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Nanoparticle Deposition onto Biofilms

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Abstract

We develop a mathematical model of nanoparticles depositing onto and penetrating into a biofilm grown in a parallel-plate flow cell. We carry out deposition experiments in a flow cell to support the modeling. The modeling and the experiments are motivated by the potential use of polymer nanoparticles as part of a treatment strategy for killing biofilms infecting the deep passages in the lungs. In the experiments and model, a fluid carrying polymer nanoparticles is injected into a parallel-plate flow cell in which a biofilm has grown over the bottom plate. The model consists of a system of transport equations describing the deposition and diffusion of nanoparticles. Standard asymptotic techniques that exploit the aspect ratio of the flow cell are applied to reduce the model to two coupled partial differential equations. We perform numerical simulations using the reduced model. We compare the experimental observations with the simulation results to estimate the nanoparticle sticking coefficient and the diffusion coefficient of the nanoparticles in the biofilm. The distributions of nanoparticles through the thickness of the biofilm are consistent with diffusive transport, and uniform distributions through the thickness are achieved in about four hours. Nanoparticle deposition does not appear to be strongly influenced by the flow rate in the cell for the low flow rates considered.

Keywords

nanoparticle transport; flow cell modeling; adhesion

I. INTRODUCTION

A biofilm is a sessile community of microorganisms embedded in a self-secreted matrix composed of extracellular polymeric substances (EPS). Patients with chronic respiratory problems often suffer from bacterial infections that develop as biofilms in the lungs. In addition to impairing respiration, bacteria and other microorganisms in lung biofilms secrete compounds that stimulate pro-inflammatory cytokines, which further damage the underlying lung tissue. The bacteria that are typically found in lung biofilms include *Pseudomonas*

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aeruginosa, *Staphylococcus aureus*, and *Haemophilus influenzae*. In a biofilm, these organisms display an increased resistance to antimicrobials and host clearance mechanisms [5, 6, 25, 45, 47, 50, 62]. Hence when biofilms form in the lung, their removal using conventional antimicrobial treatments is difficult.

Chronic respiratory infections are the primary cause of morbidity and mortality of those afflicted with cystic fibrosis (CF). CF, the most common life-shortening autosomal recessive disease among caucasians, afflicts over 30,000 persons in the United States [39]. In the CF patient, defective ion transport across the lung epithelial layer causes thick, dehydrated mucus. This hampers mucociliary clearance, causing retention and build-up of mucus in the lungs. Mucus serves as a nutrient-rich food source and physical barrier, which allows biofilms to grow, and, once established, to remain impenetrable to conventional antimicrobials [59]. CF is characterized by an exaggerated inflammatory response, progressive airway obstruction, bronchiectasis, and, eventually, respiratory failure [17]. As a result, 90% of patients die prematurely [29]. A large CF patient population and the lack of an effective treatment drive medical research to develop both new antimicrobials and effective methods to deliver drugs to biofilms in the lung.

To treat CF patients, antimicrobial is typically nebulized and delivered into the lungs by inhalation. Recently, methods have been developed to first encapsulate the antimicrobial in biodegradable polymer nanoparticles, which can then be nebulized and inhaled [7–9, 24, 27, 28, 37, 40, 56]. Once the nanoparticle is delivered to the treatment area, the antimicrobial is released by diffusion through the polymeric matrix, by degradation of the polymeric matrix, or by a combination of diffusion and degradation [11, 44]. Furthermore, some antimicrobials are not amenable to conventional methods of aerosol delivery because these drugs are degraded by the nebulization process or by the action of enzymes and immune system components. Encapsulating these drugs in nanoparticles provides protection during the delivery process, ultimately enhancing the efficacy of the treatment. Although numerous parameters affect how well particles can be delivered to the lungs, many articles have been published that seek to determine the particle sizes that are optimal for the deposition of particles onto the respiratory track. These papers all suggest that 1000 to 5000 nm is the optimal range of sizes for inhalation and deposition [14, 21]. Delivery of antimicrobial and other therapeutic agents to the lungs via nebulized nanoparticles shows great potential for the treatment of several inherited and acquired diseases such as CF, asthma, and lung cancer [18, 53].

As a potential treatment for CF patients, novel antimicrobials based on silver carbene complexes (SCC) [38, 60] have been encapsulated in L-tyrosine polyphosphate (LTP) nanoparticles. LTP has been formulated into nanoparticles and applied for lung delivery of SCCs [11, 24]. The advantages of LTP for in vivo applications and for nanoparticle formulation have been published. We mention in particular that degradation products are not cytotoxic and do not alter the local pH [11, 19, 24]. After being nebulized and inhaled, the enhanced survival percentage of mice infected with *P. aeruginosa* suggests that the LTP nanoparticles deposited in the airways of the lungs and penetrated into biofilms [24, 30]. The nanoparticles then released the SCC over a 1 to 7 day period.

A basic challenge in designing a drug delivery system based on nebulization of nanoparticles is maximizing the amount of drug delivered to the terminal bronchioles and alveoli [26]. Meeting this challenge entails answering several important questions, which include

- What is the spatial distribution of biofilm in the lung?

- How many of the inhaled nanoparticles deposit onto and penetrate into the biofilm in the lung?
- What is the final distribution of nanoparticles through the biofilm?
- Once released from the nanoparticles, does antimicrobial remain in the biofilm or leak out into either the epithelium substratum or the lung airway?
- Is there a loss of the effectiveness of the antimicrobial once in the biofilm?

In this paper, we address the second and third questions on the previous list through mathematical modeling and experiments. We develop a mathematical model of nanoparticles carried by a fluid over a biofilm growing in a parallel-plate flow cell. The basic geometry of our model, as well as of our experimental set up, is shown in Figure 1. The model describes nanoparticles depositing onto and penetrating into the biofilm. Our model assumes that adhesion by nonspecific interactions is the primary mechanism by which nanoparticles are deposited onto the biofilm. Noting the long, thin geometry of the deep passages of the lungs, we believe that our model of fluid flow between two parallel plates yields insight into nanoparticle transport and deposition by fluid in the lungs.

Once the nanoparticles are deposited, they diffuse through the biofilm. This diffusion, which we assume is the primary form of nanoparticle transport within the biofilm, distributes the nanoparticles through the EPS matrix. Several hypotheses have been suggested as to how this diffusion occurs. In [12], the authors hypothesize that microbeads diffuse through water-filled pores in the biofilm. Also, we note that some recent research characterizes biofilm as a viscoelastic material having the structure of a hydrogel [20]. These authors reference many articles that treat biofilms as viscoelastic materials. Recent modeling papers that treat biofilms as hydrogels include [5, 63]. In [5], the authors note that swelling and deswelling, a basic property of gels, has been demonstrated in biofilms. Hence pores and crevices in the biofilm may deform to allow the nanoparticles to diffuse. In [55], the authors measure the viscoelastic properties of mucoid *P. aeruginosa* biofilms.

The mathematical modeling is supported by flow-cell experiments, in which a carrier fluid containing fluorescently labeled LTP nanoparticles passes over a biofilm grown over the bottom of the flow cell. In the experiments, the distribution of nanoparticles that deposit onto and diffuse into the biofilm is studied as a function of the flow rate of the carrier fluid, the length of time the carrier fluid passes over the biofilm, and the concentration of nanoparticles in the carrier fluid. The experimental results are used to estimate several key parameters in our model.

The mathematical model is used to numerically simulate the flow cell experiments. The simulations predict nanoparticle concentrations in the biofilm over a four hour period after the nanoparticles are deposited on the surface of the biofilm. We vary parameters in the model to explore how the adhesion characteristics of the nanoparticles influence deposition onto the biofilm and how the rate of diffusion in the biofilm influences the steady-state distribution of nanoparticles through the biofilm. The sticking characteristics of polymer nanoparticles, and the average flow rates in the deep passages of the pulmonary system are unknown. We vary the magnitudes of these parameters over several orders of magnitude to illustrate the sensitivity of the results to errors in parameter measurement. Our results show that the flow rate alters the profile of the concentration plug traveling through the flow cell, but large changes in nanoparticle concentrations in the biofilm are not observed as a result of an increase in flow rate. Also, as the sticking coefficient is increased, we observe a large increase in nanoparticle concentrations in the biofilm.

