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Impact of flow on ligand-mediated bacterial flocculation

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Abstract

To understand the adhesion-fragmentation dynamics of bacterial aggregates (i.e., flocs), we model the aggregates as two ligand-covered rigid spheres. We develop and investigate a model for the attachment/detachment dynamics in a fluid subject to a homogeneous planar shear-flow. The binding ligands on the surface of the flocs experience attractive and repulsive surface forces in an ionic medium and exhibit finite resistance to rotation (via bond tilting). For certain range of material and fluid parameters, our results predict a nonlinear or hysteretic relationship between the binding/unbinding of the floc surface and the net floc velocity (translational plus rotational velocity). We show that the surface adhesion is promoted by increased fluid flow until a critical value, beyond which the bonds starts to yield. Moreover, adhesion is not promoted in a medium with low ionic strength, or flocs with bigger size or higher binder stiffness. The numerical simulations of floc-aggregate number density studies support these findings.

Keywords

adhesion; binding kinetics; micro hydrodynamics; bistability; flocculation; Smoluchowski coagulation equations

1 Introduction

The adhesion and fragmentation of particles and particle aggregates is ubiquitous in biological and colloidal process. For example, the binding/unbinding of bacterial clusters to medical implants or host cells in the presence of an external flow is a significant source of bodily infection [1]. Other examples include neutrophils binding to the walls of post-capillary venules in response to infection or injury as part of inflammatory response [2], blood-borne cells sticking to endothelial lining of blood vessels during immune response and cancer cell metastasis [3]. Medical gels coalescing with functionalized particles are candidates for targeted drug delivery [4]. The sticky properties of biological interfaces connected by multiple independent tethers are also presently inspiring the development of novel adhesives mimicking the remarkable properties of beetle and gecko feet [5]. These processes are also important unit operations in colloid-based industries such as pulp and paper-making, mineral and ceramics processing and waste water treatments [6]. Coagulation of rapidly settling aggregates are also used to explain the fate and transport of marine contaminants as well as phytoplankton explosions (also known as an *Algal bloom*) in both deep and shallow water bodies [7].

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The theoretical modeling of these processes in a fluid-borne environment presents significant challenges. The adhesive forces are composed of numerous physical processes including ligand-receptor binding kinetics [8], surface deformation [9], and flow past the surrounding surfaces [10], all of which determines the fate of the binding surfaces. Bond formation and breakage could be viewed as a statistical process modeled as a reversible chemical reaction between the population of free and bound bonds, characterized by forward and reverse reaction rates. Bell proposed a phenomenological relationship to model this effect [11], while a similar model was proposed by Dembo who treated the bonds as stretched springs [8]. A range of experimental techniques including atomic force microscopy, biomembrane force probe and optical tweezers; are being used to investigate the effect of surface charges and the strength of the ionic medium on the kinetic rate [12] and equilibrium constants [1]. Other effects like the local static shape changes in the neighborhood of adhesive surfaces was described by Evans [13], and extended by Dembo through his spring-based bond model. Further, Hammer accounted for the non-specific forces, clustering of adhesion receptor and limited ligand density [14] by introducing hydrodynamics to the problem of adhesion by exploiting classical solutions by Goldman [10]. The motion of the coalescing spheres is computed by balancing the hydrodynamic forces with those arising from a few adhesive contacts.

The aim of this study is to develop and investigate a model for ligand-mediated rigid sphere-sphere adhesion dynamics. Bacteria are modeled as ligand-covered rigid spheres, and ultimately the adhesion model we develop here will be fully incorporated into our population-level model governing the evolution of a size distribution of bacterial flocs [15]. Our model is important case from an experimental perspective. For example, consider the experiments by Sokurenko et al. which reveal the catch bond interactions of FimH proteins attached to the rigid surface of *E. coli* in stagnant conditions [16, 17]. Apropos to the length scales for bacteria and bacterial flocs, we include the ligand attachment/detachment kinetics, bond torsion, effect of surface forces in an ionic medium, and the flow hydrodynamics. These factors are incorporated into a single model for floc number density in a flowing medium. In the next section, we present the details of this adhesion mechanism, including the binder kinetic (§2.1), the long-range surface potentials, (§2.2), micro-scale forces on the floc surface (§2.3), macro-scale forces and torques arising due to the flow-hydrodynamics and the motion of the flocs in the fluid (§2.4), and the number-density conservation equation for floc-aggregation (§2.5). The macroscale-microscale force balance and the entire system of non-dimensionalized equations are listed in §2.6. In §3.1, we highlight the bifurcation results arising from the binder kinetics at steady state, while a set of simulation results of the bacterial biomass conservation (or floc-aggregation) in a planar shear flow-field are presented in §3.2. We conclude with a brief discussion of the implication of these results and the focus of our future directions.

2 Mathematical model: binder kinetics, hydrodynamics and floc-aggregation

Our aim in this article is to explore how the adhesion mechanism for rigid, micron-sized spherical flocs is governed by various geometric and fluid parameters as well as how the surface forces, the flow hydrodynamics and binding kinetics of the ligands impact a population model of flocculating spheres. Bacteria can accurately be modeled as ligand-coated rigid spheres due to the strength of the peptidoglycans in their cell walls and the binding abilities of their surface structures (fimbria, pili, etc.). Due to their relatively large micron-size scale, the binder kinetics of these flocs are significantly different from the core-shell nano-crystal interactions, which are applicable at much smaller scales [18, 19]. The next few subsections are devoted to detail the various aspects of this aggregation model.

