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Modeling antimicrobial tolerance and treatment of heterogeneous biofilms

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ABSTRACT

A multiphasic, hydrodynamic model for spatially heterogeneous biofilms based on the phase field formulation is developed and applied to analyze antimicrobial tolerance of biofilms by acknowledging the existence of persistent and susceptible cells in the total population of bacteria. The model implements a new conversion rate between persistent and susceptible cells and its homogeneous dynamics is benchmarked against a known experiment quantitatively. It is then discretized and solved on graphic processing units (GPUs) in 3-D space and time. With the model, biofilm development and antimicrobial treatment of biofilms in a flow cell are investigated numerically. Model predictions agree qualitatively well with available experimental observations. Specifically, numerical results demonstrate that: (i) in a flow cell, nutrient, diffused in solvent and transported by hydrodynamics, has an apparent impact on persister formation, thereby antimicrobial persistence of biofilms; (ii) dosing antimicrobial agents inside biofilms is more effective than dosing through diffusion in solvent; (iii) periodic dosing is less effective in antimicrobial treatment of biofilms in a nutrient deficient environment than in a nutrient sufficient environment. This model provides us with a simulation tool to analyze mechanisms of biofilm tolerance to antimicrobial agents and to derive potentially optimal dosing strategies for biofilm control and treatment.

1. Introduction

In nature, as soon as bacteria colonize on moisture surfaces, a biofilm is likely to form thereafter, consisting of the microorganisms aggregated by bacteria along with their self-produced, glue-like exopolysaccharide matrix, also known as the extracellular polymeric substance (EPS). It's commonly perceived by the medical community that biofilms are responsible for many diseases or ailments associated with chronic infections, evidenced for example by the survey that biofilms are present in the removed tissue of 80% of patients undergoing surgery for chronic sinusitis [37]. Unlike a planktonic bacterium, biofilms are hard to be eradicated by the standard antimicrobial treatment [30], which perhaps explains the frequent relapse of chronic diseases or ailments associated with bacterial infections.

Thus, an understanding of the mechanism that underlies biofilm tolerance/persistence to antimicrobial agents can greatly enhance therapeutic treatment of diseases related to biofilms. Intensive research has been conducted, primarily in experiment, to try to understand biofilm structures and dynamics, but the detailed mechanism is still not fully known. Readers may refer to the review papers [15,30] for overviews of current advances in treatment of biofilms. One essential factor for antimicrobial persistence of biofilms is perhaps the existence of persistent cells (persisters) within the biofilm colony, which are consisted of a small portion of dormant bacterial variants that are highly tolerant to antimicrobial agents [4]. Contrasting to persistent cells, the other bacteria are collectively called susceptible bacteria since they respond to antimicrobial treatment sensitively.

From the clinical point of view, understanding the mechanism of persister formation would be useful for biofilm control and eradication, which will impact on treatment of diseases related to biofilms. For reviews on mechanisms underlying the persister formation, readers are referred to the two papers by Lewis [29,30]. As dormant variants of regular bacterial cells, which don't undergo genetic changes, it is perceived that persisters are converted from regular cells due to stresses [4], such as nutrient depletion [1–3,7], existence of antimicrobial agents [33] and so on. Later, when the environment is tolerable, namely nutrient becomes sufficient or

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the concentration of antimicrobial agents drops under a threshold value, biofilms can relapse [8], which implies that persisters have converted back into susceptible bacteria for regrowth. It is a common belief that persister cells are much slow growers compared to susceptible cells.

Taking into account persister formation, researchers have conducted research on therapeutic treatment of diseases induced by biofilms. The review paper [41] provides some control strategies for biofilms. The dosing strategy when administering antibiotics to treatment of biofilms is also an important issue. There exists an evidence that a concentrated dose of biocide is more effective than using a prolonged dose of a lower concentration [22]. In addition, dosing by shocks is more effective than dosing in a persistent manner [21]. To the best of our knowledge, there have not been any optimal strategies derived for biofilm control or disease treatment related to biofilms so far. Currently, the environmental impact of biocide or side-effect of antibiotics have become common concerns, which makes the development of an optimal antimicrobial strategy even harder.

From the modeling perspective, simple mathematical models have been developed to test certain hypotheses of persister formation based on the experimental evidence that supports the concept of persisters [12,23,35,40]. For instance in [35], the author used a simple mathematical model to show that persister formation can lead to higher bacterial persistence with respect to antimicrobial agents than those grown in planktonic culture. In [23], a 3D agent-based model for biofilm dynamics under antimicrobial treatment was developed, in which it showed that the substrate limitation can contribute to persistence of biofilms with respect to antimicrobial treatment. Notably, Cogan et al. have worked on some possible mechanisms of persister formation using time-dependent, spatially homogeneous models recently [11,12,28].