There is a large literature on the the deposition patterns of aerosols inhaled into the lung. Recent examples, among numerous possibilities, include [31, 32, 57]. These studies do not look at how particles penetrate into and diffuse through lung tissue after deposition. We note that the results by Longest and Oldham [32] indicate that transient inhalation flow can be closely approximated by steady flow conditions, and hence for computational fluid dynamics simulations it is reasonable to assume steady particle flow. Most studies of aerosol deposition in the lungs do not include pathological conditions such as biofilm infection. A recent exception is Longest et al. [33], in which the authors consider the influence of inflammation due to asthma on aerosol dynamics and particle deposition patterns.

None of the studies just cited consider the diffusion of the aerosol particles into lung tissue after deposition. Drury et al. [12, 13] report on the diffusion of 1 μm beads in a biofilm. The relevance of these results for our study is discussed further at the start of Section II B below.

Several authors have studied particle-biofilm interaction in the contexts of stream ecology and waste-water treatment. Bouwer [4] published a mathematical investigation of how particles are captured by biofilms during waste-water treatment. Searcy et al. [42] studied the capture of oocysts (approximately 5 μm in diameter) by *P. aeruginosa* biofilms. Though the investigation did not include mathematical modeling, the study does bear some similarities to the deposition analysis in our experimental effort.

An issue that arises when considering the use of nanoparticles to treat lung biofilms is the penetration of nanoparticles through lung mucus. A series of works by Hanes et al. [15, 48, 49, 51, 54] provide a comprehensive review of nanoparticle penetration into mucus barriers. These authors engineer nanoparticles densely coated with poly(ethylene glycol), which allows the nanoparticles to rapidly penetrate mucus by minimizing ‘mucoadhesion’. Further, they find that it is not easy to wash the nanoparticles out of the mucus once the nanoparticles have penetrated the mucus. The surface of the LTP nanoparticle has been decorated with PEG for this reason and for improved suspension in water [11]. In both our experiments and in our modeling, there is no mucus barrier over the biofilm. The work of Hanes suggests the possibility of engineering nanoparticles that readily diffuse through mucus. Hence, our model could provide insight on how nanoparticles diffuse into lung biofilms under a mucus barrier.

II. MATERIALS AND METHODS

A. Experimental Methods

1. Fabrication of Nanoparticles—The nanoparticles used in our experiments are fabricated by water-in-oil-in-water emulsion. The initial emulsion contains L-tyrosine polyphosphate (LTP) [24] dissolved in chloroform, PEG-g-CHN (PEG grafted chitosan) dissolved in acetic acid, LPEI (linear polyethylenimine), and rhodamine dissolved in distilled deionized water at a concentration of 1% by polymer weight. (For the nanoparticle formulations, chitosan is conjugated to PEG to produce an amphiphilic polymer. During the emulsion process, the PEG interacts with the water phase and chitosan interacts with the organic phase, which contains the LTP polymer. Thus, both the PEG and LTP are shielded by the surface decoration of PEG. Characterization of nanoparticles produced using PEG-grafted onto chitosan (size distributions using dynamic light scattering) have been published [11, 24, 61].) The initial mixture was spun at 2000 rpm (Yamato LD400D) for one minute. After the addition of the 10% polyvinylpyrrolidone, the stirring speed was reduced to 1600 rpm for 3 minutes. The organic solvent was allowed to evaporate overnight. Afterward, the nanoparticles were shell frozen and freeze dried until usage (Labconco Freezone 4.5).

The morphology of the LTP nanoparticles was determined with scanning electron microscopy (SEM). The SEM samples were prepared by suspending 1 mg of nanoparticles in 1 mL of distilled and deionized water. Then, 200 μ l of the suspended nanoparticles were applied onto a stub, dehydrated, and sputter coated with silver/palladium. Dynamic laser light scattering was used to quantify the size distribution of the LTP nanoparticles. The nanoparticles were prepared by suspending 1 mg of nanoparticles in 10 ml of distilled and deionized water. Afterward, the nanoparticles were centrifuged for 10 seconds at 1000x g to remove any large aggregates and were decanted into a glass scintillation vial. A dynamic laser light scattering system (Brookhaven Instruments BI-200SM, Holtsville, NY) calculated the nanoparticle diameter by the Regularized Non-negatively Constrained Least Squares (CONTIN) method. The range of nanoparticle size was reported as differential distribution values.

2. Bacterial Strain and Culture Conditions—Biofilms were grown using *Pseudomonas aeruginosa* strain PAO1 tagged with *gfp* [58]. Liquid cultures used for flow cell inoculation were grown in a Synthetic Cystic Fibrosis sputum Media (SCFM) [36] at 37 °C shaken at 250 rpm. Biofilms were grown in 10% (vol/vol) SCFM modified with the addition of 0.04% (wt/wt) mucin (Sigma).

1 L of SCFM media is defined as follows: a buffered base is created containing: 6.5 ml 0.2 M NaH_2PO_4 , 6.25 ml 0.2 M Na_2HPO_4 , 0.348 ml 1 M KNO_3 , 1.084 ml 0.25 M K_2SO_4 , 0.122 g NH_4Cl , 1.114 g KCl , 3.03 g NaCl , 10 mM MOPS, and 779.6 ml deionized water. The following volumes of amino acids in 100 mM solutions are added to the buffered base: 8.27 ml L-aspartate in 0.5 M NaOH, 10.72 ml L-threonine, 14.46 ml L-serine, 15.49 ml L-glutamate-HCl, 16.61 ml L-proline, 12.03 ml L-glycine, 17.8 ml L-alanine, 1.6 ml L-cysteine-HCl, 11.17 ml L-valine, 6.33 ml L-methionine, 11.2 ml L-isoleucine, 16.09 ml L-leucine, 8.02 ml L-tyrosine in 1 M NaOH, 5.3 ml L-phenylalanine, 6.76 ml L-ornithine-HCl, 21.28 ml L-lysine-HCl, 5.19 ml L-histidine-HCl, 0.13 ml L-tryptophan in 0.2 M NaOH, 3.06 ml L-arginine-HCl. The pH of the solution is adjusted to 6.8 then filter sterilized. After filter sterilization, the following components are added: 1.754 ml 1 M CaCl_2 , 0.606 ml 1 M MgCl_2 , 1 ml 3.6 mM $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, 3 ml 1 M D-glucose, and 9.3 ml 1 M L-lactate (adjusted to pH of 7.0 with NaOH) [36].

3. Growth of Biofilms—Biofilms were grown in a continuous flow cell apparatus consisting of media and waste reservoirs, bubble trap, flow cell, and a peristaltic pump [52]. (The flow cell and bubble traps are designed and manufactured by BioCentrum-DTU at the Technical University of Denmark (DTU), Center for Systems Microbiology.) The chamber of the flow cell measured 40 mm long, 4 mm wide, and 1 mm deep. Media was pumped through the system in a once-through fashion at a rate of 3 ml/hr during the biofilm growth phase (18-22 hr). This corresponds to a very low wall shear stress of 0.00865 dyn/cm². (The shear stress was estimated assuming a parabolic fluid velocity and using the dynamic viscosity of water at 37 °C.) Media flow rate was altered during deposition experiments. The flow cell apparatus was inoculated with 300 μ l of a 1:100 dilution of an overnight culture of *gfp* tagged PAO1. The cells were introduced into the flow cell with a syringe and allowed to incubate for 1 hr at 37 °C for initial attachment. During the inoculation phase, media flow was stopped and tubing was clamped both up and down stream of the flow cell. Following this, media flow was restored and the flow cell was incubated for 18-22 h at 37 °C. The average biofilm thickness near the inlet was around 10 microns and the thickness near the outlet was around 5 microns, with peak thickness up to 30 microns.