2.1 Adhesion mechanism: binder kinetics

Fig. 1 illustrates the relative motion of two spheres, of radius R_1 and R_2 , respectively. The moving frame, (R_m) is fixed on the plane dividing the separation gap between the two spheres with the origin O_m at the point of minimum separation. The spheres are immersed in a fluid under planar shear flow conditions and for a given spatial point $\mathbf{x} = (x, y, z)$ in the moving frame (R_m) , $V_{\text{shear}} = (l_0 G z, 0, 0)$, where G is the shear rate. The mean rest length for the binders l_0 is chosen as the characteristic length scale. The spheres are allowed to translate (with a non-dimensional translational/propulsion velocity $\mathbf{U} = \mathbf{U}(G, x)\mathbf{e}_x$, of sphere-1 with respect to the sphere-2) and rotate (with a relative angular velocity, $\Omega = (G, x)\mathbf{e}_y$, in the anti-clockwise direction) with respect to the frame (R_m) . The relative velocities $\mathbf{U}$, Ω are functions of shear rate, G , and the horizontal displacement, x , from the center of the moving frame, O_m . The total velocity in this frame is $\mathbf{V} = \mathbf{U} + \Omega \times \mathbf{R}_1$. $\{\mathbf{e}_x, \mathbf{e}_y, \mathbf{e}_z\}$ are the corresponding unit vectors of a this frame of reference. Define $A_{\text{Tot}}g(\mathbf{x}, t)dA$ as the number of bonds in the transverse direction that are attached between the surfaces dA at time t , $D(x)$ is the distance between the two spheres, A_{Tot} is the total number of binding ligands and g is the density of bound ligands on the adhesion surface. Hence the total number of bonds formed is given by $A_c A_{\text{Tot}}g(\mathbf{x}, t)dA$, with $A_c = \pi R_c^2$ being the area of adhesion, and the radius of adhesion, $R_c = (0, \dots)$ [9].

The forward and reverse reaction rates for receptor ligand binding are then written as Boltzmann distributions, allowing highly stretched slip bonds, to be readily broken by thermal energy fluctuations. The kinetics are also influenced by the surface potential of the two charged surfaces. Further, we allow the ligands to tilt (by an angle α_0) with respect to the vertical direction. This is expressed as a Boltzmann distribution, $\mathcal{D}(\alpha_0)$, that a bond may

form between the two spheres for a given angle $\alpha_0 \in (-\frac{\pi}{2}, \frac{\pi}{2})$. With these degrees of freedom, the bond attachment / detachment rates are

$$K_{\text{on}}(\mathbf{x}) = K_{\text{on,eq}} \exp\left[\frac{\{-k_s(L(\mathbf{x}) - l_0)^2 + W(D(x))\}}{2k_B T}\right] \mathcal{D}(\alpha_0) \quad K_{\text{off}}(\mathbf{x}) = K_{\text{off,eq}} \exp\left[\frac{\{(k_0 - k_s)(L(\mathbf{x}) - l_0)^2 + W(D(x))\}}{2k_B T}\right] \quad (1)$$

where k_B is the Boltzmann constant, T is the temperature, κ_0 is the stiffness coefficient of the binders and k_s is the spring constant of the transition state used to distinguish catch ($\kappa_0 < k_s$) from slip ($\kappa_0 > k_s$) bonds [8]. The total surface potential $W(D)$ is described in §2.2. For notational simplicity, we denote $D(x) = D$. As depicted in Fig. 1, $L = \sqrt{D^2 + x^2}$ is the length of a bond in a stretched configuration. If we assume that the energy associated with tilting a bond from its vertical position is $(1/2)\kappa_\theta \alpha_0^2$, (κ_θ being the torsion constant) then $\mathcal{D}(\alpha_0)$ is given by [9]

$$\mathcal{D}(\alpha_0) = \exp\left[\frac{\kappa_\theta \alpha_0^2}{2k_B T}\right] \frac{1}{D_0} \alpha_0 = \tan^{-1}\left(\frac{x}{D}\right) \quad (2)$$

where $D_0 = \int_{-\pi/2}^{\pi/2} e^{-\frac{\kappa_\theta \alpha_0^2}{2k_B T}} d\alpha_0$ is the normalization constant for all possible tilt orientations along the flow-direction. In the limit of small binding affinity and abundant binding

receptors on the surface of adhesion (i.e., $A_{\text{Tot}} K_{\text{on,eq}} / K_{\text{off,eq}} \ll 1$), the bond ligand density evolves as the following differential equation:

$$\frac{dg}{dt} = A_{\text{Tot}} K_{\text{on}} - K_{\text{off}} g, \quad (3)$$

$$\text{where } \frac{dg}{dt} = \frac{\partial g}{\partial t} + \mathbf{V} \cdot \nabla g.$$

2.2 Long range interactions

The long-range interactions utilized in the binder attachment/detachment rates, Eqn. (1), are described via the DLVO approach, i.e., the Coulombic and Van der Waals interaction. Other interactions including hydration effects, hydrophobic attraction, short range steric repulsion and polymer bridging, which are absent in the length scales of our interest, are neglected [20].

