, \( V \), corresponding to the chosen value of \( A \) could be calculated directly from Equation A8. This volume is the maximum that a cell of area \( A \) can have and be able to pass through a pore of the given dimensions. Equation A10 can also be used to calculate permissible pore dimensions for cells with specified surface area and volume, but numerical techniques are required to obtain such values.

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