4. LTP Particles and Treatment of Biofilms—LTP nanoparticles loaded with rhodamine were suspended in water at a concentration of 0.5 mg LTP particle preparation per 1 ml of water for all the experiments. Measurement of particle number per volume was

made by filtering diluted particle suspension through a $0.22\ \mu\text{m}$ black filter to distribute particles on the filter. The filter was then mounted on a slide and imaged over 10 random fields using a Zeiss LSM 510 META confocal microscope. Images consisted of z-stacks using a vertical step size of $0.25\ \mu\text{m}$ using a 100x objective. The LTP nanoparticles were passed over the biofilm using a syringe pump at the rate of 1.5 ml/hr, 3 ml/hr, or 6 ml/hr for either 10, 20, or 30 min injection times. The peristaltic pump was halted during use of the syringe pump and restarted at a rate equal to that used for particle introduction upon completion. Media flow was continued during image capture. Approximately 30 min was allowed to pass before the first z-stacks were taken. For each experiment, 10 z-stacks of the biofilm were taken at 5 locations along the length of the flow cell using a vertical step size of $0.5\ \mu\text{m}$ and a 100x objective.

5. Data analysis—Metrics used to quantify experimental results were extracted from z-stack data. These metrics include particle count per volume of the LTP particle solution, particle count per volume of biofilm, particle location, and biofilm height. LTP particle counts and locations were found using the 3D object counter plug-in for the software ImageJ [1, 3]. Biomass volume and height were measured using COMSTAT, a biofilm image analysis program for MATLAB (MathWorks, Natick, MA) [22, 23]. Threshold values for particle and biomass detection in these programs were standardized across samples.

B. Modeling Methods

The geometry of the parallel-plate flow cell we model is shown in Figure 1. A fluid carrying nanoparticles flows between the top plate and a biofilm growing over the full length of the bottom, parallel plate. The density ρ and viscosity μ of the carrier fluid are assumed to be constants. In our simulations we use the density and viscosity of water. We assume that the interface between the biofilm and fluid is sinusoidal as a function of horizontal position and is constant over time. The average thickness of the biofilm is much smaller than the length of the flow cell. We assume the flow is parabolic, laminar, and steady state, and we impose no flux and no slip boundary conditions at the flow-cell boundaries.

The fluid contains a well-mixed, dilute concentration of nanoparticles. In our model, adhesion is the primary mechanism by which nanoparticles in the fluid attach to the biofilm. After adhering, the nanoparticles penetrate the biofilm through its water-filled pore structure. We assume that diffusion is the primary form of nanoparticle transport in the biofilm. This assumption is supported by results in Drury et al. [12, 13], who report that diffusion through the water-filled pores, rather than diffusion through the biofilm matrix or inertial impact, is the dominant mechanism for the transport of $1\ \mu\text{m}$ beads in a biofilm. The authors postulate that the surface roughness and water-filled pore structure of the biofilm allow the beads to stick and penetrate. Unfortunately, our experimental procedure does not enable us to distinguish between biofilm and water. Hence, we do not characterize the water-filled pore structure of our biofilms.

We simulate the diffusion of nanoparticles to predict the steady-state concentration of the nanoparticles in the biofilm up to four hours after the deposition of the nanoparticles on the biofilm surface.

1. Governing Equations—The horizontal and vertical velocities of the fluid flow are denoted by $\tilde{u}$ and $\tilde{v}$, respectively. Following Longest and Oldham [32], we assume a steady-state solution where the velocity fields are not time dependent. As we show below, only the volumetric flow rate and not the details of the fluid flow are needed.

Nanoparticle transport in the fluid is assumed to occur through a combination of advection and diffusion and is described by

$$\tilde{C}_{\tilde{t}} + \tilde{u}\tilde{C}_{\tilde{x}} + \tilde{w}\tilde{C}_{\tilde{z}} = D \left[\tilde{C}_{\tilde{x}\tilde{x}} + \tilde{C}_{\tilde{z}\tilde{z}} \right]. \quad (1)$$

Here D is the mass diffusivity of nanoparticles in the fluid and $\tilde{C}$ is the concentration of the nanoparticles in the fluid.

We assume that the nanoparticles transport within the biofilm only by diffusion (in particular, we neglect fluid flow in the biofilm). This transport is described by

$$\tilde{C}_{B,\tilde{t}} = D_B \left[\tilde{C}_{B,\tilde{x}\tilde{x}} + \tilde{C}_{B,\tilde{z}\tilde{z}} \right], \quad (2)$$

where D_B is the mass diffusivity of the nanoparticles in the biofilm and $\tilde{C}_B$ is the concentration of the nanoparticles in the biofilm.

At time $\tilde{t} = 0$, a well-mixed solution of nanoparticles, with known concentration A , is injected into the system continuously for a period of minutes, denoted by t_{inj} . Following this time period, injection ceases and the nanoparticle plug is allowed to flow through the flow cell. We describe this inlet boundary condition at $\tilde{x} = 0$ by

$$\tilde{C}(0, \tilde{z}, \tilde{t}) = A - AH(\tilde{t} - t_{inj}), \quad (3)$$

where A is the nanoparticle concentration of the fluid and $H(\tilde{t} - t_{inj})$ is the Heaviside function.

Because the time scale for significant growth of the biofilm is on the order of days, we assume that the interface between the biofilm and the fluid is described by a function $\tilde{h}$ of the horizontal position but not of time. The flux of nanoparticles from the liquid through the fluid-biofilm interface at $\tilde{z} = \tilde{h}$ is

$$D\nabla\tilde{C} \cdot \hat{n} = \frac{D \left[-\tilde{C}_{\tilde{x}}\tilde{h}_{\tilde{x}} + \tilde{C}_{\tilde{z}} \right]}{\sqrt{1 + \tilde{h}_{\tilde{x}}^2}} = S_c \tilde{C}, \quad (4)$$

where $\nabla = \frac{\partial}{\partial \tilde{x}} \hat{i} + \frac{\partial}{\partial \tilde{z}} \hat{k}$, $\hat{n}$ is the unit outward normal to the interface, and S_c is the sticking coefficient of the nanoparticles. A non-zero sticking coefficient implies that some of nanoparticles that contact the interface adhere or stick; a sticking coefficient of 0 implies that no nanoparticles stick. We comment that the sticking coefficient, S_c , is a generic term representing unspecified interactions leading to the deposition of nanoparticles onto the biofilm surface. We make deposition linear in concentration, as described by (4), for mathematical convenience. It is possible that a nonlinear form is more appropriate and that biofilm surface curvature effects should be included to describe the deposition process.

The flux of nanoparticles into the biofilm at $\tilde{u}\tilde{h}(\tilde{x})$ is

$$D_B = \frac{\left[-\tilde{C}_{B,\tilde{x}}\tilde{h}_{\tilde{x}} + \tilde{C}_{B,\tilde{z}} \right]}{\sqrt{1 + \tilde{h}_{\tilde{x}}^2}} = S_c \tilde{C}. \quad (5)$$

Coupling this equation with (3) for the inlet concentration, we see that the nanoparticle flux into the biofilm takes on two distinct features. Up until the time t_{inj} there is a non-zero inlet source of nanoparticles into the flow chamber. This leads to a non-zero flux of nanoparticles into the biofilm. After time t_{inj} the inlet source concentration is zero. Eventually, depending

upon the flow rate, which is defined below, the nanoparticles in the fluid flow are convected downstream past the biofilm, after which a flow containing no nanoparticles passes over the biofilm. According to (5), this results in a no-flux condition at the biofilm surface. In other words, once deposited and diffusing through the biofilm, the nanoparticles do not escape the biofilm. For the four hour time frame considered here, this is a reasonable assumption and agrees with the observations of [12, 15]. We note that Drury et al. ultimately observe microbeads slowly leaking out of the biofilm over a time period of days. We would need to adjust equation (5) to account for this slow flux if we were to simulate longer time periods. It is also worth noting that when used in treatment, the nanoparticles would deposit, penetrate into the biofilm and begin releasing antimicrobial. Hence, that the nanoparticles escape the biofilm at a slow rate is advantageous for the drug delivery scheme.