For two charged spheres, with radii R_1, R_2 , the repulsive Coulombic forces in the gap, D , is given by

$$W_C(D) = 2\pi\epsilon_0\epsilon\psi_0^2 \frac{2R_1R_2}{R_1+R_2} e^{-kD} \quad (4)$$

where κ is the Debye length, ϵ_0 are the dielectric constant of vacuum and the medium, respectively, ψ_0 is the average *zeta potential* or the electric potential of the diffuse cloud of charged counterions. The attractive Van der Waal forces for these spherical flocs in the regime of close contact ($D \rightarrow R_1, R_2$), is

$$W_{vw}(D) = -\frac{A}{6D} \frac{R_1R_2}{R_1+R_2} \quad (5)$$

where A is the Hamaker constant, measuring the van der Waal ‘two-body’ pair-interaction for macroscopic objects.

2.3 Micro-scale forces

Consider one individual bond formed between the points, O_m on sphere 1 (which is also the origin of the frame (R_m)) and P on sphere 2 (Fig. 1). The instantaneous force it exerts on the two spheres has three components: an extensional force related to bond stretching by Hooke’s law: $\mathbf{f}_E = \frac{\kappa_0}{L}(L - l_0)\mathbf{e}_L$, force due to surface-charges: $\mathbf{f}_C = \frac{1}{4\pi\epsilon_0} \frac{Q_1Q_2}{D^2}\mathbf{e}_D$ and a torsional force proportional to the angle formed by the bond with the vertical:

$\mathbf{f}_T = (\kappa_\theta \frac{\alpha_0}{L})\mathbf{e}_{L\perp}$, where $\mathbf{e}_L = -(\mathbf{x}\mathbf{e}_x + D\mathbf{e}_z)/L$ and $\mathbf{e}_{L\perp} = (-D\mathbf{e}_x + \mathbf{x}\mathbf{e}_z)/L$ are the radial and orthogonal unit vectors as shown in Fig. 1. The total micro-scale extensional force, $\mathbf{F}_E$, surface force due to charges, $\mathbf{F}_C$ and torsional force, $\mathbf{F}_T$ arising from all bonds are given by [9]

$$\mathbf{F}_i(\mathbf{x}, t) = A_{\text{Tot}} \int_{A_c} g(\mathbf{x}, t) \mathbf{f}_i(\mathbf{x}, t) dA(\mathbf{x}, t), i = \{E, C, T\}. \quad (6)$$

2.4 Macro-scale forces

O’Neill derived the expression for the force, $\mathbf{F}^t$, and torque, $\mathbf{T}^t$, exerted on two spheres moving past one another (and without relative rotation) in a viscous quiescent fluid [21]. At leading order in $\epsilon = D/R_1 \ll 1$, using lubrication theory, they obtained

$$\mathbf{F}^{t*} = \frac{\mathbf{F}^t}{6\pi\mu R_1 U} = -\frac{4}{15} \frac{\beta(2+\beta+2\beta^2)}{(1+\beta)^3} \ln(\epsilon) \mathbf{e}_x, \mathbf{T}^{t*} = \frac{\mathbf{T}^t}{8\pi\mu R_1^2 U} = \frac{1}{10} \frac{\beta(4+\beta)}{(1+\beta)^2} \ln(\epsilon) \mathbf{e}_y, \quad (7)$$

where starred quantities are the non-dimensional counterparts of the force and the torque, $U = |\mathbf{U}|$, $\beta = \frac{R_2}{R_1}$ (being the magnitude of a vector quantity), μ is the dynamic viscosity

of the (Newtonian) fluid, $\beta = \frac{R_1}{R_2}$, $\mathbf{e}_x$ and $\mathbf{e}_z$ are the horizontal and vertical unit vectors, and $\mathbf{e}_y = \mathbf{e}_z \times \mathbf{e}_x$. The force and torque produced by the relative rotation (but no relative translation) of sphere 2 with respect to sphere 1, about the y-axis in stagnant fluid conditions, are given in [22]

$$\mathbf{F}^{r*} = \frac{\mathbf{F}^t}{8\pi\mu R_1^2 \Omega} = -\frac{1}{10} \frac{\beta(4+\beta)}{(1+\beta)^2} \ln(\varepsilon) \mathbf{e}_x, \mathbf{T}^{r*} = \frac{\mathbf{T}^r}{8\pi\mu R_1^3 \Omega} = -\frac{2}{5} \frac{\beta}{(1+\beta)} \ln(\varepsilon) \mathbf{e}_y. \quad (8)$$

