A hyperelastic model for simulating cells in flow

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Abstract

In the emerging field of 3D bioprinting, cell damage due to large deformations is considered a main cause for cell death and loss of functionality inside the printed construct. Those deformations, in turn, strongly depend on the mechano-elastic response of the cell to the hydrodynamic stresses experienced during printing. In this work, we present a numerical model to simulate the deformation of biological cells in arbitrary three-dimensional flows. We consider cells as an elastic continuum according to the hyperelastic Mooney-Rivlin model. We then employ force calculations on a tetrahedralized volume mesh.

To validate our model, we perform a series of FluidFM® compression experiments with REF52 cells demonstrating that our hyperelastic model provides a very good description of the experimental data even at very large deformations up to 80%. In addition, we validate the model by comparing to axisymmetric simulations and to previous AFM experiments on bovine endothelial cells and artificial hydrogel particles. To investigate cell deformation in flow, we incorporate our model into Lattice Boltzmann simulations via an Immersed-Boundary algorithm. In linear shear flows, our model shows excellent agreement with analytical calculations and previous simulation data.

Keywords: hyperelasticity, cell deformation, Mooney-Rivlin, atomic force microscopy, shear flow, Lattice-Boltzmann

1 Introduction

The dynamic behavior of flowing cells is central to the functioning of organisms and forms the base for a variety of biomedical applications. Technological systems that make use of the elastic behavior of cells are, for example, cell sorting [1], real-time deformability cytometry [2, 3] or probing techniques for cytoskeletal mechanics [4–15]. In most, but not all of these applications cell deformations typically remain rather small. A specific example where large deformations become important is 3D bioprinting. Bioprinting is a technology which, analogously to common 3D printing, pushes a suspension of cells in highly viscous hydrogels – a so-called bioink – through a fine nozzle to create three-dimensional tissue structures. A major challenge in this process lies in the control of large cell deformations and cell damage during printing. Those deformations arise from hydrodynamic stresses in the printer nozzle and ultimately affect the viability and functionality of the cells in the printed construct [16–19]. How exactly these hydrodynamic forces correlate with cell deformation, however, strongly depends on the elastic behavior of the cell and its interaction with the flowing liquid. Theoretical and computational modeling efforts in this area have thus far been restricted to pure fluid simulations without actually incorporating the cells [17, 20, 21] or simple 2D geometries [22, 23]. The complexity of cell mechanics and the diversity of possible applications make theoretical modeling of cell mechanics in flow a challenge which, to start with, requires reliable experimental data for large cell deformations.

The most appropriate tool to measure cellular response at large deformations is atomic force microscopy (AFM) [8, 24–31]. AFM cantilevers with pyramidal tips, colloidal probes, or flat geometries are used to indent or compress cells. Therefore, a common approach to characterize the elasticity of cells utilizes the Hertzian theory, which describes the contact between two linear elastic solids [32, p. 90-104], but is limited to the range of small deformations [33]. Experimental measurements with medium-to-large deformations typically show significant deviations from the Hertz prediction, e.g. for cells or hydrogel particles [34]. Instead of linear elasticity, a suitable description of cell mechanics for bioprinting applications requires more advanced hyperelastic material properties. While for simple anucleate fluid-filled cells such as, e.g., red blood cells, theoretical models abound [35–39], the availability of models for cells including a complex cytoskeleton is rather limited. In axisymmetric geometries, [40] and [41] used an axisymmetric finite element model with hyperelasticity to model biological cells. In three dimensions, [42] utilized a DPD technique based on a bead-spring model while [43] and [44] considered viscoelastic and hyperelastic finite element models, respectively, in shear flow.

In this work, we present an alternative model for fully three-dimensional simulations of cells in flow based on a Mooney-Rivlin strain energy functional. To demonstrate the appropriateness of our model even at large cell deformation up to 80%, we provide extensive validation by car-
The calculation below:

Together with the right Cauchy-Green deformation tensor, our perelastic Mooney-Rivlin model implemented in this work. This section provides a brief overview of the hyperelastic models which usually require large matrix operations. Furthermore, it is easily extensible and allows, e.g., the inclusion of a cell nucleus by the choice of different elastic moduli for different parts of the volume.

We finally present simulations of our cell model in different flow scenarios using an Immersed-Boundary algorithm to couple our model with Lattice Boltzmann fluid calculations. In a plane Couette (linear shear) flow, we investigate the shear stress dependency of single cell deformation, which we compare to the average cell deformation in suspensions with higher volume fractions, and show that our results in the Neo-Hookean limit are in accordance with earlier elastic cell models [43, 44].

2 Theory

In general, hyperelastic models are used to describe materials that respond elastically to large deformations [46, p. 93]. Many cell types can be subjected to large reversible shape changes. This section provides a brief overview of the hyperelastic Mooney-Rivlin model implemented in this work.