We assume no flux of nanoparticles through the upper and lower plates of the flow cell. Hence

$$\tilde{C}_{\tilde{z}}(\tilde{x}, d, \tilde{t})=0, \quad \tilde{C}_{B\tilde{z}}(\tilde{x}, 0, \tilde{t})=0. \quad (6)$$

We describe the volumetric flow rate Q of the carrier fluid by

$$Q = \int_{\tilde{h}}^d \tilde{u} d\tilde{z}. \quad (7)$$

We supplement the equations above with initial conditions

$$\tilde{C}(\tilde{x}, \tilde{z}, 0)=0, \quad \tilde{C}_B(\tilde{x}, \tilde{z}, 0)=0, \quad (8)$$

so that the initial concentrations of nanoparticles in the carrier fluid and in the biofilm are zero.

2. Non-dimensionalization—The height, d , of the flow cell is assumed to be much smaller than the length, L , of the cell. Hence, we define the small-aspect ratio parameter $\epsilon = d/L$, where $\epsilon \ll 1$. We nondimensionalize the governing equations with the scalings given in Table I. We then identify several non-dimensional groupings. For the fluid, the Peclet number $P_e = QD$ is a ratio measuring the strength of transport by advection to transport by diffusion. The nanoparticle diffusivity in the biofilm is assumed to be much less than that in the fluid and so we define

$$\epsilon \widehat{D} = \frac{D_B}{D}. \quad (9)$$

Here $\widehat{D}$ is an $\mathcal{O}(1)$ constant. Finally, the ratio of sticking time of the nanoparticles onto the biofilm surface to diffusion through the biofilm is

$$K = \frac{S_c d}{D_B}. \quad (10)$$

After rescaling, the fluid domain is $x \in [0, 1]$ and $z \in [h(x), 1]$ and the governing equation for the concentration of nanoparticles in the fluid is

$$\widehat{D}\epsilon C_t + \epsilon P_e [uc_x + wc_z] = \epsilon^2 C_{xx} + C_{zz}, \quad (11)$$

with boundary conditions

$$C_z(x, 1, t) = 0, \quad (12)$$

$$\frac{-\epsilon^2 C_x h_x + C_z}{\sqrt{1 + \epsilon^2 h_x^2}} = K \epsilon \widehat{D} C \quad \text{at } z = h, \quad (13)$$

$$C(0, z, t) = 1 - H(d^2 D_B^{-1} t - t_{inj}), \quad (14)$$

and initial condition

$$C(x, z, 0) = 0. \quad (15)$$

After rescaling, the biofilm occupies $x \in [0, 1]$ and $z \in [0, h(x)]$, and the concentration of nanoparticles in the biofilm is governed by

$$C_{B,t} = \epsilon^2 C_{B,xx} + C_{B,zz} \quad (16)$$

with the boundary conditions

$$\frac{-\epsilon^2 C_{B,x} h_x + C_{B,z}}{\sqrt{1 + \epsilon^2 h_x^2}} = K C \quad \text{at } z = h \quad (17)$$

$$C_{B,z}(x, 0, t) = 0, \quad (18)$$

and initial condition

$$C_B(x, z, 0) = 0. \quad (19)$$

3. Asymptotic Techniques—We now introduce the expansions

$$u = u_0 + \epsilon u_1 + \cdots \quad (20)$$

$$w = w_0 + \epsilon w_1 + \cdots \quad (21)$$

$$C = C_0 + \epsilon C_1 + \cdots \quad (22)$$

$$C_B = C_{B,0} + \epsilon C_{B,1} + \cdots \quad (23)$$

Straightforward computations yield the $O(1)$ equation

$$C_{0,zz} = 0 \quad (24)$$

with boundary conditions

$$C_{0,z}(x, 1, t) = 0 \quad (25)$$

$$C_{0,z}(x, h, t) = 0 \quad (26)$$

$$C_0(0, z, t) = 1 - H(d^2 D_B^{-1} t - t_{inj}) \quad (27)$$

and initial condition

$$C_0(x, z, 0) = 0 \quad (28)$$

for transport within the fluid. The $\mathcal{O}(1)$ equation for transport of nanoparticles in the biofilm is

$$C_{B0,t} = C_{B0,zz} \quad (29)$$

with boundary conditions

$$C_{B0,z}(x, h(x), t) = KC_0, \quad (30)$$

$$C_{B0,z}(x, 0, t) = 0, \quad (31)$$

and initial condition

$$C_{B0}(x, z, 0) = 0. \quad (32)$$

From (24), (25), and (26), we see that

$$C_0 = C_0(x, t), \quad (33)$$

which is independent of z .

To determine $C_0(x, t)$ we need only part of the $\mathcal{O}(\epsilon)$ system of equations. These are

$$\widehat{D}C_{0,t} + P_e u_0 C_{0,x} = C_{1,zz}, \quad (34)$$

$$C_{1,z}(x, 1, t) = 0, \quad (35)$$

$$C_{1,z}(x, h, t) = K\widehat{D}C_0(x, t). \quad (36)$$

Integrating (34) with respect to z and using (35) and (36), we find

$$\widehat{D}C_{0,t} [1 - h(x)] P_e C_{0,x} + K\widehat{D}C_0(x, t) = 0 \quad (37)$$

4. Numerical Solution of the System of Equations—In summary, to determine the concentration of nanoparticles in the fluid we solve (37) with boundary and initial conditions

$$C_0(0, t) = 1 - H(d^2 D_B^{-1} t - t_{inj}), \quad (38)$$

$$C_0(x, 0) = 0. \quad (39)$$

Once known, C_0 is then used to determine the concentration of nanoparticles in the biofilm by solving equations (29)–(32).

For later discussion, it is instructive to examine an approximate solution to (37) for the nanoparticle concentration in the fluid flow over the biofilm, when $h(x) = 0$. Letting $C_0(0, t)$ in (38) be represented by $F(t)$, we find

$$C_0(x, t) = F\left(t - \frac{D_B L}{dQ} x\right) e^{-\left(\frac{S_C L}{Q} x\right)}. \quad (40)$$

This expression admits the following interpretation. The first term describes the convective transport of the inlet nanoparticle concentration downstream. As the flow rate Q increases, the inlet concentration is convected further downstream. This means that more nanoparticles flow over the biofilm surface for larger flow rates. As a consequence, the flux of nanoparticles into the biofilm increases per (30). The second term, of exponential form, describes the deposition characteristics of the nanoparticles. If the flow rate is slow, the nanoparticles tend to deposit near the inlet, while at larger flow rates more nanoparticles are convected downstream, which leads to a more spatially (in the x -direction) uniform profile.

Since x dependence has been removed, (29) can be solved for C_{B0} as a function z using a one-dimensional Crank-Nicolson scheme at fixed locations in x . Equation (37) is solved using a modified Lax-Wendroff scheme. For details, see [35].

III. RESULTS

A. Experimental Results

Scanning electron microscopy (SEM) of the LTP nanoparticles shows spherical conformation, a smooth surface morphology, and a heterogeneous size distribution with a mean diameter of approximately 500 to 2500 nm. See Figures 2 and 3. From the Stokes-Einstein equation, the diffusion coefficient of particles of this size in water is estimated to be

$D = 2 \times 10^{-9} \frac{\text{cm}^2}{\text{s}}$. We use this value in our numerical simulations. In testing, it was observed that blank nanoparticles degraded completely over a period of 7 days [10], which is consistent with the degradation profiles observed for LTP films and capsules [11, 43]. The suitability of LTP nanoparticles encapsulated with a hydrophobic functional group for nebulization has been established by an in vivo study [24].