The shear-flow of the fluid in which the spheres are immersed induces a third set of hydrodynamic force and torque [10, 23]. In the absence of any relative motion between the spheres (i.e. either relative translation or relative rotation), these components (also known as the far-field solutions, obtained by matching the inner and the outer series expansion of the solution to the Stoke's flow [23]) are given by

$$\mathbf{F}^{s*} = \frac{\mathbf{F}^s}{12\pi\mu R_1^2 G} = -\frac{25\beta+16}{16\beta} \left(\frac{\beta}{1+\beta}\right)^2 \mathbf{e}_x, \mathbf{T}^{s*} = \frac{\mathbf{T}^s}{8\pi\mu R_1^3 G} = \left(\frac{\beta}{1+\beta}\right)^3 \left(1 - \frac{3}{16} \left(\frac{\beta}{1+\beta}\right)^3\right) \mathbf{e}_y, \quad (9)$$

where G is the shear rate of the fluid. In each of the above expressions for forces and torques, the next term is of the order $\mathcal{O}(\ln(\varepsilon))$ and $\mathcal{O}(\varepsilon)$ in Eqns.(7, 8) and Eqn.(9), respectively, and these are therefore neglected [22]. By principle of linear super-position, the total forces and the torques on two moving spheres in a shear flow are the sum of the contributions from Eqns.(7,8,9); $\mathbf{F} = \mathbf{F}^t + \mathbf{F}^r + \mathbf{F}^s$, and $\mathbf{T} = \mathbf{T}^t + \mathbf{T}^r + \mathbf{T}^s$.

2.5 Bacterial floc aggregation

In this section, we briefly outline the floc aggregation model developed in [15]. We start with the definition of the aggregate number-density:

$b(t, v, \mathbf{x})$ = number of aggregates having volumes between v & $v + \Delta v$ in time t at a spatial point $\mathbf{x}$.

In volumes between v_1 and v_2 , the total number of flocs B_0 is given by

$$B_0(t, v_1, v_2, \mathbf{x}) = \int_{v_1}^{v_2} b(t, v, \mathbf{x}) dv, \quad (10)$$

for $[v_1, v_2] \in [\underline{v}, \bar{v}]$, where $\underline{v}$ and $\bar{v}$ are the minimum and maximum aggregate volume sizes, respectively. A finite nutrient supply and the duration of the experiment allow us to assume that $\bar{v}$ is finite. Furthermore, $\underline{v}$ is the minimum volume because the extracellular structures must be actively maintained and thus the minimal size of a floc is a single bacteria. The conservation of the aggregate biomass or the governing equation for b in a homogeneous fluid-flow (e.g. a planar shear flow) is

$$b_t + \mathbf{V} \cdot \nabla b = A(v, b), \quad (11)$$

where $\mathbf{V}$ is the net relative velocity of one floc with respect to the other in the moving frame of reference (see Fig. 1). $A = A_{\text{in}} - A_{\text{out}}$, where A_{in} is the rate with which flocs of size in $[v, v + \Delta v]$ are created and A_{out} is the rate a floc of size in $[v, v + \Delta v]$ joins with another floc, to form a volume greater than $v + \Delta v$. These rates are given by

$$A_{\text{in}}(v, b) = \frac{1}{2} \int_{\underline{v}}^{v-\underline{v}} K_A(v', v-v') b(t, v', \mathbf{s}) b(t, v-v', \mathbf{s}) dv', v \in [2\underline{v}, \bar{v}] \quad (12a)$$

$$A_{\text{out}}(v, b) = b(t, v, \mathbf{s}) \int_{\underline{v}}^{v-\underline{v}} K_A(v-v') b(t, v', \mathbf{s}) dv', v \in [\underline{v}, v-\underline{v}]. \quad (12b)$$

K_A is the aggregation kernel, describing the rate with which flocs of volume v and v combine to form a floc of volume $v + v$. In a Stokes flow, this rate depends on the binder kinetics via the microscale forces, $\mathbf{F}_{\text{Tot}} = \mathbf{F}_E + \mathbf{F}_C + \mathbf{F}_T$, as follows [24]

$$K_A = \gamma_A A_c F_{\text{Tot}} / \zeta, \quad (13)$$

where $F_{\text{Tot}} = |\mathbf{F}_{\text{Tot}}|$, is the drag coefficient, A_c is the aggregation contact efficiency parameter.

2.6 Non-dimensionalized system

Since the slope of the spherical surface at the contact point of the binders (point P, Fig. 1) is $\mathcal{O}(x/R_1)$ (where 'x' is the horizontal distance from origin O_m of the moving frame) and the adhesion radius has a span $\mathcal{O}(l_0)$, the surface of the two spheres are considered to be locally flat, i.e., $D(x) \approx D$. We anticipate that the length-scales will be $\mathcal{O}(l_0)$, and hence introduce the following dimensionless variables and denote them with starred quantities:

$$\begin{aligned} x &= l_0 x^*, D = l_0 D^*, L = x = l_0 L^* \\ K_{\text{on}} &= K_{\text{on}}^* K_{\text{on,eq}}, K_{\text{off}} = K_{\text{off}}^* K_{\text{off,eq}}, g = g^* K_{\text{eq}} \\ t &= t^* / G, U = U^* l_0 K_{\text{off,eq}}, \Omega = \Omega^* l_0 K_{\text{off,eq}} / R_1, \text{ and } V^* = U^* + \Omega^*, \end{aligned} \quad (14)$$

where $K_{\text{eq}} = A_{\text{Tot}} \frac{K_{\text{on,eq}}}{K_{\text{off,eq}}}$. Note that we have introduced two time-scales, one associated with the fluid velocity, G^{-1} , and the other with the reaction chemistry, $K_{\text{off,eq}}$. This is done to neglect the lower order terms in the non-dimensional form of equations (and in the limit of slow time-scales), as shown below. Further, we introduce the following non-dimensional parameters,

$$r = \frac{\kappa_0 l_0^2}{2k_B T}, \kappa_\theta^* = \frac{\kappa_\theta}{2k_B T}, \kappa_s^* = \frac{\kappa_s}{\kappa_0} \quad (15)$$

