Electronic Supplementary Material (ESI) for Lab on a Chip

Mechanical disruption of mammalian cells in a microfluidic system and its numerical analysis based on computational fluid dynamics

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Supplements

This supplementary material is to explain the derivation of formulas and essential analytical expressions, which were used to calculate values for membrane bursting tension, the cummulative probability for cell disruption, and the critical energy dissipation rate required for cell disruption.

Supplement 1 — Derivation of compressed cell volume according to the Guldinius Law

The hemicircular curve of the toroidal rim cross-section of the compressed cell can be described by the function [1]

$$y = R_i + \sqrt{\frac{1}{4}d^2 - x^2}\,, \tag{1}$$

where R_i is the y-coordinate of the base of the toroidal rim and d is its width. The cell volume is obtained by rotating this function around the x-axis at $y = 0$. Following the first Guldinius Law for rotation bodies [1], this volume is described by

$$\begin{aligned} V_c &= \pi \int \left(R_i + \sqrt{\frac{1}{4}d^2 - x^2} \right)^2 dx \\ &= \pi \left[xR_i^2 + \frac{1}{4}R_i r^2 \arcsin\left(\frac{2x}{d}\right) + x\sqrt{\frac{1}{4}d^2 - x} + \frac{1}{4}xd^2 - \frac{1}{3}x^3 \right]_{x=-\frac{d}{2}}^{x=+\frac{d}{2}} \\ &= \pi d R_i^2 + \frac{1}{4}\pi^2 R_i d^2 + \frac{1}{6}\pi d^3\,. \end{aligned} \tag{2}$$

Supplement 2 — Correlation of laminar shear stress and cell membrane tension

The deformation of a cell is correlated to the laminar shear stress by[2]

$$T = \frac{\xi \eta_0 r_0}{D} \nabla \mathbf{v} \,. \tag{3}$$

Substitution with

$$T = K \left(\frac{A - A_0}{A_0} \right) \tag{4}$$

gives

$$D \left(\frac{A - A_0}{A_0} \right) = \frac{\xi \eta_0 r_0}{K} \nabla \mathbf{v} \,, \tag{5}$$

which in full form can be written as

$$\frac{\xi \eta_0 r_0}{K} \nabla \mathbf{v} = \frac{D}{2} \left(\frac{1-D}{1+D} \right)^{2/3} + \frac{D}{2} \left(\frac{1-D}{1+D} \right)^{-1/3} \left[\frac{\arccos\left(\frac{1-D}{1+D}\right)}{\sqrt{1 - \left(\frac{1-D}{1+D}\right)^2}} \right] - D \,. \tag{6}$$

The right side of this equation with the cell deformation as the only variable can be solved numerically and fitted by an exponential expression, $F(x)$, according to

$$D \approx F(x) = 1 - \exp\left(a x^b + c x^d \right) \tag{7}$$

with high accuracy. The relative error of this fitting is $< 1\,\%$ (see Fig. 1) for the range of critical shear stresses for cell disruption, which is $250 - 450\ \mathrm{N\,m^{-2}}$. The fitting function $F(x)$ describes the correlation of D to $\nabla \mathbf{v}$ and according to equation 6:

$$x = \frac{\xi \eta_0 r_0}{K} \nabla \mathbf{v} = D \left(\frac{A - A_0}{A_0} \right) \,. \tag{8}$$

After substitution of D with $F(x)$ in equation 3, using the fitting parameters $a = c = -1.2$, $b = 0.8$, and $d = 1/3$, the final correlation of T to $\nabla \mathbf{v}$ can be expressed by

$$T = \frac{\xi \eta_0 r_0}{F(x)} \nabla \mathbf{v} \tag{9}$$

or in full form by

$$T = \xi \eta_0 r_0 \nabla \mathbf{v} \left[1 - \exp\left(-1.2 \left(\frac{\xi \eta_0 r_0}{K} \nabla \mathbf{v} \right)^{4/5} - 1.2 \left(\frac{\xi \eta_0 r_0}{K} \nabla \mathbf{v} \right)^{1/3} \right) \right]^{-1} \,. \tag{10}$$

