Supplementary Information

Picoliter Agar Droplet Breakup in Microfluidics Meets Microbiology Application: Numerical and Experimental Approaches

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SI 1.1. Geometry of microfluidic chips

Figure S1a show the design of the microfluidic chip which comprises three parallel inlets (each has a width of 200 μ m) that meet at a nozzle (50 μ m × 50 μ m). The downstream channel has a width of 200 μ m. The channels have a uniform depth of 80 μ m. The dimensions of configuration A are selected similar to the fabricated chip used in our experimental chip for producing agar droplets in mineral oil where an external heating source is applied to the inlet of the microchannels. Configuration B (**Fig S1b**) is similar to the chip fabricated in Ref.¹ where bacterial cells are encapsulated in agar droplets emulsified in mineral oil while heating the inlets at 37 °C.



Figure S1 Schematic diagram of two different microfluidic chips. (a) Configuration A is our fabricated chip used in the experimental study to validate the numerical model. It combines three-parallel inlet channels with the 50 μ m × 50 μ m nozzle. The microchannels have a constant depth of 80 μ m, (b) Configuration B is similar to the chip used in the experimental study presented in Ref.¹.

SI 1.2. Governing Equations

The volume of fluid (VOF) method, proposed by Hirt and Nichols,² is used to trace the interface between two immiscible phases by solving the phase indicator function " ϕ " defined as the ratio of the volume occupied by one phase to the total volume of the cell.^{3, 4}

$$\frac{\partial \phi}{\partial t} + \nabla .(U\phi) = \phi(\nabla .U) \tag{1}$$

From vector calculus;

$$\nabla .(U\phi) = \phi(\nabla .U) + U. \nabla \phi$$
⁽²⁾

From equations (1) and (2), the phase indicator function for incompressible fluids will be obtained as below.

$$\frac{\partial \phi}{\partial t} + U.\,\nabla \phi = 0 \tag{3}$$

The volume fraction of the dispersed phase is directly computed from $(1 - \phi)$. $\phi = 1$ when the mesh cell is full of one phase and $\phi = 0$ if the mesh cell is full of the other phase. $0 < \phi < 1$ if the cell contains an interface of the two phases.

• Mass conservation equation

$$\frac{\partial \rho}{\partial t} + \nabla .(\rho U) = 0 \tag{4}$$

• Conservation of linear momentum

$$\frac{\partial(\rho U)}{\partial t} + \nabla (\rho U U) = -\nabla p + \nabla T + F_{\sigma}$$
(5)

where U is velocity vector, **T** is viscous stress tensor, p is pressure, and F_{σ} is force per unit volume resulted from surface tension at the interface.

For the momentum equation, the surface tension at the liquid-liquid interface generates an additional pressure gradient resulting in a force that is evaluated per unit volume using the continuum surface stress (CSS) method^{2, 5, 6} and is given in equation (6).

$$F_{\sigma} = \nabla \left[\sigma \left[|\nabla \phi| \ I - \frac{\nabla \phi \otimes \nabla \phi}{|\nabla \phi|} \right] \right]$$
(6)

where *I* is the unit tensor, σ is surface tension coefficient, $\nabla \phi$ volume fraction gradient, and \otimes tensor product between two vectors of the original and transformed normally. The two immiscible fluids are reflected as one effective fluid within the whole domain.

The strain tensor is decomposed into a more convenient form for discretization as equation (7).

$$\nabla T = \nabla (\mu \nabla U) + (\nabla U) \cdot \nabla \mu = \nabla (\mu (\nabla U + \nabla^T U))$$
(7)

Rearranging the momentum equation results in equation (8).

$$\frac{\partial(\rho U)}{\partial t} + \nabla .(\rho U U) = -\nabla p + \nabla .(\mu (\nabla U + \nabla^T U)) + F_{\sigma}$$
(8)

The viscosity of oil is presented only as a function of temperature. For the agar, the apparent viscosity η , which replaces μ in equations (7) and (8), is described as non-Newtonian power-law temperature dependent and is modeled as equation (9).

$$\eta_{min} < \eta = m\dot{\gamma}^{n-1}e^{\frac{T_0}{T}} < \eta_{max}$$
⁽⁹⁾

where η_{min} and η_{max} are zero and infinite shear viscosities, respectively; *m* is empirical curve-fitting parameters and known as the fluid consistency coefficient (as a measure of the average viscosity of the fluid); and n is flow behavior index (as a measure of the deviation of the fluid from Newtonian), respectively. Although the agar exhibits both shear-thinning and viscoelastic behavior, the viscoelastic behavior is neglected in this study as a simplification and due to the fact that fluids are exposed to high shear rates in microchannels, consequently the shear-thinning behavior is the only consideration where n < 1. η_{min} , η_{max} , m_{r} , and n have different values depending upon the agar concentration.