The non-dimensional form of the reaction rates (1), bond-density evolution equation (3) along with the boundary conditions become

$$K_{\text{on}}^* = \exp[-\kappa_s^* r (L-1)^2 + W^*(D) - \kappa_\theta^* \alpha_0^2] / P_0 \quad (16)$$

$$K_{\text{off}}^* = \exp[(1 - \kappa_s^*) r (L-1)^2 + W^*(D)] \quad (17)$$

$$\frac{G}{K_{\text{off,eq}}} \frac{\partial g^*}{\partial t^*} + V^* \frac{\partial g^*}{\partial x^*} = K_{\text{on}}^* - K_{\text{off}}^* g^* \quad (18)$$

$$g^* \rightarrow 0 \text{ as } |x^*| \rightarrow \infty, \quad (19)$$

where $W^*(D) = W(D) / 2k_B T$. In the limit of $\frac{G}{K_{\text{off, eq}}} \ll 1$, we neglect the first term in Eqn. (18) that accounts for the unsteady binding effects and solve for the bond-density at quasi-equilibrium

$$V^* \frac{\partial g^*}{\partial x^*} = K_{\text{on}}^* - K_{\text{off}}^* g^*, \quad (20)$$

whose analytical solution (along with the boundary condition Eqn. 19) is given by

$$g^*(x^*) = \frac{1}{V^*} \int_{x^*}^{\infty} K_{\text{on}}^*(s_2) e^{-\frac{1}{V^*} \int_{x^*}^{s_2} K_{\text{off}}^*(s_1) ds_1} ds_2. \quad (21)$$

Similarly, the micro-scale forces arising from all bonds in the non-dimensional form, are

$$\begin{aligned} \mathbf{F}_E^*(U, D) &= - \int_{A_c^*} g^*(1 - 1/L^*) [x^* \mathbf{e}_x + D^* \mathbf{e}_z] dA^*, \\ \mathbf{F}_C^*(U, D) &= -(1/l_0 r) \mathbf{e}_z \int_{A_c^*} g^*(\pi \varepsilon_0 \varepsilon \psi_0^2 \kappa \frac{2R_1 R_2}{R_1 + R_2} \left(e^{-\kappa D} - \frac{A}{24D^2} \right) dA^*, \\ \mathbf{F}_T^*(U, D) &= (\kappa_\theta^* / r) \int_{A_c^*} (g^* / L^{*2}) \alpha_0 [-D^* \mathbf{e}_x + x^* \mathbf{e}_z] dA^*, \end{aligned} \quad (22)$$

with $F_i^* = F_i / F_0$, and $F_0 = (A_{\text{Tot}} K_{\text{eq}} \kappa_0 l_0^3)$. The subscripts E, C, T denote *extension*, *surface charges* and *torsion*, respectively. The coupling between the macro-scale and the micro-scale is obtained via the global force balance on the spheres in the horizontal and vertical directions, and the torque balance about the center of mass of the spheres. Assembling these components from the fluid, Eqns. (7–9), and the binders, Eqn. (22), we obtain

$$(\mathbf{F}_E^* + \mathbf{F}_C^* + \mathbf{F}_T^*) \cdot \mathbf{e}_z = 0, \quad (\mathbf{F}_E^* + \mathbf{F}_C^* + \mathbf{F}_T^*) \cdot \mathbf{e}_x + (F^{r*} + F^{t*} + F^{s*}) = 0, \quad \frac{\beta R_1}{\beta + 1} (\mathbf{F}_E^* + \mathbf{F}_C^* + \mathbf{F}_T^*) \cdot \mathbf{e}_x + (T^{r*} + T^{t*} + T^{s*}) = 0. \quad (23)$$

The above system of equations solve for the unknowns U , D . Finally, Eqns (16, 17, 21–23), together with the boundary condition Eqn. (19) is the system of equations that completely describes the complete binder kinetics in the non-dimensional form. The binder kinetic equations are then utilized to solve the non-dimensional form of the conservation of floc aggregation, Eqn. (11). The numerical results for both of these systems is described in the next section.

3 Results and discussion

The values of the parameter used in our numerical calculations are listed in Table 1. Since we do not wish to study the tilting effects [9] or the effect of catch-vs-slip bonds [8], the corresponding material properties are fixed at $\kappa_\theta^* = 1.0$ and $\kappa_s^* = 0.5$, respectively. The dielectric constant in vacuum is $\varepsilon_0 = 8.854 \times 10^{-12}$ Farad m^{-1} , while the permittivity of water at $T = 25^\circ\text{C}$ is $\varepsilon = 78.5$. The Boltzmann factor is taken as $k_B T = 4 \times 10^{-21}$ J. The salt dissolved in the fluid is assumed to be a 1–1 electrolyte with the corresponding *zeta potential* of $\psi_0 = 25$ mV (this value corresponds to the surface potential studies in [20], Chap-3. We assume that the solute concentration in the fluid only effects the Debye length, λ_D).