The displacement of a point is given by

\[ u_i = y_i - x_i, \]

where \( x_i \) (\( i = 1, 2, 3 \)) refers to the undeformed configuration (material frame) and \( y_i \) to the deformed coordinates (spatial frame). We define the deformation gradient tensor and its inverse as [46, p. 14,18]

\[ F_{ij} = \frac{\partial y_i}{\partial x_j} = \frac{\partial u_i}{\partial x_j} + \delta_{ij} \quad \text{and} \quad F^{-1}_{ij} = \frac{\partial x_i}{\partial y_j}. \]

Together with the right Cauchy-Green deformation tensor, \( C = F^T F \) (material description), we can define the following invariants which are needed for the strain energy density calculation below:

\[ J = \det F \] (3)
\[ I = T_C J^{-2/3} \] (4)
\[ K = \frac{1}{2} (T_C - T_C^2) J^{-4/3} \] (5)

Here,

\[ T_C = \text{tr} C \quad \text{and} \quad T_C^2 = \text{tr} (C^2) \]

are the trace of the right Cauchy-Green deformation tensor and its square, respectively. The non-linear strain energy density of the Mooney-Rivlin model is given by [47, 48]

\[ U = \left[ \frac{\mu_1}{2} (I-3) + \frac{\mu_2}{2} (K-3) + \kappa \frac{1}{2} (J-1)^2 \right], \] (7)

where \( \mu_1, \mu_2, \) and \( \kappa \) are material properties. They correspond – for consistency with linear elasticity in the range of small deformations – to the shear modulus \( \mu = \mu_1 + \mu_2 \) and bulk modulus \( \kappa \) of the material and are therefore related to the Young’s modulus \( E \) and the Poisson ratio \( \nu \) via [46, p. 74]

\[ \mu = \frac{E}{2(1+\nu)} \quad \text{and} \quad \kappa = \frac{E}{3(1-2\nu)}. \] (8)

Through the choice \( \mu_2 = 0 \) in (7), we recover the simpler and frequently used [44, 49] Neo-Hookean strain energy density:

\[ U_{NH} = \left[ \frac{\mu}{2} (I-3) + \frac{\kappa}{2} (J-1)^2 \right] \] (9)

As we show later, this can be a sufficient description for some cell types. To control the strength of the second term and quickly switch between neo-Hookean and Mooney-Rivlin strain energy density, we introduce a factor \( w \in [0, 1] \) and set

\[ \mu_1 = w\mu \quad \text{and} \quad \mu_2 = (1-w)\mu \] (10)

such that \( w = 1 \), which equals setting \( \mu_2 = 0 \) in (7), corresponds to the purely neo-Hookean description in (9), while \( w < 1 \) increases the influence of the \( \mu_2 \)-term.

In contrast to the Mooney-Rivlin and neo-Hookean description, which both describe non-linear deformations, the strain energy density of a linear elastic material is given by [46, p. 76]

\[ U_L = \frac{1}{2} \sigma_{ij} e_{ij}, \] (11)

where \( \sigma \) denotes the Cauchy stress tensor and where we imply Einstein summation over repeated indices. The infinitesimal strain tensor \( \varepsilon \) is related to the Cauchy stress tensor linearly via Hooke’s law

\[ e_{ij} = \frac{1 + \nu}{E} \sigma_{ij} - \frac{\nu}{E} \sigma_{kk} \delta_{ij}, \] (12)

with the Young’s modulus \( E \) and the Poisson ratio \( \nu \).

3 Tetrahedralized cell model

In this section we apply the hyperelastic theory of section 2 to a tetrahedralized mesh as shown in figure 1.

3.1 Calculation of elastic forces

We consider a mesh consisting of tetrahedral elements as depicted in figure 1. The superscript \( \alpha \) refers to the four vertices of the tetrahedron. The elastic force acting on vertex \( \alpha \) in direction \( i \) is obtained from (7) by differentiating the strain energy density \( U \) with respect to the vertex displacement as

\[ f^\alpha_i = -V_0 \frac{\partial U}{\partial u^\alpha_i}, \] (13)
where $V_0$ is the reference volume of the tetrahedron. In contrast to [44], the numerical calculation of the force in our model does not rely on the integration of the stress tensor, but on a differentiation where the calculation of all resulting terms involves only simple arithmetics. Applying the chain rule for differentiation yields:

$$f_i^\alpha = -V_0 \left[ \left( \frac{\partial U}{\partial \xi^1} \right) \frac{\partial T_C}{\partial F_{kl}} \frac{\partial T_C}{\partial F_{kl}} + \left( \frac{\partial U}{\partial \xi^2} \right) \frac{\partial T_C}{\partial F_{kl}} \frac{\partial T_C}{\partial F_{kl}} \right] \frac{\partial J}{\partial F_{kl}}$$

(14)

The evaluation of (14) requires the calculation of the deformation gradient tensor $F$, which is achieved by linear interpolation of the coordinates and displacements inside each tetrahedral mesh element as detailed in the next section.