Some mathematical models on dosing strategies for treating diseases related to biofilms have also been derived. For instance, Cogan et al. discussed effective dosing strategies using a simple mathematical model in [12,28]. In [14], he discussed the effect of periodic disinfection using a one-dimensional model. In [44], the adaptive response to dosing protocols for biofilm controls was analyzed, which provided some sufficient conditions for eradicating biofilms using a constant dosing approach. In addition, models analyzing other impact factors, which may contribute to biofilm's persistence to antimicrobial treatment were also proposed. For instance, the author in [17] analyzed and simulated diffusive resistance of bacterial biofilms to penetration of antibiotics.

Most of the modeling efforts in the past focused on reactive kinetics of biofilm persistence and treatment. Very few considered the hydrodynamic effect and the spatio-temporal heterogeneous structures of biofilms in 3D space and time. It is well-known that biofilms are of highly heterogeneous spatial structures and rich temporal dynamics. The spatial heterogeneity can significantly impact on biofilm formation and its function, especially, concerning biofilm's persistence to antimicrobial agents. In this paper, we develop a multiphasic hydrodynamic model for biofilms of multiple bacterial phenotypes; in particular, we limit the phenotypes to the persister and susceptible type. This model extends our previous model of biofilms based on biomass-solvent mixtures [45] by distinguishing between the persister cell and the susceptible cell when biofilms are treated by antimicrobial agents. In this model, the interplay among the various biomass components such as various bacterial types, EPS and solvent is carefully taken into account both hydrodynamically and chemically [46]. The model shows that the dynamical interaction between persistent and susceptible phenotypes can impact dramatically on overall dynamics of the biofilm. It provides the spatio-temporal resolution that is needed for more details about antimicrobial treatment against biofilm colonies in space and time than the previous models can, providing more insight into hydrodynamics of biofilms under antimicrobial treatment.

The paper is organized as follows. In Section 2, we formulate the hydrodynamic theory for the biofilm system based on the phase field formulation. Then, an efficient numerical solver for the governing partial differential equation system is developed using the semi-implicit finite difference strategy in Section 3. In Section 4, numerical results are presented and discussed. Finally, we summarize the result and draw a conclusion.

2. Mathematical model formulation

We model the biofilm together with its surrounding aqueous environment as a multiphase complex fluid. The biofilm consists of the mixture of biomass and solvent, in which biomass is made up of bacteria of various phenotypes and their products like exopolysaccharide (EPS). Nutrient and antimicrobial agents are small molecule substances dissolved in solvent. Their mass and volume fractions are negligibly small, which will therefore be neglected in this model. However, their chemical effects are important and will therefore be retained. Let ϕ_{bs} be the volume fraction of the bacteria that are susceptible to antimicrobial agents, ϕ_{bp} the volume fraction of the bacteria that are persistent to the agent, ϕ_{bd} the volume fraction of the dead bacteria, ϕ_b the volume fraction of the bacteria, and ϕ_p the volume fraction of EPS. We in addition denote the concentration of the nutrient and the antimicrobial agent as c and d, respectively, and define ϕ_n the volume fraction of the biomass, consisting of all the volume fractions for the bacteria as well as EPS,

$$\phi_n = \phi_p + \phi_{bs} + \phi_{bp} + \phi_{bd}. \tag{1}$$

In addition to the volume fractions introduced above, the volume fraction of the solvent is denoted as ϕ_s. The incompressibility of the complex fluid mixture then implies

$$\phi_s + \phi_n = 1. \tag{2}$$

To help the reader to better understand the structure of our biofilm model, we show a schematic for the biofilm colony in Fig. 1.

In this model, we make a simplifying assumption that all components in the biomass including the bacteria and EPS share the same mass density, which is roughly correct. We denote ρ_n and ρ_s the density of the biomass and solvent, and η_n and η_s the viscosity of the biomass and solvent, respectively. Then, the volume averaged viscosity and density are given, respectively, by

$$\eta = \phi_n\eta_n + \phi_s\eta_s, \quad \rho = \phi_n\rho_n + \phi_s\rho_s. \tag{3}$$

We assume the bacteria, regardless whether they are alive or dead, and the EPS mix with the solvent owing to the osmotic pressure. Then, we adopt the modified free energy introduced in [45] and denote it by F:

$$F = \int_\Omega d\mathbf{x}\left[\frac{\gamma_1}{2}k_BT|\nabla\phi_n|^2 + \gamma_2 k_BT\left(\frac{\phi_n}{N}\ln\phi_n + (1-\phi_n)\ln(1-\phi_n) + \chi\phi_n(1-\phi_n)\right)\right]. \tag{4}$$

This is the modified Flory–Huggins free energy with a conformational entropy, in which γ_1 and γ_2 parametrize the strength of the conformational entropy and the bulk mixing free energy, respectively, χ is the mixing parameter, N is the extended polymerization index for the biomass, k_B is the Boltzmann constant and T is the absolute temperature.

2.1. Transport equations for biomass components

Given the free energy density functional f in Eq. (4), the 'extended' chemical potentials with respect to biomass components