3.1 Binder kinetics: steady state analysis

We solve the system of equation involving binder kinetics using the adaptive Lobatto quadrature (via Matlab function **quadl**) to evaluate the integral in Eqn. (21), which is then coupled with the system of algebraic equations, Eqn. (23).

Fig. 2a presents the collision function, $g^*(x^*, z^* = 1)$, vs. the horizontal distance, x^* , from the origin of the axes (refer Fig. 1), at different ionic-fluid compositions. In stagnant fluid (i.e., $\mathbf{V}^* = 0$), $g^*(x^*) = K_{\text{on}}^*/K_{\text{off}}^*$, which has a symmetric, Gaussian profile with a maximum at $x^* = 0$, $z^* = 1$ (Substitute $V^* = 0$ in Eqns.(16–18)) [9]. However, this symmetry about the y-axis is broken for non-zero shearing velocities. A non-zero fluid flow induces a tilt among the binders ($\theta_0 \neq 0$) which leads to a skewed adhesion (i.e., g_{max}^* is away from the y-axis). Further, this collision impact (or the overall value of g^*) is increased for smaller Debye lengths. Lower screening length implies that the adhering spheres can come closer and coalesce, leading to a higher collision factor.

Next, we explore the flow kinematics of these identically binding spheres in a uniform shear flow. The subfigures in Fig. 3 depict the steady-state solution in the phase-space (g^* , V^*) at the horizontal distance from the center, $x^* = 1$; at different flow parameter, θ_0 , and geometric parameter, R , respectively. The material properties are fixed at $\kappa_\theta^* = 1.0$, $\kappa_s^* = 0.5$. An *adhesion* phase is defined when the majority of the binders (inside the adhesion area, A_c) are hooked with each other, i.e., $g^* > 0.5$; otherwise the spheres are in the *fragmentation* phase. A third, *bistable* phase, in which the spheres exhibit a stable steady-state adhesion as well as fragmentation, exists in-between these two phases.

Three different types of adhesion-fragmentation kinematics are found. For example, in weak ionic conditions (refer Fig. 3a, $\theta_0 = 2.5$ curve), the transition from adhesion to fragmentation phase (and vice-versa) is irreversible and discontinuous. For this curve, the adhesive effects are strong for low fluid velocities (i.e., g^* has a stable steady-state branch with $g^* > 0.5$ in the velocity range $V^* < 2.0$). As the velocity is increased to a critical value ($V_0^* \approx 2.0$), the system abruptly jumps to a steady-state value in the fragmentation phase.

It remains in this phase even if the fluid velocity is subsequently reduced below this critical value. In stronger ionic conditions (Fig. 3a, $\theta_0 = 1.0, 1.5$ curves), this transition is reversible with flow, and either changes continuously ($\theta_0 = 1.0$ curve) or discontinuously through a bistable region ($\theta_0 = 1.5$ curve). Similar flow-kinematic transition curves are observed for different binder stiffness, θ_0 , and floc-sizes, R .

Another observation is that surface-adhesion is comparatively stronger in highly ionic fluids, represented by a shorter Debye length, κ_s^* (e.g., see how the g^* -curve changes for different Debye lengths, κ_s^* , Fig. 3a). A short screening length implies a smaller separation distance between the interacting surface, and hence a strong adhesion (Fig. 4b). Stiff binders (e.g. see g^* -curves for different θ_0 , Fig. 3b) as well as large floc-sizes (e.g. g^* -curves for different R , Fig. 3c) hinder adhesion, as well.

Physically, we can explain these abrupt hysteretic transitions (when it occurs) between the adhesion and the fragmentation solutions, by noting the relation between the micro-scale forces and the fluid velocity. Fig. 4a depicts the horizontal component of these forces, $F_{Ex}^* + F_{Tx}^*$ between the binding ligands vs. the fluid velocity, for different ionic compositions respectively. For small velocities ($V^* < 0.1$), the total force due to the stretching and tilting of the ligands along the flow direction, increases as V^* increases. However, with stronger flow ($V^* > 1.0$), the bonds rupture and there is a rapid decay of these forces. As the fluid flow is increased, the bound ligands are unable to prevent some degree of fragmentation

between the surfaces and start to yield. This in turn decreases the total adhesive forces, eventually leading to a state where the spheres are free from nearly all adhesive bonds.

The force-velocity curves exhibit a non-linear dependence for different ionic compositions of the fluid (or the Debye length). As the binders are advected away from the vertical alignment, the z-component of the torsion force, F_T as well as the surface forces, F_S , pushes the spheres farther away. For sufficiently large separation distances, however, the bonds stretch, and the extension forces, $F_E(D^*)$, tend to pull the spheres close to each other. All these forces depend on the minimum separation, $D^* = D^*(V^*)$, (Fig. 4b), which explains the non-linear variation versus the Debye length.