The two immiscible fluids are considered as one effective fluid throughout the domain, the physical properties of which are calculated as weighted averages based on the distribution of the liquid volume fraction.

$$\rho = \rho_c \alpha + (1 - \alpha) \rho_d \tag{10}$$

$$\mu = \mu_c \alpha + (1 - \alpha)\mu_d \tag{11}$$

where ρ_c and ρ_d are densities of the continuous and dispersed phases, respectively; and μ_c and μ_d are viscosities of the continuous and dispersed phases, respectively.

Since the immiscible fluids cannot be blended, the following criteria need to be met⁷; (i) For each fluid, the local normal component of the velocities must be equal to the interface velocity; (ii) The velocities tangent to the interface inside and outside the droplet must be equal; and (iii) The tangential shear stresses, introduced by equation (12) need to be balanced at the interface.

$$\mu_{d} \frac{\partial u_{t}}{\partial r} \Big|_{d} = \left. \mu_{c} \frac{\partial u_{t}}{\partial r} \right|_{c} \tag{12}$$

where $\frac{\partial u_t}{\partial r}\Big|_d$ and $\frac{\partial u_t}{\partial r}\Big|_c$ are derivative of the tangential velocity with respect to *r*-direction inside and outside the droplet, respectively. Equation (12) presents the importance of the viscosity ratio $\frac{\mu_d}{\mu_c}$ in formation and motion of droplets.

• Energy conservation equation

$$\frac{\partial(\rho h)}{\partial t} + \nabla .(\rho U h) = \nabla .(k \nabla T) + S$$
(13)

where h is sensible heat represented by equation (14), k is thermal conductivity, and S is the source term as equation (15).

$$h = C_p dT \tag{14}$$

$$S = S_{\Phi} + S_h + S_g \tag{15}$$

The source term S represents the submission of enthalpy source term S_h , viscous dissipation term S_{Φ} , and heat generation term S_g . The viscous dissipation term of each phase S_{Φ} is described as follow.⁸

$$S_{\Phi} = \tau_{xx}\frac{\partial u}{\partial x} + \tau_{xy}\frac{\partial u}{\partial y} + \tau_{xz}\frac{\partial u}{\partial z} + \tau_{yx}\frac{\partial v}{\partial x} + \tau_{yy}\frac{\partial v}{\partial y} + \tau_{yz}\frac{\partial v}{\partial z} + \tau_{zx}\frac{\partial w}{\partial x} + \tau_{zy}\frac{\partial w}{\partial y} + \tau_{zz}\frac{\partial w}{\partial z}$$

For Newtonian fluids (oil), using the following correlations (17) to obtain viscous dissipation term in equation (18).

$$\tau_{xx} = 2 \,\mu \frac{\partial u}{\partial x} \qquad \qquad \tau_{yy} = 2 \,\mu \frac{\partial v}{\partial y} \qquad \qquad \tau_{zz} = 2 \,\mu \frac{\partial w}{\partial z}$$

$$\tau_{xy} = \tau_{yx} = \mu \left(\frac{\partial u}{\partial y} + \frac{\partial v}{\partial x} \right) \qquad \tau_{xz} = \tau_{zx} = \mu \left(\frac{\partial u}{\partial z} + \frac{\partial w}{\partial x} \right) \qquad \tau_{yz} = \tau_{zy} = \mu \left(\frac{\partial w}{\partial y} + \frac{\partial v}{\partial z} \right) \tag{17}$$

$$S_{\Phi_{i}} = \mu_{i} \left\{ 2 \left[\left(\frac{\partial u_{i}}{\partial x} \right)^{2} + \left(\frac{\partial v_{i}}{\partial y} \right)^{2} + \left(\frac{\partial w_{i}}{\partial z} \right)^{2} \right] + \left(\frac{\partial u_{i}}{\partial y} + \frac{\partial v_{i}}{\partial x} \right)^{2} + \left(\frac{\partial u_{i}}{\partial z} + \frac{\partial w_{i}}{\partial x} \right)^{2} + \left(\frac{\partial w_{i}}{\partial y} + \frac{\partial v_{i}}{\partial z} \right)^{2} \right\}$$