### 3.2 Interpolation of the displacement field

Following standard methods, e.g. [46], we start by interpolating a point $x_i$ inside a single tetrahedron using the vertex positions $x_i^\alpha \ (\alpha = 1, 2, 3, 4)$. The interpolation uses an inscribed, dimensionless coordinate system, denoted by $(\xi_1, \xi_2, \xi_3)$ with $0 \leq \xi_j \leq 1$, as depicted in figure 1a. One vertex defines the origin while the remaining three indicate the coordinate axes. A set of shape functions, i.e. interpolation functions, $N^\alpha (\xi_1, \xi_2, \xi_3)$ is employed to interpolate positions inside the tetrahedron volume. An arbitrary point $x_i$ inside the element is interpolated as

$$x_i = \sum_{\alpha=1}^{4} N^\alpha (\xi_1, \xi_2, \xi_3) x_i^\alpha \quad (15)$$

where the shape functions are defined as [46, p. 483]:

$$N^1 (\xi_1, \xi_2, \xi_3) = \xi_1 \quad (16)$$

$$N^2 (\xi_1, \xi_2, \xi_3) = \xi_2 \quad (17)$$

$$N^3 (\xi_1, \xi_2, \xi_3) = \xi_3 \quad (18)$$

$$N^4 (\xi_1, \xi_2, \xi_3) = 1 - \xi_1 - \xi_2 - \xi_3 \quad (19)$$

According to (1), the displacement of vertex $\alpha$ in $i$-direction is given by

$$u_i^\alpha = y_i^\alpha - x_i^\alpha \quad (20)$$

Therefore similar to (15), the displacement at an arbitrary point in the volume can also be expressed in terms of the shape functions and the vertex displacements as

$$u_i = \sum_{\alpha=1}^{4} N^\alpha (\xi_1, \xi_2, \xi_3) u_i^\alpha \quad (21)$$

The calculation of the deformation gradient tensor according to (2) requires the spatial derivative of the displacement:

$$F_{ij} \equiv \frac{\partial u_i}{\partial x_j} = \frac{\partial u_i}{\partial \xi_k} \frac{\partial \xi_k}{\partial x_j} = A_{ik} \delta_{kj}$$

(22)

By inserting (21) into (22) and evaluating the shape functions, the components of the matrix $A$ are easily determined to be the difference of the displacements between the origin (vertex 4) and the remaining vertices 1, 2 and 3:

$$A_{ik} = u_k^i - u_4^i \quad (23)$$

Note that due to the linear interpolation $A_{ik}$ is constant inside a given tetrahedron. The matrix $B = J^{-1}$ is the inverse of the Jacobian matrix, obtained similarly to (23) as

$$J_{ik} = \frac{\partial x_i}{\partial \xi_k} = x_i^k - x_4^k \quad (24)$$

Since $x_i$ refers to the reference coordinates, the calculation of the matrices $J$ and $B$ has to be performed only once at the beginning of a simulation. With the interpolation of the displacement in each tetrahedron, we can write all derivatives occurring in (14), as listed in the following:

$$\frac{\partial u}{\partial \xi^1} = \frac{\mu_1}{2} \frac{\partial \mu_1}{\partial T_C} = J^{-\frac{5}{2}}$$

$$\frac{\partial u}{\partial \xi^2} = \frac{\mu_2}{2} \frac{\partial \mu_2}{\partial T_C} = T_C J^{-\frac{3}{2}}$$

$$\frac{\partial u}{\partial \xi^3} = 2F_{kl} \frac{\partial F_{kl}}{\partial T_C} = -\frac{1}{2} T_C J^{-\frac{3}{2}}$$

$$\frac{\partial u}{\partial \xi^4} = 4F_{ik} C_{kl} \frac{\partial F_{ik}}{\partial T_C} = \delta_{ik} \delta_{ml} \delta_{lm}$$

$$\frac{\partial u}{\partial \xi^1} = J^{-\frac{5}{2}}$$

### 3.3 Taylor Deformation parameter

As a measure for the cell deformation, we use the Taylor deformation parameter [44, 50, 51]

$$D = \frac{a_3 - a_1}{a_3 + a_1} \quad (25)$$

where $a_1$ and $a_3$ are respectively the minor and major semi axis of an ellipsoid corresponding to the inertia tensor of the cell. The Taylor deformation is a good measure for approximately elliptic cell deformations, as they occur in shear flow (cf. section 6).

To calculate $D$, first the components of the inertia tensor

$$\Theta_{ij} = \int_V x_k x_l \delta_{ij} - x_i x_j dV \quad (26)$$

where $\bar{x}$ is a vector inside the volume $V$, are calculated using our discretized cell with $N_{tetra}$ tetrahedra as

$$\Theta_{ij} = \sum_{l=1}^{N_{tetra}} V_l (r_i^l r_j^l + r_j^l r_i^l) \quad (27)$$
4 Comparison of the numerical model to FluidFM® measurements on REF52 cells

In this section, we validate compression simulations of our cell model with FluidFM® compression experiments of REF52 cells [45]. These experiments provide as an output the required force to produce a certain deformation of the cell, which can be directly compared to our model. We start with a detailed description of the experiments and show the suitability of our model to describe the elastic behavior of REF52 cells afterwards.