LTP nanoparticles were observed embedded within the biofilms. Figure 4 shows a representative slice of a z -stack. The LTP nanoparticles (red) are clearly visible and easily distinguished from the bacteria (green). Figure 5 shows a three-dimensional reconstruction of a single z -stack. This figure demonstrates the scale and morphology typical of the biofilms observed in these experiments. Figures 4 and 5 represent nanoparticles in a biofilm 1 hr after deposition at a flow rate of 1.5 ml/hr for 30 min. The z -slice in Figure 4 was taken at location $z = 17.5 \mu\text{m}$ in a $35 \mu\text{m}$ stack. A quantitative representation of particle location within the biofilm is provided below in Figures 6 and 7.

The average concentrations of LTP nanoparticles in the bulk fluid and the biofilm for each combination of flow rate and injection duration parameters are shown in Table II. Metrics calculated for each set of parameter values in Table II are averaged over 3 replicates. Each replicate consists of 10 z -stacks taken at 5 discrete locations along the length of the flow-cell

0.5 to 2 hrs after particle deposition. A slight increase in nanoparticle concentration within the biofilm was observed in correlation with flow rate. Note that the experimental results indicate that it is possible to deposit 10^9 nanoparticles per ml of biofilm. Later, we relate this quantity to the amount of drug delivered to the biofilm as well as to the efficacy of this amount. Since the relevant equations in the model are linear in particle concentration, we calculate the proportion of nanoparticle concentration in the biofilm to concentration in the bulk fluid. It is this metric that is used to guide our estimates of parameter values in the model. From the data in Table II, we estimate a value of 2.0×10^{-5} cm/s for the sticking coefficient used in the mathematical model. We discuss this further in the modeling section below.

To understand the spatial distribution of nanoparticles in the biofilm, we consider histograms of relative particle depth. Relative depth is defined as the ratio between a particle's vertical position and the vertical position of the fluid-biofilm interface at that location. Hence a relative depth of 0-5% corresponds to a particle that is near the biofilm substratum, while a relative depth of 95-100% corresponds to a particle that is near the fluid-biofilm interface. For some nanoparticles, a relative depth greater than 100% was measured, placing these nanoparticles above the fluid-biofilm interface. These data occur because the location of the interface is defined as the location above the substratum at which the fluorescent signal drops below a certain threshold. Hence there are still some bacteria above the interface. In the analysis of nanoparticle distribution through the biofilm depth that we present next, data on nanoparticles with a relative depth of greater than 100% are not reported.

Figure 6 shows a representative histogram of nanoparticle relative depth 0.5 to 2 hours after deposition. This figure represents data aggregated over 11 samples using a flow rate of 3 ml/hr and injection duration of 20 min. Particle depth is plotted on the horizontal axis with the left side representing the biofilm substratum and the right side representing the fluid-biofilm interface. This histogram suggests that, over the time period during which the positions are measured, most particles are located near the fluid-biofilm interface with an exponential distribution moving toward the bottom of the biofilm. Nanoparticle concentration was observed to be largely unaffected by local biofilm thickness, though fewer particles were observed in areas of minimal thickness. This is to be expected, as there is little biofilm for particles to interact with in these locations. No significant differences in nanoparticle distribution were observed for different flow rates or injection durations.

Figure 7 shows a similar representation of the nanoparticle distribution 2 to 4 hours after the deposition using the same flow rate and injection duration parameters. These data are aggregated over 15 samples. Unlike the distribution represented in Figure 6, this distribution appears more uniform. Taken together, our data suggests that nanoparticles diffuse to a uniform distribution through the biofilm within 2 to 4 hours after deposition. This leads to an estimate of the nanoparticle diffusion coefficient, $D_B = 1.0 \times 10^{-9}$ cm²/s. We note that this value is about half the diffusivity for nanoparticle transport in water. Hence, the pegylated structure of the nanoparticles allows for diffusive transport of the nanoparticles through the structure of the biofilm at a rate that is comparable in magnitude to nanoparticle transport through water. This is consistent with findings in the Hanes investigations for transport of pegylated particles through mucus [15, 48, 49, 51, 54]. We postulate that the reduction in diffusivity is due to hindrances by and adhesion to biofilm components. Finally, for the four hour period during which we observed the biofilm in the flow cell, we did not see any noticeable leakage of nanoparticles out of the biofilm. This supports the use of the no-flux component in (5) during the time period after the nanoparticles have been convected downstream.

These experimental results quantify the extent to which LTP nanoparticles stick to the biofilm surface, penetrate the biofilm, and then distribute throughout the depth of the biofilm. The nanoparticles adhere to the surface sufficiently well to attain a concentration of nanoparticles within the biofilm that is 2 orders of magnitude higher than concentration of nanoparticles in the bulk fluid. In addition, at times soon after the nanoparticle deposition, the distribution of nanoparticles shows an exponential decay as a function of distance from the fluid-biofilm interface. This observation supports the assumption that diffusion is the dominant transport mechanism for nanoparticles within the biofilm. Our data suggest that the nanoparticles are distributed more uniformly through the thickness of the biofilm in as little as 2 to 4 hrs after nanoparticle deposition.

B. Simulation Results

We use numerical simulations to characterize the distribution of nanoparticles through the biofilm as several parameters in the model are varied. The flow rate Q can be controlled, and the mass diffusivity in the carrier fluid, D , is well known. However, the mass diffusivity in the biofilm, D_B and the sticking coefficient, S_c , are not known; the experimental results are used to calibrate values for those parameters. These calibrated values provide a default case, described in Table III, that is used as the basis for a parametric study. Because the system of equations underlying the simulations is linear, we do not define the input concentration A in the presentation of the results. Rather, all results are presented as the ratio of nanoparticle concentration in the biofilm to nanoparticle concentration in the bulk fluid. For this relative concentration, the experimental results in Table II indicate final values of 100 to 900, and the numerical simulations predict relative concentrations between 300 and 900. The experiments suggest that diffusive equilibrium takes 120 to 240 minutes, while the simulations typically predict equilibrium in 70 to 100 minutes.

Default Parameters—To match the experimental setup, the height d and length L of the flow cell are set at 4 cm and 0.1 cm, respectively. For the biofilms grown in the experiments, the dependence of the height on position is irregular; the height averages 10 microns near the inlet, decreasing to about 5 microns near the outlet, with peaks up to 30 microns. The function

$$h(x) = 0.015(1 - x/2) + 0.01(1 - x/4)\sin(50\pi x) \quad (41)$$

is used to model the fluid-biofilm interface. The expression $h(x)$ for the fluid-biofilm interface is chosen as a simple, mathematically convenient model that captures two features of our biofilm morphology, namely, that the thickness of the biofilm varies significantly relative to the average height of the biofilm and that the average thickness of the biofilm decreases from the inlet to the outlet of the flow cell. In particular, the constants in $h(x)$ were picked based on our observations of the general features of the biofilms we grew and were not fit from data. A 30 minute injection duration is used in all the simulations. The flow rate Q was set at $5 \times 10^{-4} \text{ cm}^3/\text{s} = 1.8 \text{ ml/hr}$, which is consistent with a subset of the experiments.

The model is linear with respect to the sticking coefficient S_c and the mass diffusivity in the biofilm D_B , so values for these parameters can be calibrated sequentially. First, the default value of the sticking coefficient, $S_c = 2 \times 10^{-5} \text{ cm/s}$, was obtained by requiring that roughly half of the injection concentration in the fluid be absorbed by the biofilm by the time the input plug reached the outlet at $x = L$. The default value of the diffusivity in the biofilm, $D_B = 1 \times 10^{-9} \text{ cm}^2/\text{s}$, was then obtained by requiring that the final relative concentration be about 500.

Parameter Study—Holding the flow rate Q fixed at $5 \times 10^{-4} \text{ cm}^3/\text{s}$ and varying S_c and D_B from their default values by $\pm 20\%$ shows that the dependence of the final relative concentration on those parameters is nearly linear. Table IV summarizes the results. Here relative concentration is defined as the nanoparticle concentration averaged over the 40 mm extent of the biofilm in the x -direction. As discussed in the approximation given by (40), an exponential decrease in deposition is predicted along the lengthwise extent of the biofilm. For the parameters chosen in the numerical simulations, this results in about a 50% reduction in nanoparticle concentration over the 40 mm length of the biofilm.