For agar-in-oil, $S_g = 0$ and S_h indicates the effect of latent heat due to the phase change of the agar.⁹

$$S_h = \frac{\partial(\rho\Delta H)}{\partial t} + \nabla .(\rho U \Delta H) \tag{19}$$

where ΔH is the latent heat of the agar phase.

The enthalpy of the material H is the sum of the sensible enthalpy and the latent heat.

$$H = h + \Delta H \tag{20}$$

Rearranging the energy equation for the agar results in equation (21).

$$\frac{\partial(\rho H)}{\partial t} + \nabla .(\rho U H) = \nabla .(k \nabla T) + S_{\Phi}$$
(21)

Although most materials have identical melting and solidifying temperature, the agar material shows different melting and freezing temperatures depending upon its concentration. Consequently, temperatures of solidification T_{solid} and liquification T_{liquid} should be determined and used as input parameters for calculating the latent heat ΔH as equation (22).

$$\Delta H = \beta \, LH \tag{22}$$

LH is the latent heat of the agar material and β is liquid fraction and is defined as equation (23).

$$\beta = \begin{cases} 0 & T \leq T_{solid} \\ 1 & T \geq T_{liquid} \\ \frac{T - T_{solid}}{T_{liquid} - T_{solid}} & T_{solid} < T < T_{liquid} \end{cases}$$
(23)

SI 1.3. Dimensionless parameters

During droplet formation, the continuous and dispersed phases meet at a junction to form a large deformation at the interface and then break into disconnected droplets. Therefore, the interfacial forces play a key role in droplet generation. The physical properties and flow rates of the two phases as well as the dimensions of the microchannels are key parameters to control the size of the generated droplets and the transition from one regime to another.¹⁰ Four different dimensionless numbers are used in this study as explained below.

<u>Capillary number</u> (Ca) (equation (24)) is the ratio of local shear stresses (viscous forces) to capillary pressure (interfacial forces). In other words, it expresses the contest between viscous shear stresses (causes the deformation of the liquid interface) and capillary pressure (resists the deformation).

$$Ca = \frac{\mu_c U_c}{\sigma} \tag{24}$$

Different expression of U_c for each geometry are used particularly for the flow-focusing configurations: U_c is defined as equations (25) and (26) in terms of characteristic deformation rate *G* and characteristic droplet radius $a_{o,11,12}$

$$G = \frac{Q_c}{b\Delta z} \left(\frac{1}{w_o} - \frac{1}{2w_c} \right) \tag{25}$$

$$a_o = \frac{w_d}{2} \tag{26}$$

where Q_c is volumetric flow rate of the continuous phase, *b* is depth of the device; Δz is the axial distance between the end of the inlet channels and the orifice with the width w_o . w_c and w_d are width of the inlet channels of the continuous phase and dispersed phase, respectively. The capillary number is obtained by rewriting equation (24) for the flow-focusing geometry (equation 27).

$$Ca = \frac{\mu_c \, Ga_o}{\sigma} \tag{27}$$

<u>Weber number (We)</u> is defined as the ratio between inertia forces and capillary pressure. The inertia forces are dominant for the jetting mode. *Ca* is commonly calculated for the continuous phase, while *We* is calculated for the dispersed phase. We < 1 for most fluid flows in microfluidic systems.^{13, 14}

$$We = \frac{\rho_d {u_d}^2 L}{\sigma}$$
(28)

Volumetric flow rates ratio is the ratio between volumetric flow rate of the dispersed phase to

volumetric flow rate of the continuous phase $\varphi = \frac{Q_d}{Q_c}$.

<u>Agar concentration C</u>: is the ratio of agar added to a specific amount of distilled water. This concentration is measured in w/w, w/v, or v/v.