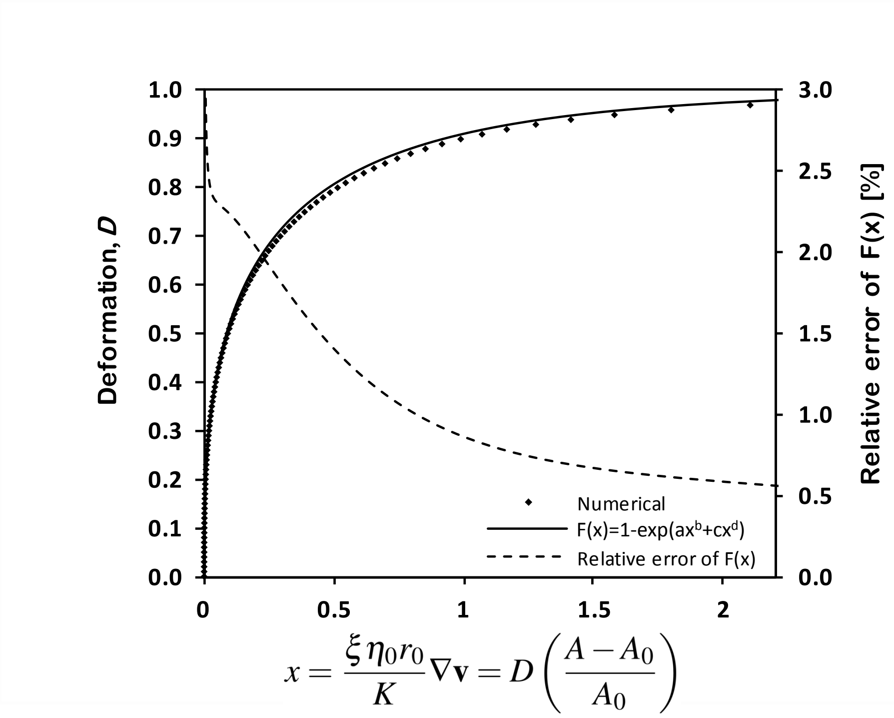

Figure 1 Fitting of the function D with a fitting function, $F(x)$. First, the expression $D\Delta A$ (right side of equation 6) was solved numerically and plotted for $0 \leq D \leq 1$. This type of curve can be fitted with an exponential function. According to equation 8, $x = \frac{\xi \eta_0 r_0}{K} \nabla \mathbf{v}$, which leads to the final expression in equation 10.

Supplement 3 — Fitting of the cummulative probability distribution

To obtain an analytical expression for the probability distribution of cell bursting at a given membrane tension, the integral

$$f(z) = \int_z^{\infty} e^{-u^2/2} du \tag{11}$$

was approximated over the full range of $-\infty \leq z \leq +\infty$ with an approximation function, $G(z)$, which was reported previously[3] to be

$$G(z) \approx \frac{\exp(-z^2/2)}{z} \left[1 - \frac{(1+bz^2)^{1/2}/(1+az^2)}{C_0 z + \left[C_0^2 z^2 + \exp(-z^2/2)(1+bz^2)^{1/2}/(1+az^2) \right]^{1/2}} \right], \tag{12}$$

where $C_0 = (\pi/2)^{1/2}$, $a = \left[1 + (1 - 2\pi^2 + 6\pi)^2\right]^{1/2}$, and $b = 2\pi a^2$. Substitution of equation 12 with $z = (T - T_m)/\sigma_T$ gives the analytical expression

$$P_s(z) = 1 - \frac{G(z)}{\sqrt{2\pi}}, \tag{13}$$

which can be used to calculate the probability for cell bursting from the results of CFD-analysis, where the membrane tension was obtained according to equation 3 in supplement 2.

Supplement 4 — Calculation of the fraction of disrupted cells in a CHO cell population

The integral

$$P_s^{\Sigma} = \frac{1}{\sigma_r \sqrt{2\pi}} \int_{r_{0_1}}^{r_{0_2}} e^{-\frac{1}{2}\left(\frac{r_0 - r_m}{\sigma_r}\right)^2} P_s(T \propto r_0) dr_0 \tag{14}$$

or a cell size distribution which is normally distributed can be approximated according to the trapezoidal rule for integral approximation[1] with

$$P_s^{\Sigma} \approx \frac{1}{\sqrt{8\pi}} \sum_{i=2}^{N} (r_i - r_{i-1}) \left(e^{-\frac{1}{2}\left(\frac{r_i - r_m}{\sigma_r}\right)^2} P_s(r_i) + e^{-\frac{1}{2}\left(\frac{r_{i-1} - r_m}{\sigma_r}\right)^2} P_s(r_{i-1}) \right) \tag{15}$$

and for a cell size distribution which cannot be approximated by a normal distribution

$$P_s^{\Sigma} \approx \frac{1}{\sqrt{8\pi}} \sum_{i=2}^{N} (r_i - r_{i-1}) \left(q_i P_s(r_i) + q_{i-1} P_s(r_{i-1}) \right) \tag{16}$$

with q_i being the fraction of cells with a radius r_i.