The dependence of the final relative concentration on the sticking coefficient S_c is nearly linear, as seen in Figure 8. For these results, the value of S_c is varied with the other parameters held at their default values.

As seen in Figure 9, the final relative concentration is also nearly linear in D_B , the mass diffusivity in the biofilm. Here, the value of D_B is varied while the other parameters are held at their default values.

The effect of the flow rate Q on the final relative concentration is described in Figure 10. As noted above where (40) was introduced, the inlet concentration is convected further downstream as the flow rate Q increases. This means that more nanoparticles flow over the biofilm surface for larger flow rates. As a consequence, the flux of nanoparticles into the biofilm increases per (30), which leads to an increase in nanoparticle concentration in the biofilm as shown in Figure 10. Ultimately a plateau in concentration is approached as predicted by the limiting behavior of the exponential term in (40) as Q increases.

Overall, the final relative concentrations computed by the model lie within the range seen in the experiments, indicating that the model can be used in a predictive manner.

IV. DISCUSSION

The effectiveness of an antimicrobial is often described by the minimum inhibitory concentration (MIC) of the antimicrobial needed to stop the growth of the biofilm. If one assumes the MIC of a drug, C_{MIC} , is known, then one question to be answered is how many nanoparticles need to be deposited into the biofilm in order to achieve a concentration C_{MIC} in the lung biofilm after M days of drug release from the nanoparticles? We developed a simple approximation to answer this question for nanoparticle-delivered medication to the pulmonary region [34]. These results predicted that about 10^9 nanoparticles per ml of biofilm are sufficient to achieve an MIC consistent with experiments [38]. Likewise, in a more sophisticated model of biofilm growth and decay [46] we predicted that similar quantities of nanoparticles are needed to kill the biofilm. Our experimental results (see Table II) and the model in this manuscript demonstrate the feasibility of depositing 10^9 nanoparticles per ml into the biofilm.

In our experiments, $3 \mu\text{m}$ sized particles travel at an average velocity of 75 cm/hr, or $2 \cdot 10^{-4} \text{ m/s}$, within the flow-cell with volumetric flow rate 3 ml/hr. Particles of this size are expected to deposit in the lower regions of the lung, generations 15 to 20 [2]. Within the lung, particles deposit onto the mucus-liquid lining of the lung. Deposited particles are then pulled downward by gravity [16]. This resulting velocity due to settling may cause particles to contact the lung epithelial layer or supposed biofilms present in the lung. Frijlink and De Boer place the particle velocity by air transport in generations 15 to 20 between 0.1 and 0.01 m/s, respectively, and sedimentation velocity around $1 \cdot 10^{-4} \text{ m/s}$ [16]. Therefore, our model of particle deposition may be a reasonable approximation of the sedimentation process, in which particles travel in a liquid environment at a flow rate of $1 \cdot 10^{-4} \text{ m/s}$. While we

envision our experimental setup as a model of the lung, it was necessary to deviate from the in vivo system in certain aspects. The mucin concentration used in our experiments, in particular, is far lower than that of the lung. This variation is necessary to operate the flow cell appropriately. An injection time of 10 to 30 min represents the approximate amount of time a patient can be expected to use a nebulizer (C. Cannon, professional experience).

The basic goal of our long term research effort is to develop an effective drug delivery system and treatment strategy to kill bacteria growing in biofilms in the lung. By developing an improved understanding of biofilm structure and the efficacy of different treatments, our investigations will contribute to the more general problem of controlling harmful biofilms. This manuscript focuses on the basic challenge of designing a drug delivery system to deliver antimicrobial (in our case silver carbene complexes) to the pulmonary region of the lung. We present experiments and a simple mathematical model to determine the deposition of biodegradable polymer nanoparticles onto a biofilm. We demonstrate that it is possible to deposit concentrations of nanoparticles that are needed to achieve a desired MIC level within the biofilm.

We derive a two dimensional model of nanoparticle transport in a flow cell, adhesion to a biofilm's surface and the subsequent transport into a biofilm. The governing equations are simplified by the following assumptions to produce a system of coupled equations which we solve numerically:

1. The carrier fluid is a well mixed dilute concentration of nanoparticles.
2. Fluid flow is parabolic, laminar, and steady state.
3. No flux and no slip boundary conditions are imposed at flow cell boundaries.
4. Nanoparticle adhesion is the main mode of capture at a biofilm surface.
5. Diffusion is the dominant form of particle transport in the biofilm.

The nanoparticles are assumed to remain in the biofilm after capture, and we show they diffuse to a uniform concentration after the flow plug has passed. System parameters are varied. The flow rate Q alters the profile of the concentration plug traveling through the flow cell, but large changes in nanoparticle concentrations in the biofilm are not observed as a result of an increased flow rate, for the low (ml/hour) flow rates considered. The sticking coefficient S_c parameter limits the flux of nanoparticles passing through the fluid/biofilm interface. As the sticking coefficient is increased, a large increase in nanoparticle concentrations in the biofilm is observed. The model can also be easily generalized to describe a more accurate surface morphology of a growing biofilm.

The simple model presented here makes use of these many assumptions to balance the need for accuracy of delivery predictions and the desire to make the model computationally efficient. Our future models will relax these restrictions, to achieve more realistic results, and will consider combination therapies that have been shown to be effective [41]. Alternatively, a single dose of nanoparticles with multiple erosion rates may suffice. Further, we note that while nanoparticles may contain a sufficient amount of total antimicrobial to raise the concentration above the MIC level, these doses may not lead to biofilm decay because of the slow release of the antimicrobial from each nanoparticle. Hence, the biofilm continues to grow. This results in a decrease in the concentration level due to the diffusion of the antimicrobial over a larger volume, and so the concentration remains below the MIC. Making such predictions and including multiple pharmacokinetic options entail developing a much more comprehensive model, which is in progress.