SI 1.4. Thermo-physical properties of the carrier and dispersed fluids

The physical properties of the carrier and dispersed fluids utilized in this study are temperature dependent. **Table S1** summarizes the thermo-physical properties of mineral oil and surface tension between the agar and oil.¹⁵

Table S1 Physical properties of the oil used in the numerical simulations¹⁵ (Temperature in Kelvin, T_{ref} =298 K)

Properties	Mineral oil
Density (ρ) [kg m ⁻³]	-0.524 T + 1002.4
Viscosity (µ) [Pa s]	9 ⁻⁰⁶ T ² - 0.0061 T + 1.0478
Thermal conductivity (k) [W m ⁻¹ K ⁻¹]	0.14
Specific heat $({}^{C}p)$ [J kg ⁻¹ K ⁻¹]	2500
Surface tension between agar and oil (σ) [N m ⁻¹]	-0.0001T + 0.048

The values of thermal conductivity¹⁶ and latent heat¹⁷ of different agar concentrations are collected from the literature. The density (**Figs S2**) and viscosity (**Figs S3**) of different agar samples are measured in our BioMEMS Laboratory, University of Calgary. The change in density of the agar is negligible for temperature varying between 25 to 50° C. However, agar density is strongly dependant on the agar concentration. **Figs S3a** and **S3b** delineate the measurements of agar viscosity at different shear rates (0.001-1000 1/s) for 0.5% and 1% agar concentrations within the temperature range of 35-45 °C. The agar at 2% concentration is completely gel at temperatures less than 40 °C which enables the measurement of its viscosity at 40 °C and 45 °C (**Fig. S3c**). Fitting correlations that discribe thermal conductivity and viscosity as a function of temperature are developed for each agar concentration. These correlations are used as input polynomials for computational simulations in ANSYS.



Figure S2 Agar density as a function of agar concentration.

(b)



Figure S3 Agar viscosity as a function of temperature at different agar concentrations (a) 0.5%, (b) 0.75%, (c) 1%, (d) 1.5%, and (e) 2%

Table S2 Physical properties of the agar used in our numerical simulations (Temperature in Kelvin, T_{ref} =298 K). Agar 0.75% and 1.5% have been simulated to study the effect of agar concentration on the droplet formation droplets to increase the reliability of the simulations.

Properties	Agar 0.5%	Agar 1%	Agar 2%	
ρ [kg m ⁻³]	950.87	954.14	1003.2	
η [Pa s]	$0.0506 \dot{\gamma}^{0.564-1} e^{\frac{273.15}{T}}$	$0.092 \dot{\gamma}^{0.47-1} e^{\frac{273.15}{T}}$	$0.372 \dot{\gamma}^{0.487-1} e^{\frac{273.15}{T}}$	
η _{min [Pa s]}	0.000705	0.000705	0.000705	
η _{max [Pa s]}	100	2000	10000	
<i>k</i> [W m ⁻¹ K ⁻¹]	0.0015 T + 0.1655	0.0015 T + 0.1629	$3^{-06} \mathrm{T}^2$ - 0.0003T + 0.3848	
$C_{p [\mathrm{J} \mathrm{kg}^{-1} \mathrm{K}^{-1}]}$	4182	4182	4182	
LH [J kg ⁻¹]	573.6	814.4	1200	
T _{solid [K]}	300	300	300	
T _{liquid [K]}	313	318	323	

Properties	Agar 0.75%	Agar 1.5%
ρ [kg m ⁻³]	953.67	972
η [Pa s]	$0.087 \dot{\gamma}^{0.553-1} e^{\frac{273.15}{T}}$	$0.242\dot{\gamma}^{0.48-1}e^{\frac{273.15}{T}}$
η _{min [Pa s]}	0.000705	0.000705
η _{max [Pa s]}	400	10000
$k [{ m W} { m m}^{-1} { m K}^{-1}]$	0.0015 T + 0.163	0.0015 T + 0.159
$C_{p [\mathrm{J kg}^{-1} \mathrm{K}^{-1}]}$	4182	4182
<i>LH</i> [J kg ⁻¹]	573.6	814.4
T _{solid [K]}	300	300
T _{liquid [K]}	313	320