Supplement 5 — Calculation of the critical energy dissipation rate required for cell disruption

The energy dissipation rate is defined by[4]

$$EDR = \tau : \nabla \mathbf{v} \tag{17}$$

and describes the irreversible conversion of mechanical energy to heat per volume (in a viscous fluid). τ is the stress tensor, $\nabla \mathbf{v}$ is the velocity gradient tensor and both can be calculated from CFD-analysis. To calculate the critical energy dissipation rate, EDR_{crit}, which is required for cell disruption in the micronozzle geometry, P_s was plotted against EDR (both obtained from CFD-analysis) for the range of cell diameters of the CHO cell population with $d_c = 8 - 25$ µm. These curves (see Fig. 9) were approximated for the relevant range of EDR values with

$$P_s = a \cdot \log(EDR) + b\,, \tag{18}$$

where $a = 0.2547$ is the constant slope of the curves and $b = f(d_c)$ is a function of the cell diameter. These values for b for each curve (each cell diameter) can be plotted against the cell diameter to obtain an expression for b with

$$b = f(d_c) = -2.4039 \cdot (d_c)^{-0.1369}\,, \tag{19}$$

which after substitution into equation 18 yields

$$P_s = 0.2547 \cdot \log(EDR) - 2.4039 \cdot (d_c)^{-0.1369}\,. \tag{20}$$

This expression can be used to calculate the level of cell disruption at a known energy dissipation rate. After rearrangement for EDR, it can also be used to estimate the energy dissipation rate required to obtain a certain level of cell disruption, e.g. 50 %, for a given cell diameter. Finally, the mean critical energy dissipation rate for the whole cell population with known size distribution was calculated. For this purpose, the trapezoidal rule for integral approximation was used to describe the cell size distribution in a similar way as demonstrated for the determination of P_s^{Σ} (supplement 4).

Supplement 6 — Parameters and conditions for CFD-analysis

Comsol Multiphysics 3.5a was used for numerical simulations. The micronozzle geometry of a single nozzle was created in a 2D drawing area and extruded to a height of 50 µm, which corresponds to the etch depth. The Incompressible Navier-Stokes application mode ('mmglf2') of the MEMS-module was used for numerical simulation within this domain with default values of the application mode properties. The density of the fluid was set to $\rho = 1000\,\mathrm{kg\,m^{-3}}$ and the viscosity was set to $\eta = 1$ Pa·s.

The governing equations (described in the Comsol MEMS User's Guide) in all subdomains were

$$\rho\frac{\delta\mathbf{v}}{\delta t} - \nabla\cdot\left[-p\mathbf{I} + \eta\left(\nabla\mathbf{v} + (\nabla\mathbf{v})^T\right)\right] + \rho\mathbf{v}\cdot\nabla\mathbf{v} = \mathbf{F} \tag{21}$$

and

$$\nabla\cdot\mathbf{u} = 0 \tag{22}$$

with ρ being the fluid density, $\mathbf{v}$ as the velocity vector, p being the pressure, η is the dynamic viscosity, F is a body force term (N/m^3) and I is the identity matrix.

The boundary conditions were defined as follows:

Inlets: Laminar inflow, volumetric flow rate V_0 set to the experimentally given flow rates, entrance length $L_{entr} = 10^{-4}$ m and outer edges constrained to zero.

$$L_{entr}\nabla_t\cdot\left[p\mathbf{I} - \eta\left(\nabla_t\mathbf{v} + (\nabla_t\mathbf{v})^T\right)\right] = -\mathbf{n}p_{entr} \tag{23}$$

and

$$\nabla_t\mathbf{v} = 0 \tag{24}$$

where $\mathbf{n}$ is the normal vector of the inlet.

Outlets: No pressure ($p_0 = 0$) and no viscous stress.

$$\eta\left(\nabla\mathbf{v} + (\nabla\mathbf{v})^T\right)n = 0, p_0 = 0 \tag{25}$$

and

$$p_0 = 0. \tag{26}$$

Walls: No slip boundary condition. $\mathbf{v} = 0$

No artificial diffusion was used for the Navier-Stokes equation.

The equation system was solved (stationary) using the PARDISO direct solver with nested dissection as preordering algorithm and using row preordering.

After a solution has been obtained, yielding the velocity vector field, equation 10 was used to calculate the probability for cell disruption on the basis of this vector field using Comsol's postprocessing capabilities.

References

[1] *Kleine Enzyklopädie Mathematik*, VEB Bibliographisches Institut Leipzig.

[2] C. Born, Z. Zhang, M. Al-Rubeai and C. R. Thomas, *Biotechnology and Bioengineering*, 1992, **40**, 1004–1010.

[3] R. G. Hart, *Mathematics of Computation*, 1966, **20**, 600–602.

[4] R. B. Bird, W. E. Stewart and E. N. Lightfoot, *Transport Phenomena*, John Wiley & Sons, Inc., 2007, vol. 2.