In Fig. 5 we have identified the domain of adhesion / bistability / fragmentation, within a range of parameters used in our numerical calculations. A variety of experiments, especially those involving cell-cell or cell-wall adhesion [25, 26], highlight similar transition behaviors. Cellular adhesion bistability occurs from a tug-of-war between two kinetic processes taking place within the contact area: (a) bond formation, and (b) bond rupture due to shear flow [27]. Consider the dynamic adhesion behavior of a rolling silica particle over a flat charged surface, performed by Duffadar and his colleagues [28]. The spheres have a uniform surface charge distribution, hence we consider the fraction of the surface covered by the charges, $\phi = 1.0$ in their experiments. Further, the separation distances are small compared to the particle size, so the curvature effects of the spheres are neglected. A rolling spherical particle of radius $R = 1\mu\text{m}$ in a flow-field $G = 50\text{ s}^{-1}$, exhibits rolling/skipping, a behavior similar to adhesion (Fig. 9b, [28]) which corresponds to the vertical slice $V^* = 0.8$ in Fig. 5a (The solution $V^* = 0.8$, corresponding to the flow-field $G = 50\text{ s}^{-1}$, is obtained by solving the system of equations involving the binder kinetics, Eqns 16, 17, 19, 21, 22, 23). Similarly, other regimes can be identified for different shear rates.

3.2 Bacterial floc aggregation studies

In this section, we use the aggregation kernel (developed above) in our bacterial flocculation model and simulate the overall population dynamics (Eqn. (11)) for few sets of material parameters and floc velocities. In this simulation, we employed the discretization scheme developed by Banks et al. [29] and adopted by Prigent [30] and others [15, 31]. The parameters used in the simulations are listed in Table 1. As reported in [15], the convergence of this first order approximation scheme was tested and a linear relationship was found between the L₂-error of the solution and the mesh-size, Δv (results not reported here). The initial number density is chosen as the exponential curve $b_0(v) = 3.89 \times 10^9 e^{-1.56v} + 7.67 \times 10^{-4} e^{-0.00676v}$, where the coefficients were fit to the experimental data from [15]. All solutions are marched in time until non-dimensional $T=100$. We chose 1 femtoliters (fL) as a lower bound $\underline{v}$ in our simulations, since the radius of the smallest aggregate (consisting of a single bacteria) was of the order of a micron. In our discrete aggregation model we chose the upper bound, $v = 2000$, the volume resolution of $N = 1000$ bin per fL and a temporal resolution of $\Delta t = 10^{-3}$.

Fig. 6 highlights the floc population at different ionic conditions, κ_θ^* , and fixed material properties $\kappa_\theta^* = 1.0$, $\kappa_s^* = 1.5$ and binder stiffness, $\gamma_0 = 10^{-5}\text{ Nm}^{-1}$. We depict results inside the window $1 \leq v \leq 200\text{ fL}$, for clarity. At steady-state inter-floc relative velocity $V^* = 1.0$ (see Fig. 6a), the binding/unbinding mechanism of the ligands corresponds to the adhesion domain of Fig. 5a. The number of small (or large) floc aggregates are lower (or higher) than the initial conditions at the end of the simulation time. Within the fluids at different ionic conditions, larger aggregates are favored in highly ionic fluids (or fluids represented by shorter Debye length). A short screening length implies a smaller separation distance between the interacting floc-surfaces, resulting in a closer interaction or a stronger adhesion (see results in Fig. 3a) and hence a greater number of large flocs. Conversely, large

aggregates are not favored in the fragmentation domain (e.g., when $V^* = 6.0$, Fig. 6b) or in weak ionic conditions, due to the same reasons mentioned above.

4 Conclusions and discussion

We have developed a comprehensive model for the coalescence of two spherical, rigid bacterial flocs via tiltable, elastic ligands in an ionic fluid subject to a homogeneous shear flow. We have demonstrated that the transition between the adhesion and the fragmentation phases can be reversibly continuous, reversibly discontinuous, or irreversible, depending on the ionic strength of the fluid medium, stiffness of the binding ligands and the size of the adhering flocs. Although different aspects of the adhesion mechanism have been studied separately by several groups, we have successfully combined these features in a single bacterial biomass conservation model. The numerical simulations of this model, subject to a homogeneous, planar shear flow, confirm that when adhesion dominates, the population is characterized by a greater number of large floc aggregates. The reverse is true when fragmentation dominates.

Simplifications of this model leads to several important cases of adhesion which are experimentally verified. For example, a negligible shearing effect of the fluid flow on the binders may cause the finite resistance to rotation (or the tiltability effects) to disappear. This is a reasonable approximation provided the torque due to thermal fluctuations

dominates the torque on the binders due to the streaming flow (i.e. $\frac{\kappa_\theta}{k_B T} \ll 1$ in Eqn.(2)) [24]. The same effect could be achieved in the absence of flow hydrodynamics (i.e. setting $\mathbf{V} = 0$ in Eqn.(3, 11)), thus causing the binders to adhere normal to the binding surface [32]. Similarly, cell-cell adhesion in the absence of surface charges have been reported in the medical literature as well [33].