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References

- [1]. Abramoff M, Magelhaes P, Ram S. J Biophotonics. 2004; 11:36.
- [2]. Asgharian B, Hofmann W, Bergmann R. Aerosol Science and Technology. 2001; 34(4):332. URL <http://www.tandfonline.com/doi/abs/10.1080/02786820119122>.
- [3]. Bolte S, Cordelières F. J Microscopy. 2006; 224:213.
- [4]. Bouwer EJ. Water Research. 1987; 21(12):1489. ISSN 0043-1354, URL <http://www.sciencedirect.com/science/article/pii/0043135487901321>.
- [5]. Cogan N, Keener J. Mathematical Medicine and Biology-A Journal of the IMA. 2004; 21(2):147. [PubMed: 15228104]
- [6]. Costerton J, Stewart S, Greenberg E. Science. 1999; 284(5418):1318. [PubMed: 10334980]
- [7]. Dailey L, Kleemann E, Wittmar M, Gessler T, Schmehl T, Roberts C, Seeger W, Kissel T. Pharmaceutical Research. 2003; 20:2011. [PubMed: 14725368]
- [8]. Dailey L, Schmehl T, Gessler T, Wittmar M, Grimminger F, Seeger W, Kissel T. Journal of Controlled Release. 2003; 86:131. [PubMed: 12490379]
- [9]. Desai T, Hancock R, Finlay W. Journal of Controlled Release. 2002; 84:69. [PubMed: 12399169]
- [10]. Ditto, A. DNA-LPEI Complexes Encapsulation in LTP nanospheres as a non-viral gene therapy vector. Biomedical Engineering, The University of Akron; Akron: 2006. Technical Report2006
- [11]. Ditto AJ, Shah PN, Lopina ST, Yun YH. International Journal of Pharmaceutics. 2009; 368(1-2): 199. URL <http://www.ncbi.nlm.nih.gov/pubmed/19026734>. [PubMed: 19026734]
- [12]. Drury W, Stewart P, Characklis W. Biotechnology and Bioengineering. 1993; 42(1):111. ISSN 1097-0290, URL <http://dx.doi.org/10.1002/bit.260420115>. [PubMed: 18609654]
- [13]. Drury WJ, Characklis WG, Stewart PS. Water Research. 1993; 27(7):1119. ISSN 0043-1354, URL <http://www.sciencedirect.com/science/article/pii/004313549390003Z>.
- [14]. Emami J, Hamishehkar H, Najafabadi AR, Gilani K, Minaian M, Mahdavi H, Mirzadeh H, Fakhari A, Nokhodchi A. Journal of Microencapsulation. 2009; 26(1):1. URL <http://informahealthcare.com/doi/abs/10.1080/02652040802083900>. [PubMed: 18465287]
- [15]. Ensign LM, Cone R, Hanes J. Advanced Drug Delivery Reviews. 2012; 64(6):557. ISSN 0169-409X, ;ce:title;Advances in Oral Drug Delivery: Improved Bioavailability of Poorly Absorbed Drugs by Tissue and Cellular Optimization;ce:title;, URL <http://www.sciencedirect.com/science/article/pii/S0169409X11003036>. [PubMed: 22212900]
- [16]. Frijlink HW, De Boer AH. Expert Opinion On Drug Delivery. 2004; 1(1):67. URL <http://www.ncbi.nlm.nih.gov/pubmed/16296721>. [PubMed: 16296721]
- [17]. Gibson R, Burns J, Ramsey B. Am J Respir Crit Care Med. 2003; 168(8):918. [PubMed: 14555458]
- [18]. Griesenbach U, Ferrari S, Geddes D, Alton E. Gene Therapy. 2002; 9:1344. [PubMed: 12364999]
- [19]. Gupta AS, Lopina ST. Journal of Biomaterials Science (Polymer Edition). 2002; 13(10):1093. URL <http://www.ingentaconnect.com/content/vsp/bsp/2002/00000013/00000010/art00003>.
- [20]. Hall-Stoodley L, Costerton JW, Stoodley P. Nat Rev Micro. 2004; 2(2):95. ISSN 1740-1526, URL <http://dx.doi.org/10.1038/nrmicro821>.
- [21]. Heyder J. Proceedings of the American Thoracic Society. 2004; 1(4):315. URL <http://pats.atsjournals.org/content/1/4/315.abstract>. [PubMed: 16113452]
- [22]. Heydorn A, Ersbøll B, Hentzer M, Parsek M, Givskov M, Molin S. Microbiology. 2000; 146:2409. [PubMed: 11021917]
- [23]. Heydorn A, Nielsen A, Hentzer M, Sternberg C, Givskov M, Ersbøll B, Molin S. Microbiology. 2000; 146:2395. [PubMed: 11021916]

- [24]. Hindi K, Ditto A, Panzner M, Medvetz D, Hovis C, Han D, Hilliard J, Taylor J, Yun Y, Cannon C, Youngs W. *Biomaterials*. 2009; 30:3771. [PubMed: 19395021]
- [25]. Jackson K, Keyser R, Wozniak D. *Semin Respir Crit Care Med*. 2003; 24(6):663. [PubMed: 16088582]
- [26]. Katz S, Adatia I, Louca E, Leung K, Humpl T, Reyes J, Coates A. *Pediatric Pulmonology*. 2006; 41:666. [PubMed: 16703580]
- [27]. Kleemann E, Dailey L, Abdelhady H, Gessler T, Schmehl T, Roberts C, Davies M, Seeger W, Kissel T. *Journal of Controlled Release*. 2004; 100:437. [PubMed: 15567508]
- [28]. Koping-Hoggard M, Issa M, Kohler T, Varum K, Artursson P. *J. Gene. Med.* 2005; 7(9):1215. [PubMed: 15895386]
- [29]. Lambiasi A, Raia V, Pezzo M, Sepe A, Carnovale V, Rossano F. *BMC Infectious Diseases*. 2006; 6(1):4. URL <http://journals.ohiolink.edu/ejc/article.cgi?issn=14712334&issue=v06i0001&article=4+moadiacopwcf&search+term=%28Microbiology+of+airway+disease+in+patients+with+cystic+fibrosis%29>. [PubMed: 16405721]
- [30]. Leid JG, Ditto AJ, Knapp A, Shah PN, Wright BD, Blust R, Christensen L, Clemons CB, Wilber JP, Young GW, Kang AG, Panzner MJ, et al. *Journal of Antimicrobial Chemotherapy*. 2011 <http://jac.oxfordjournals.org/content/early/2011/10/03/jac.dkr408.full.pdf+html>. URL <http://jac.oxfordjournals.org/content/early/2011/10/03/jac.dkr408.abstract>.
- [31]. Li Z, Kleinstreuer C, Zhang Z. *Journal of Aerosol Science*. 2007; 38(6):625. ISSN 0021-8502, URL <http://www.sciencedirect.com/science/article/pii/S002185020700050X>.
- [32]. Longest P, Oldham M. *Journal of Aerosol Science*. 2008; 39(1):48.
- [33]. Longest PW, Vinchurkar S, Martonen T. *Journal of Aerosol Science*. 2006; 37(10):1234. ISSN 0021-8502, URL <http://www.sciencedirect.com/science/article/pii/S0021850206000231>.
- [34]. Nassar, D.; Stine, A.; Clemons, C.; Miller, K.; Wilber, J.; Young, G.; Deblock, M.; Panzner, M.; Youngs, W.; Ditto, A.; Yun, Y.; Milsted, A., et al. 2012. In Preparation
- [35]. Neubig, R. Masters Thesis. The University of Akron; 2011.
- [36]. Palmer K, Aye L, Whiteley M. *J Bacteriol*. 2007; 189:8079. [PubMed: 17873029]
- [37]. Pandey R, Sharma A, Zahoor A, Sharma S, Khuller G, Prasad B. *Journal of Antimicrobial Chemotherapy*. 2003; 52:981. [PubMed: 14613962]
- [38]. Panzner M, Deeraksa A, Smith A, Wright B, Hindi K, Kascatan-Nebioglu A, Torres A, Judy B, Hovis C, Hilliard J, Mallett R, Cope E, et al. *Eur J Inorg Chem*. 2009; 13:1739. [PubMed: 20160993]
- [39]. Ramsey B. *New England Journal of Medicine*. 1996; 335(15):1167.
- [40]. Rudolph C, Muller R, Rosenecker J. *The Journal of Gene Medicine*. 2002; 4:66. [PubMed: 11828389]
- [41]. Rybak M. *American Journal of Infection Control*. 2006; 34(5):S38. [PubMed: 16813981]
- [42]. Searcy K, Packman A, Atwill E, Harter T. *Appl. Environ. Microbiol*. 2006; 72(9):6242. [PubMed: 16957251]
- [43]. Sen Gupta A, Lopina S. *Polymer*. 2005; 46:2133.
- [44]. Soppimath K, Aminabhavi T, Kulkarni A, Rudzinski W. *Journal of Controlled Release*. 2001; 70:1. [PubMed: 11166403]
- [45]. Stewart P, Costerton J. *Lancet*. 2001; 358:135. [PubMed: 11463434]
- [46]. Stine, A.; Nassar, D.; Clemons, C.; Miller, K.; Wilber, J.; Young, G.; Youngs, W.; Yun, Y.; Milsted, A.; Leid, J.; Cannon, C. 2012. In Preparation
- [47]. Sufya N, Allsion D, Gilbert P. *J Appl Microbiol*. 2003; 95:1261. [PubMed: 14633000]
- [48]. Suh J, Choy K, Lai S, Suk J, Tang B, Prabhu S, Hanes J. *International Journal of Nanomedicine*. 2007; 2(4):735. [PubMed: 18203439]
- [49]. Suk J, Lai S, Wang Y, Ensign L, Zeitlin P, Boyle M, Hanes J. *Biomaterials*. 2009; 30(13):2591. ISSN 0142-9612, URL <http://www.sciencedirect.com/science/article/B6TWB-4VG5P9S-4/2/783eaa908e8fd29ba9c77f714719f3e6>. [PubMed: 19176245]
- [50]. Szomolay B, Klapper I, Dockery J, Stewart P. *Environmental Microbiology*. 2005; 7(8):1186. [PubMed: 16011755]