4.1 FluidFM® indentation measurements

We perform a series of compression measurements of REF52 cells with a Flex FPM (Nanosurf GmbH, Germany) system that combines the AFM with the FluidFM® technology (Cytosurge AG, Switzerland). In contrast to conventional AFM techniques, FluidFM® works in a liquid surrounding and uses flat cantilevers that possess a microchannel. By applying a suction pressure, cells can be aspirated and retained at the aperture of the cantilever’s tip. A more detailed description of the setup and its functionality is already reported in [30]. All experiments are based on a cantilever with an aperture of 8µm diameter and a nominal spring constant of 2 N m⁻¹. In order to measure the cellular deformation, a suspended cell was sucked onto the tip and compressed between the cantilever and the substrate until a setpoint of 100 nN was reached.

An example micrograph of the experiment before compression is shown in figure 2. Analogously to AFM, primary data in form of cantilever position (in m) and deflection (in V) has to be converted to force and deformation through the deflection sensitivity (in mV⁻¹) and the cantilevers’ spring constant. The cellular deformation further requires the determination of the contact point, which we choose as the cantilever position where measured force starts to increase. The undeformed cell size is obtained as mean from a horizontal and vertical diameter measurement using the software ImageJ.

4.2 Simulation setup

The experimental setup of the previous section is easily transferred and implemented for our cell model: the undeformed spherical cell rests on a fixed plate while a sec-
ond plate approaches from above to compress the cell as depicted in figure 3 (a,b). In section 5.2 below we will also use a slightly modified version where a sphere indents the cell as shown in figure 3 (c,d). A repulsive force prevents the cell vertices from penetrating the plates or the spherical indenter. The elastic restoring forces (cf. section 3) acting against this imposed compression are transmitted throughout the whole mesh, deforming the cell.

We use meshes consisting of 3000 to 5000 vertices and about 20000 to 30000 tetrahedra to build up a spherical structure. More details of the mesh and its generation (section S-2.3) as well as the algorithm (section S-3) are provided in the SI.

4.3 Results

In our FluidFM® experiment series with REF52 cells, the cell radii lie between 7.1 µm and 10.4 µm with an overall average of 8.6(7) µm. In figure 4 we depict the force as function of the non-dimensionalized deformation, i.e. the absolute compression divided by the cell diameter. The experimental data curves share general characteristics: the force increases slowly in the range of small deformations up to roughly 40%, while a rapidly increasing force is observed for larger deformations. Although the variation of the cell radius in the different measurements is already taken into account in the deformation, the point of the force upturn differs significantly which indicates a certain variability in the elastic parameters of the individual cells.

We use the compression simulation setup as detailed in section 4.2 to calculate force-deformation curves of our cell model. The Poisson ratio is chosen as \( v = 0.48 \). A best fit approach is used to determine the Young’s modulus and the ratio of shear moduli \( \omega \) and leads to very good agreement between model prediction and experimental data as shown in figure 4 as well as section S-1 of the SI. While the general range of force values is controlled using the Young’s modulus, \( \omega \) especially defines the point of the force upturn. We find Young’s moduli in the range 110Pa to 160Pa and a Poisson ratio of 0.48 in all simulations and the Young’s modulus is determined using a best fit to the experimental data points. Since the neo-Hookean description appears to be sufficient for these data sets, we further set \( r = 1 \).

In figure 6a, we show the experimental data for suspended, round, bovine endothelial cells of five separate measurements from [40] together with the prediction of the Hertz theory for a Young’s modulus of 1000Pa. Fitting our data with Young’s moduli in the range of 550Pa to 2400Pa, we find good agreement between our calculations and the experimental data. We note that [40] observed similarly good agreement for their axisymmetric incompressible neo-Hookean FEM simulations which however cannot be coupled to external flows in contrast to the approach presented here. The same procedure is applied to the colloidal probe indentation data of hydrogel particles from [34], showing in figure 6b the experimental data and the prediction of the Hertz theory from [34]. We find excellent agreement between our model calculations for Young’s moduli in the range of 580 ± 100Pa and the experimental data. For both systems, figure 6 shows large deviations between the Hertzian theory and the experimental data for medium-to-large deformations. Our model provides a significant improvement in this range.

5 Comparison of our numerical model to other micromechanical setups

In this section, we compare our simulations to axisymmetric calculations using the commercial software Abaqus and validate our cell model with further experimental data for bovine endothelial cells from [40] and very recent data for hydrogel particles from [34].

5.1 Validation with axisymmetric simulations

To validate our model numerically, we compare our simulated force-deformation curves to calculations using the commercial software Abaqus [52] (version 6.14).