Conversely, there are a number of limitations to our approach. For example, nonlinearity of the micro-scale forces and the effects of cell deformability (significant in many cell-adhesion applications) and can significantly modify the microscale-macroscale force balance thereby modifying the adhesion region. As mentioned above, bacteria do not deform while aggregating, however, a possible extension of our model to deformable cells would make our results applicable to mammalian cells. Our approach also excludes spatial inhomogeneity arising through the material parameters, the effects of catch behavior ($\kappa_s^* > 1.0$), non-equilibrium binding effects, stochasticity and the discrete number of bonds [1], the electro-viscous drag on the sphere surface surrounded by ionic solution (which modifies the fluid velocity across the separation distance between the spheres) [34], as well as forces large enough to tear the binding ligands from their anchoring surface [5]. All these effects can lead to several non-trivial behavior (including the absence of hysteretic behavior in binder-kinetics) that deserves deeper investigation in the future.

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Following are the highlights of this work

- A unified model for bacterial floc-aggregate number density has been developed.
- Features include: binding/unbinding of surface ligands, bond torsion, surface potential, flow hydrodynamics.
- Different adhesion types seen w.r.t flow velocity: reversibly continuous, reversibly discontinuous or irreversible.
- It is seen that surface adhesion is aided by the fluid flow only until a critical value.
- Adhesion domain characterized by greater number of large floc-aggregates.

Figure 1.

An illustration of two rigid, spherical flocs which are coated with binding ligands and translating and rotating in uniform flow. (R_m) is the moving frame of reference, with the origin O_m fixed on the surface of sphere 1 at the point of minimum separation.

Figure 2.

g^* vs x^* , with the fluid velocity set at $V^* = 10$. Material parameters fixed at $\kappa_\theta^* = 1.0$, $\kappa_s^* = 0.5$. Note, g^* is not symmetric about $x = 0$ due to a non-zero shearing velocity.

Figure 3. Plot of $g^*(x^* = 1)$ vs. V^* for (a) different screening length, κ , $R = 1\mu\text{m}$, $\kappa_0 = 10^{-3}\text{ Nm}^{-1}$ (b) different binder stiffness, κ_0 , and $R = 1\mu\text{m}$, $\kappa = 1.5$ (c) different sphere radius, R , $\kappa = 1.5$ and $\kappa_0 = 10^{-3}$; for identically colliding spheres. Material properties are fixed at $\kappa_\theta^* = 1.0$, $\kappa_s^* = 0.5$. Three different adhesion-fragmentation transition are detected: (1) continuous, reversible transition (green curve), (2) discontinuous reversible transition (red curve), and (3) discontinuous, irreversible transition (black curve).

Figure 4.

(a) Horizontal component of the micro-scale forces, $F_{E_x}^* + F_{T_x}^*$, and (b) Minimum vertical separation between the spheres, D^* , vs the fluid velocity, V^* , at a horizontal distance, $x^* = R_c^*$. Radius of the identically colliding spheres is $R = 1\mu\text{m}$. Material properties are fixed at $\kappa_\theta^* = 1.0$, $\kappa_s^* = 0.5$. The force-velocity curves show a non-linear dependence at different ionic compositions of the fluid, due to non-linear dependence of the force on the minimum separation distance, D^* , which is a variable.

Figure 5.

Regions of adhesion/fragmentation/bistability in $\log_{10}(\kappa_0) - V^*$ plane for (a) different ionic conditions, κ , and $R = 1.0\mu\text{m}$, and (b) different floc size, R , and $\kappa = 1.5$. The horizontal distance from the center is fixed at $x^* = R_c^*$, and the material properties set at $\kappa_\theta^* = 1.0$, $\kappa_s^* = 0.5$. The adhesion / fragmentation regions are characterized by $g^* > 0.5$ and $g^* < 0.5$, respectively. The boundary of the bistable region is the locus of the limit points of the hysteretic $g^* - V^*$ curves in Fig. 3.

(a)

(b)

Figure 6.

Floc number density distribution versus floc-volume for different screening lengths at simulation time $T=100$, binder stiffness $\gamma_0 = 10^{-5} \text{ Nm}^{-1}$, and (a) $V^* = 1.0$ (i.e. inside the adhesion domain, $g^* > 0.5$), (b) $V^* = 6.0$ (i.e. inside the fragmentation domain, $g^* < 0.5$), see Fig. 5a. The dash-dot curve in these figures is the initial conditions ($b_0(x)$).

Table 1

Parameters common to all numerical results. The first 8 parameters are used in the system of Eqns (16, 17, 19, 21, 22, 23) at steady-state, while the last 2 parameters are utilized in the time-dependent Eqn. (11).

Parameter	Value	Units	Source
l_0	10^{-8}	m	[24]
σ	$10^{-5} - 10^{-2}$	N m^{-1}	[24]
μ	10^{-3}	$\text{N s} / \text{m}^2$	[9]
A_{Tot}	10^9	m^{-2}	[24]
$K_{\text{on, eq}}$	10^{-10}	s^{-1}	[11]
$K_{\text{off, eq}}$	10^2	s^{-1}	[11]
A	$2.44 k_B T$	J	[20]
G	$1 - 100$	s^{-1}	[9]
A	2.7×10^{-30}	fL^{-1}	[15]
	1.0	$\text{N m}^{-1} \text{s}$	[9]