SI 1.5. Implementations of the mathematical model and simulation software

The mathematical equations described in this work are solved using ANSYS-Fluent software based on the finite volume method (FVM). The software contains solvers to simulate laminar and turbulent fluid flows and consider Newtonian and non-Newtonian fluids. evaporation/condensation and solidification/melting physics, and chemical reactions. The computational domain is divided into a number of cells based on the element size or number of cells for each face. The governing equations are described in differential forms and then integrated over each cell. The problem is solved iteratively until the residuals in continuity, velocity components, and energy between two successive iterations are less than a predetermined tolerance (error<1e⁻⁰⁸). The solution algorithm is the PISO, a part of the SIMPLE family of the algorithms used mainly for transient flow calculations. The PISO algorithm improves the convergence of velocity and pressure calculations by performing two skewness and neighbor corrections. The momentum and energy equations are discretized using the second-order UPWIND scheme. For agar-in-oil, "multiphase", "Energy", and "solidification & melting" models of Fluent are employed for the simulations. All simulations are carried out on 60 processors using WestGrid utility provided by Compute Canada.

SI 1.6. Mesh dependency tests

A mesh-independency test is conducted for both configurations to verify the numerical simulations and the solver. For configuration A, a mesh size of 2 μ m × 2 μ m × 2 μ m was selected for each cell which resulted in 739,506 cells. It required a continuous computational time of 235 hrs (~10 days) run parallelly on 144 processors. Due to the limitation of computational sources and the large running time required for each case, four numerical cases similar to the available experimental geometries are selected and run to verify the solver. For the design of configuration B, the mesh dependency test is conducted to determine the appropriate grid resolution (detailed in **Table S3**). A mesh size of 1 μ m ×1 μ m× 1 μ m (shown in **Fig S4**) is selected for each case which required a continuous computational time of 75 hrs (~3 days), run parallelly on 60 processors.

Table S3 Mesh dependency tests for configuration **B** indicating the number of nodes for each mesh size and calculated droplet diameter, the percentage error between the droplet size obtained in two successive meshes, and the thickness of the interface at each mesh.

Test	Mesh size (µm	Number of	Droplet diameter	% Error in	Interphase
	×μm ×μm)	cells	(µm)	droplet	thickness
				diameter	(μm)
Test 1	$2.5 \times 2.5 \times 2.5$	41,240	48.5		3.75
Test 2	$1.25\times1.25\times1.25$	329,920	46.5	4.12	1.88
Test 3	$1 \times 1 \times 1$	644,375	45.1	3.12	1.50
Test 4	$0.625 \times 0.625 \times$	2,639,360	45.0	0.22	0.94
	0.625				



Figure S4 The selected mesh of size 1 μ m × 1 μ m × 1 μ m for the design configuration B.

SI 1.7. Fabrication of microfluidic chips

The photo-lithography technique is utilized to fabricate SU-8 molds per standard protocols.¹⁸ To perform soft-lithography for making polymer chips, polydimethylsiloxane (PDMS) and curing agent (Sylgard 184 from Dow Corning) are thoroughly mixed with a ratio of 10:1. The mixture is then degassed in a vacuum chamber for 10 min to eliminate trapped air bubbles in the PDMS

sample. The PDMS mixture is poured onto the pre-silanized SU-8 mold and cured in an oven for 2 hrs at 100 °C. The cured PDMS layer is then peeled off from the mold and punched to create the inlets and outlet holes. The fluidic PDMS layers are then bonded to a regular microscope slide glass (VWR international Inc.) through standard oxygen plasma activation (Electro-Technic Products). The glass substrate is cleaned with acetone before bonding.

SI 1.8. Simulation and experimental results

Table S4 Comparison between the numerical results of agarose droplet diameter for the current study versus experimental results presented in Ref.¹. φ is flow rate ration of dispersed phase to continuous phase.

φ	Average droplet diameter (experimental) ¹	Droplet diameter (numerical)	% Error
1.0	44.89	44.30	1.32
1.5	38.06	38.24	0.47
2.0	34.65	34.50	0.43
3.0	27.31	28.35	3.80
3.5	23.52	26.23	11.52





Figure S5 The effect of agar concentration on droplet size for the flow rate ration of 0.1, capillary number of 0.16257, and for (a) 2%, (b) 1%, (c) 0.5% agar concentration.



Figure S6 The numerical simulation results of three-dimensional (3D) interphase of (a) dripping regime, (b) jetting-dripping and (c) threading regimes.



Figure S7 Simulation results of (a) temperature distribution, (b) enthalpy of oil and agar and (c) the liquid fraction showing the liquid and solid regimes.

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