In Abaqus, we use a rotationally symmetric setup consisting of a two-dimensional semicircle, which is compressed between two planes, similar to our simulation setup in section 4.2 and the finite element model utilized in [40]. The semicircle has a radius \( r = 15 \mu m \), a Young’s modulus of \( E = 2.25kPa \) and a Poisson ratio of \( v = 0.48 \). We choose a triangular mesh and the built-in implementation of the hyperelastic neo-Hookean model. In figure 5 we see very good agreement between the results of the two different numerical methods.

5.2 Validation with AFM experiments

To compare with the AFM experiments of [40], we simulate a cell with radius 15 µm using the setup of section 4.2. For the hydrogel particle indentation [34] we use the setup depicted in figure 3 (c,d) with a particles radius of 40µm and a radius of the colloidal probe of 26.5µm. The Poisson ratio is chosen as 0.48 in all simulations and the Young’s modulus is determined using a best fit to the experimental data points. Since the neo-Hookean description appears to be sufficient for these data sets, we further set \( w = 1 \).

In figure 6a, we show the experimental data for suspended, round, bovine endothelial cells of five separate measurements from [40] together with the prediction of the Hertz theory for a Young’s modulus of 1000Pa. Fitting our data with Young’s moduli in the range of 550Pa to 2400Pa, we find good agreement between our calculations and the experimental data. We note that [40] observed similarly good agreement for their axisymmetric incompressible neo-Hookean FEM simulations which however cannot be coupled to external flows in contrast to the approach presented here. The same procedure is applied to the colloidal probe indentation data of hydrogel particles from [34], showing in figure 6b the experimental data and the prediction of the Hertz theory from [34]. We find excellent agreement between our model calculations for Young’s moduli in the range of 580 ± 100Pa and the experimental data. For both systems, figure 6 shows large deviations between the Hertzian theory and the experimental data for medium-to-large deformations. Our model provides a significant improvement in this range.

6 Application in shear flow

We now apply our model to study the behavior of cells in a plane Couette (linear shear) flow setup and compare the steady cell deformation to other numerical and analytical
6.1 Single cell simulation

The first simulation setup, a single cell in infinite shear flow, is realized by choosing a simulation box of the dimensions $10 \times 15 \times 10$ (x x y x z) in units of the cell radius. The infinite shear flow is approximated by applying a tangential velocity $u_{\text{wall}}$ on the x-z-planes at $y=0$ in negative and at $y=15$ in positive x-direction, as depicted in figure 7. The tangential wall velocity is calculated using the distance $H$ of the parallel planes and the constant shear rate $\dot{\gamma}$ via

$$u_{\text{wall}} = \frac{1}{2} H \dot{\gamma}. \quad (29)$$

The box is periodic in x and z. A single cell is placed at the center of the simulation box corresponding to a volume fraction of $\phi = 0.0003$. We choose the following parameters: fluid mass density $\rho = 10^3 \text{kg/m}^3$, dynamic viscosity $\eta = 10^{-3} \text{Pa s}$, and shear rate $\dot{\gamma} = 4 \text{s}^{-1}$. The capillary number is defined by [43, 44]

$$Ca = \frac{\eta \dot{\gamma}}{\mu}, \quad (30)$$

and is used to set the shear modulus $\mu$ of our cell relative to the fluid shear stress $\eta \dot{\gamma}$. Simulation snapshots of the steady state deformation of a single cell in shear flow are depicted in dependency of the capillary number in figure 8a. We compare the Taylor deformation parameter $D$ to previous approximate analytical calculations of [49] for a two-dimensional elastic solid in infinite shear flow in figure 8b and see reasonable agreement.

A possibly even more intuitive way to measure cell deformation is the net strain of the cell which we define as

$$\Delta \varepsilon = \frac{(d_{\text{max}} - d_{\text{ref}})}{d_{\text{ref}}}. \quad (31)$$

It describes the relative stretching of the cell using the maximum elongation $d_{\text{max}}$, i.e. the maximum distance of two cell vertices, and its reference diameter $d_{\text{ref}} = 2R$. A strain of $\Delta \varepsilon = 1$ thus corresponds to an elongation of the cell by an additional 100% of its original size. In figure 8c, we depict the $\Delta \varepsilon$ as function of Ca. For small capillary numbers, i.e. small shear stresses, a linear stress-strain dependency
6.2 Multiple cell simulations

The second simulation setup, implemented to investigate the multiple particle aspect of our model, consists of 4 (8) cells in a $5 \times 8 \times 4$ simulation box (in units of the cell radius), corresponding to a volume fraction of $\phi = 0.11$ ($\phi = 0.22$) occupied by cells. The cells are inserted at random initial positions in the box and the flow parameters are the same as in the first setup (cf. section 6.1).

Figure 9a shows simulation snapshots of the cells in suspensions with volume fraction $\phi = 0.11$ and $\phi = 0.22$ for $Ca = 0.2$. The Taylor deformation of the suspensions, depicted in figure 9b, is calculated as an average over all cells and over time after an initial transient timespan. We find good agreement when comparing the averaged cell deformation in suspension with [43, 44].