- [51]. Tang B, Dawson M, Lai S, Wang Y, Suk J, Yang M, Zeitlin P, Boyle M, Fu J, Hanes J. Proceedings of the National Academy of Sciences. 2009; 106(46):19268. URL <http://www.pnas.org/content/106/46/19268.abstract>.
- [52]. Tolker-Nielsen T, Sternberg C. Chapter 1. Curr Protoc Microbiol. 2011 Unit 1B.2.
- [53]. Vadolas J, Williamson R, Ioannou P. Pulm. Pharmacol. Ther. 2002; 15:61. [PubMed: 11969364]
- [54]. Wang Y, Lai S, Suk J, Pace A, Cone R, Hanes J. Angewandte Chemie International Edition. 2008; 47(50):9726. ISSN 1521-3773, URL <http://dx.doi.org/10.1002/anie.200803526>.
- [55]. Wloka M, Rehage H, Flemming H-C, Wingender J. Biofilms. 2005; 2(4):275. ISSN 1479-0505, URL <http://journals.ohiolink.edu/ejc/article.cgi?issn=14790505&issue=v02i0004&article=275sarbotnompab>.
- [56]. Wong J, Yang H, Blasetti K, Schnell G, Conley J, Schofield L. Journal of Controlled Release. 2003; 92:265. [PubMed: 14568408]
- [57]. Xi J, Longest P. Annals of Biomedical Engineering. 2007; 35(4):560. URL <http://www.ingentaconnect.com/content/klu/abme/2007/00000035/00000004/00009245>. [PubMed: 17237991]
- [58]. Yang L, Barken K, Skindersoe M, Christensen A, Givskov M, Tolker-Nielsen T. Microbiology. 2007; 153:1318. [PubMed: 17464046]
- [59]. Yoon S, Hennigan R, Hilliard G, Ochsner U, Parvatiyar K, Kamani M, Allen H, DeKievit T, Gardner P, Schwab U, Rowe J, Iglewski B, et al. Developmental Cell. 2002; 3(4):593. ISSN 1534-5807, URL <http://www.sciencedirect.com/science/article/B6WW3-473W0CW-K/2/ed6166c0cc48f5c1807d634af67f2b7a>. [PubMed: 12408810]
- [60]. Youngs W, Tessier C, Garrison J, Quezada C, Melaiye A, Durmus S, Panzner M, Kascatan-Nebioglu A, Sessler J, Lippard S. Medicinal Inorganic Chemistry. 2005; 903:414.
- [61]. Yun YH, Jiang H, Chan R, Chen W. Journal of Biomaterials Science (Polymer Edition). 2005; 16(11):1359. URL <http://www.ingentaconnect.com/content/vsp/bsp/2005/00000016/00000011/art00003>.
- [62]. Zacarias G, Ferreira C, Velasco-Hernandez J. Journal of Theoretical Biology. 2005; 233:245. [PubMed: 15619364]
- [63]. Zhang T, Cogan N, Wang Q. SIAM Journal on Applied Mathematics. 2008; 69(3):641. URL <http://epubs.siam.org/doi/abs/10.1137/070691966>.

FIG. 1.

Flow cell geometry. The height of the biofilm $\tilde{h}$ is a known function of horizontal position $\tilde{x}$. The length of the flow cell is $L = 4.0$ cm and the distance d between the top and bottom plates is 0.1 cm.

FIG. 2.
SEM image of Blank LTP nanoparticles.

FIG. 3.
Distribution of nanoparticle sizes based on dynamic light scattering.

FIG. 4. Rhodamine-labeled LTP nanoparticles (red) embedded within *P. aeruginosa* PAO1 biofilm expressing GFP (green). Arrow indicates the position of one of the nanoparticles.

FIG. 5.

Three-dimensional reconstruction of *P. aeruginosa* PAO1 biofilm expressing GFP from confocal microscope data. Cells appear green, rhodamine-labeled LTP nanoparticles appear as red points. Arrows indicates the positions of two of the nanoparticles.

FIG. 6.
LTP nanoparticle distribution between 0.5 and 2 hrs after the deposition of nanoparticles.

FIG. 7.
LTP nanoparticle distribution between 2 and 4 hrs after the deposition of nanoparticles.

FIG. 8. The dependence of the final relative concentration (nanoparticle concentration in the biofilm/nanoparticle concentration in the bulk fluid) on the sticking coefficient S_C , with other parameters held at default values.

FIG. 9.

The dependence of the final relative concentration (the ratio of nanoparticle concentration in the biofilm to nanoparticle concentration in the bulk fluid) on the mass diffusivity in the biofilm D_B , with other parameters held at default values.

FIG. 10.

The dependence of the final relative concentration (ratio of nanoparticle concentration in the biofilm to nanoparticle concentration in the bulk fluid) on the flow rate Q , with other parameters held at default values.

TABLE I

System variables, characteristic scalings and their units

Description	Variable	Rescaling	Units
horizontal coordinate	$\tilde{x}$	L	cm
vertical coordinate	$\tilde{z}$	d	cm
biofilm height	$\tilde{h}$	d	cm
time	$\tilde{t}$	$\frac{d^2}{D_B}$	s
x-velocity	U	$\frac{Q}{d}$	$\frac{\text{cm}}{\text{s}}$
z-velocity	W	$\epsilon \frac{Q}{d}$	$\frac{\text{cm}}{\text{s}}$
particle concentration, biofilm	$\tilde{C}_B$	A	$\frac{\text{g}}{\text{cm}^2}$
particle concentration, fluid	$\tilde{C}$	A	$\frac{\text{g}}{\text{cm}^3}$

TABLE II
Concentration of LTP nanoparticles observed in bulk fluid and biofilm under varying flow rates and injection durations

Flow Rate (ml/hr)	Duration (min)	Bulk Fluid Mean (# particles/ml)	Bulk Fluid Std Dev	Biofilm Mean (# particles/ml)	Biofilm Std Dev	Proportion Biofilm/Bulk concentrations
1.5	10	8.08E6	2.27E6	1.86E9	1.97E9	230.5
1.5	20	5.64E6	2.33E6	9.65E8	1.05E9	171.2
1.5	30	6.91E6	1.22E6	2.65E9	4.49E8	384.2
3	10	9.12E6	3.61E6	1.66E9	7.21E8	182.5
3	20	6.90E6	3.23E6	6.35E9	9.78E8	919.5
3	30	4.42E6	5.78E5	2.41E9	9.77E8	545.1
6	10	5.93E6	2.66E6	3.46E9	2.69E9	583.3
6	20	4.90E6	7.77E5	4.52E9	2.39E8	921.5
6	30	5.26E6	1.53E5	2.63E9	1.48E9	499.5

TABLE III

Default parameter values

Description	Parameter	Value	Units
Flow Rate	Q	5.0×10^{-4}	$\frac{\text{cm}^3}{\text{s}}$
Mass Diffusivity in Fluid	D	2.0×10^{-9}	$\frac{\text{cm}^2}{\text{s}}$
Mass Diffusivity in Biofilm	D_B	1.0×10^{-9}	$\frac{\text{cm}^2}{\text{s}}$
Sticking Coefficient	S_c	2.0×10^{-5}	$\frac{\text{cm}}{\text{s}}$
Length of the Flow Cell	L	4.0	cm
Height of the Flow Cell	d	0.1	cm

TABLE IV

Relation between final relative concentration, mass diffusivity in the biofilm, and sticking coefficient. Table entries show the final relative concentration as D_B and S_c are varied together with percentage changes compared to the baseline result.

S_c cm/s	0.8×10^{-9} (−20%)	D_B cm ² /s 1.0×10^{-9}	1.2×10^{-9} (+20%)
1.6×10^{-5} (−20%)	391 (−35%)	489 (−19%)	587 (−2%)
2.0×10^{-5}	481 (−20%)	602 (baseline)	722 (+20%)
2.4×10^{-5} (+20%)	569 (−5%)	711 (+18%)	853 (+42%)