7 Conclusion

We presented a simple but accurate numerical model for cells and other microscopic particles for the use in computational fluid-particle dynamics simulations.

The elastic behavior of the cells is modeled by applying Mooney-Rivlin strain energy calculations on a uniformly tetrahedralized spherical mesh. We performed a series of FluidFM® compression experiments with REF52 cells as an example for cells used in bioprinting processes and found excellent agreement between our numerical model and the measurements. In addition, we showed that the model compares very favorably to force versus deformation data from previous AFM compression experiments on bovine endothelial cells [40] as well as colloidal probe AFM indentation of artificial hydrogel particles [34]. At large deformations, a clear improvement compared to Hertzian contact theory has been observed.

By coupling our model to Lattice Boltzmann fluid calculations via the Immersed-Boundary method, the cell deformation in linear shear flow as function of the capillary number was found in reasonable agreement with approximate analytical calculations [49] on isolated cells as well as previous simulations of neo-Hookean and viscoelastic solids [43, 44] at various volume fractions.

The presented method together with the precise determination of model parameters by FluidFM® /AFM experiments may provide a greatly improved set of tools to predict cell deformation - and ultimately cell viability - in strong hydrodynamic flows as occurring, e.g., in bioprinting applications.

Acknowledgements

Funded by the Deutsche Forschungsgemeinschaft (DFG, German Research Foundation) - Project number 326998133 - TRR 225 "Biofabrication" (subproject B07). We gratefully acknowledge computing time provided by the SuperMUC system of the Leibniz Rechenzentrum, Garching. We further acknowledge support through the computational resources provided by the Bavarian Polymer Institute.
Figure 8: (a) Converged shapes of a single cell in a $10 \times 15 \times 10$ ($x \times y \times z$) simulation box (in units of the cell radius) with a shear flow in $x$-direction as function of the capillary number $Ca$. (b) Comparison of our model predictions for a single cell in shear flow to the analytical calculations of [49] in the range of $Ca \in [0.01, 2.0]$. (c) The relative stretch $\Delta \varepsilon$ of our cell model as function of the capillary number $Ca$. A linear behavior is found for small capillary numbers up to $Ca = 0.3$, while increasing stress is required for larger deformations due to the strain-hardening quality of the neo-Hookean model. Lines are a guide to the eye.

Figure 9: (a) Multiple cells in a $5 \times 8 \times 4$ ($x \times y \times z$) simulation box (in units of the cell radius) with a confined shear flow in $x$-direction for a capillary number of $Ca = 0.2$ and 4 cells corresponding to a volume fraction of $\phi = 0.11$, and 8 cells corresponding to $\phi = 0.22$. (b) Averaged deformation of multiple cell simulations with $\phi = 0.11$ and $\phi = 0.22$ in comparison to data from [43] and [44].

Christian Bächer thanks the Studienstiftung des deutschen Volkes for financial support and acknowledges support by the study program "Biological Physics" of the Elite Network of Bavaria. Furthermore, we thank the laboratory of professor Alexander Bershadsky at Weizmann Institute of Science in Israel for providing the REF52 cells stably expressing paxillin-YFP.

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SUPPLEMENTARY MATERIAL FOR THE MANUSCRIPT

A hyperelastic model for simulating cells in flow

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S-1 Supplementary Material for the cell experiments

Additional force-deformation curves for our FluidFM® measurements on REF52 cells are shown in figure S-1. Compared to the curves depicted in the manuscript in figure 4, these measurements show an earlier upturn of the force. Thus, our model overestimates the force necessary for a small deformation of the cell and slightly underestimates the force for larger deformations. Nevertheless, all measurements fit in the simulated range of $E = 220 \pm 100\text{Pa}$ for $w = 0.25$ and an averaged cell radius of $8.6(7)\mu\text{m}$, as figure S-1 shows. The cell radii and Young’s moduli for all measurements are listed in table S-1.

Table S-1: Measured cell radii $R$ and fitted Young’s moduli $E$ and $w$ for our FluidFM® experiments.

| Number | 1   | 2   | 3   | 4   | 5   | 6   | 7   | 8   | 9   |
|--------|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| $R$ [µm] | 7.1 | 9.2 | 8.3 | 8.0 | 9.5 | 9.1 | 8.4 | 9.4 | 8.3 |
| $E$ [Pa]  | 160 | 190 | 220 | 170 | 210 | 290 | 210 | 220 | 125 |
| $w$       | 1   | 0.25| 0.25| 0.5 | 0.25| 0.25| 0.25| 0.25| 0.25|

Figure S-1: Our numerical model in comparison to our FluidFM® measurements on REF52 cells. The ratio of the shear moduli is chosen as $w = 0.25$ for all curves. The gray area shows the simulation of a cell with an averaged cell radius of $8.6(7)\mu\text{m}$ and Young’s modulus range $220 \pm 100\text{Pa}$.
S-2 Supporting Information for the numerical model

S-2.1 Convergence of single cell deformation in shear flow

The temporal development of the deformation \( D \) of a single cell in a Couette flow can be seen in figure S-2. Starting from a spherical shape \( (D = 0) \), the cell experiences a shape change during an initial transient timespan, after which it assumes a steady shape. For capillary numbers \( \text{Ca} > 0.2 \), we first find an overrelaxation of the deformation before it converges towards a constant value.

![Figure S-2: Single cell deformation in Couette flow for different capillary numbers. After an initial transient timespan, the deformation converges to a constant value.](image)

S-2.2 Translational and rotational invariance of the force calculation

As a very direct test for the correct behavior of our model, we consider a single tetrahedron and examine the behavior of the volume and the elastic force for an initially applied translation, rotation and stretching. In figure S-3a, the behavior of the volume under these deformations is shown over the first time steps. While the volume remains constant under pure translation, pure rotation, and a combination of both, it quickly relaxes towards its reference value after an initial stretch is applied. The same behavior is observed for the elastic force acting on one tetrahedron vertex, in figure S-3b.

![Figure S-3: The behavior of (a) the volume and (b) the elastic force on a single vertex of a tetrahedron after an initial rotation, translation or stretching.](image)
Table S-2: Statistics of meshes created using different built-in algorithms of Gmsh [1]. Listed are edge length $L$, triangle area $A$, and tetrahedron volume $V$ providing average, standard deviation, minimum and maximum value for each mesh.

| Algorithm    | Frontal2D | MeshAdapt | Delaunay2D | Delaunay3D | Frontal3D |
|--------------|-----------|-----------|------------|------------|-----------|
| $\bar{L}$    | 1.252     | 1.362     | 1.292      | 1.362      | 1.484     |
| $\sigma_L$   | 0.243     | 0.301     | 0.299      | 0.301      | 0.530     |
| $L_{\text{min}}$ | 0.616     | 0.588     | 0.592      | 0.588      | 0.510     |
| $L_{\text{max}}$ | 2.138     | 2.345     | 2.462      | 2.345      | 3.622     |
| $\bar{A}$    | 0.348     | 0.422     | 0.382      | 0.422      | 0.565     |
| $\sigma_A$   | 0.377     | 0.473     | 0.436      | 0.473      | 0.837     |
| $A_{\text{min}}$ | 0.218     | 0.228     | 0.192      | 0.228      | 0.204     |
| $A_{\text{max}}$ | 1.577     | 1.851     | 1.709      | 1.851      | 4.444     |
| $\bar{V}$    | 0.218     | 0.291     | 0.252      | 0.291      | 0.473     |
| $\sigma_V$   | 0.078     | 0.121     | 0.112      | 0.121      | 0.405     |
| $V_{\text{min}}$ | 0.049     | 0.051     | 0.043      | 0.051      | 0.049     |
| $V_{\text{max}}$ | 0.600     | 0.881     | 0.840      | 0.881      | 2.353     |

S-2.3  Mesh generation and mesh independence

The tetrahedral mesh of our spheroid is generated using the software gmsh (version 4.3.0) [1]. The Frontal2D meshing algorithm produced a mesh with highest uniformity considering edge length, triangle area and tetrahedron volume distribution. Nevertheless, all other available meshing algorithms produce likewise uniform meshes, with one exception being the Frontal3D algorithm, as listed in table S-2. We demand the uniformity of the mesh to increase the accuracy of our coupled Immersed-Boundary Lattice Boltzmann simulations. Figure S-4 shows the force-deformation curves for meshes with increasing number of tetrahedra, which are converged and thus prove sufficient sampling of the volume mesh.

S-2.4  Hertz theory

Although originally designed for the contact between two linear elastic spheres, the Hertz theory can be applied to the contact between a linear elastic sphere and a flat plate [2]. The general assumptions for the Hertz-theory are the following [3, p. 91-92]:

- frictionless, smooth contact surfaces
- contact area small compared to sphere dimension
- homogeneous, isotropic and linear elastic material

S-2.4.1  Sphere-sphere contact

The following quantities are necessary to describe the normal contact of two elastic spheres. The radii $R_1$ and $R_2$ of the spheres define the effective radius of curvature $R$ of the bodies by

$$\frac{1}{R} = \frac{1}{R_1} + \frac{1}{R_2}.$$  (S-1)
Figure S-4: Force-deformation behavior of meshes with increasing number of tetrahedra. All meshes are already sufficiently sampled, such that they produce the same converged output. The following parameters were used: cell radius $R = 1$, Young’s modulus $E = 2.250$, and Poisson ratio $\nu = 0.48$.

Through their Young’s moduli and the Poisson ratios, $E_1$, $E_2$ and $\nu_1$, $\nu_2$, the effective stiffness $K$ is defined as:

$$\frac{1}{K} = \frac{1 - \nu_1^2}{E_1} + \frac{1 - \nu_2^2}{E_2}$$

(S-2)

The displacement $\delta$, which measures the distance that the sphere centers approach each other due to a normal force $N$ acting on each sphere, can be expressed in terms of the above parameters [2]:

$$\delta = \left( \frac{9N^2}{16KR^2} \right)^{\frac{1}{3}}$$

(S-3)

Therefore, the force–displacement relation according to the Hertzian theory for a sphere-sphere contact is given by

$$N(\delta) = \frac{4}{3}KR^2 \delta^\frac{3}{2}.$$  

(S-4)

### S-2.4.2 Sphere-plane contact

The analytical solution for the force–displacement relation according to the Hertzian theory for the contact of a linear elastic sphere with a rigid plane can be obtained from (S-4) by applying the following modifications: the plane has no curvature, thus $R_2 \to \infty$ and (S-1) simply yields $R = R_1$. Since the plane is assumed rigid, i.e. $E_2 \gg E_1$, (S-2) reduces to $K = \frac{E_1}{1-\nu_1^2}$. In this case, $N$ is the force acting on the sphere and $\delta$ is the distance between the center of the sphere and the plane.
S-3 Compression and indentation simulations

After initialization, each time step of our overdamped relaxation simulation consists of the following two steps: the movement of the upper wall to compress – or the sphere to indent – the cell and the integration of the equation of motion of the cell vertices,

\[ \dot{\vec{y}}^\alpha = \gamma^{-1} (\vec{f}^\alpha + \vec{f}_{\text{probe}}^\alpha). \]  

(S-5)

The vertex velocity \( \dot{\vec{y}}^\alpha \) is obtained from the elastic restoring forces (\( \vec{f}^\alpha \) (14) and the probe repulsion \( \vec{f}_{\text{probe}}^\alpha \)), considering a friction factor \( \gamma \). Since here we are only looking at a sequence of equilibrium states, the value of \( \gamma \) is irrelevant for the resulting force-deformation curves and only influences the performance and stability of the simulations. The equation of motion is integrated using a fourth order Runge-Kutta algorithm. The repulsive cell-probe interaction, preventing the cell vertices from penetrating the plates or the indenter, has the form

\[ \vec{f}_{\text{probe}} (d) = \frac{c_F}{d^2} \vec{n}, \]  

(S-6)

with the cell-probe distance \( d \) and a proportionality factor \( c_F \). The force points normal to the probe, resulting in a compression between two plates and a radial displacement away from the indenter.
This section briefly summarizes the Lattice Boltzmann method implemented in the open-source package ESPResSo [4]. For an introduction into the Lattice Boltzmann method we refer the interested reader to the book by Krüger et al. [5]. The Lattice Boltzmann equation for the multiple relaxation time scheme used in ESPResSo reads:

$$ f_i(\vec{x} + \vec{c}_i \Delta t, t + \Delta t) - f_i(\vec{x}, t) = \sum_{j=0}^{18} (M^{-1} \omega M)_{ij} \left( f_j(\vec{x}, t) - f^{eq}_j(\vec{x}, t) \right) $$  \hspace{1cm} (S-7)

It describes the collision and streaming of the population distribution $f_i$ ($i = 0, \ldots, 18$) during one time step $\Delta t$. Here, $\vec{c}_i$ are the discretized lattice velocities, $M$ denotes transformation matrix that maps the populations onto moment space, $\omega$ is the diagonal relaxation frequency matrix, and $f^{eq}_i$ denote the equilibrium population distributions. The relaxation frequency for the shear moments $\omega_S$ is related to the dynamic viscosity of the fluid via [6]

$$ \eta = \rho c_s^2 \left( \frac{1}{\omega_S} - \frac{1}{2} \right) \Delta t, $$  \hspace{1cm} (S-8)

with the fluid mass density $\rho$ and the lattice speed of sound $c_s$. In order to ensure simulation stability, we choose the time step globally according to Krüger et al.[5, p. 273] as

$$ \Delta t = c_s^2 \left( \tau - \frac{1}{2} \right) \frac{\Delta x^2}{\nu} = \frac{\Delta x^2}{6\nu}, $$  \hspace{1cm} (S-9)

with $c_s^2 = \frac{1}{3}$, a global relaxation parameter $\tau = 1$, and the kinematic viscosity $\nu$.

At the boundaries of the channel a bounce-back algorithm is applied to realize a no-slip boundary condition. For the plane Couette setup, the bounce-back algorithm additionally allows for a fixed tangential velocity component.

We use a combined CPU/GPU implementation which enables the calculation of the flow field on the GPU, while the calculation of the cell motion is done in parallel on multiple (4 to 20) CPUs. In lattice units, our simulation box for the single cell in shear flow setup (cf. section 6.1) has the dimensions $100 \times 150 \times 100$ ($x \times y \times z$), for the multiple cell simulation (cf. section 6.2) it is $50 \times 80 \times 40$. The dynamic viscosity, chosen as $1.0 \times 10^{-3}$ Pas in physical and $\nu = 1$ in simulation units, determines the time step in our simulations as $\Delta t = 1.67 \times 10^{-1}$ which corresponds to $1.67 \times 10^{-7}$ s.
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