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Modeling the Response of a Biofilm to Silver-Based Antimicrobial

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Abstract

Biofilms are found within the lungs of patients with chronic pulmonary infections, in particular patients with cystic fibrosis, and are the major cause of morbidity and mortality for these patients. The work presented here is part of a large interdisciplinary effort to develop an effective drug delivery system and treatment strategy to kill biofilms growing in the lung. The treatment strategy exploits silver-based antimicrobials, in particular, silver carbene complexes (SCC). This manuscript presents a mathematical model describing the growth of a biofilm and predicts the response of a biofilm to several basic treatment strategies. The continuum model is composed of a set of reaction-diffusion equations for the transport of soluble components (nutrient and antimicrobial), coupled to a set of reaction-advection equations for the particulate components (living, inert, and persister bacteria, extracellular polymeric substance, and void). We explore the efficacy of delivering SCC both in an aqueous solution and in biodegradable polymer nanoparticles. Minimum bactericidal concentration (MBC) levels of antimicrobial in both free and nanoparticle-encapsulated forms are estimated. Antimicrobial treatment demonstrates a biphasic killing phenomenon, where the active bacterial population is killed quickly followed by a slower killing rate, which indicates the presence of a persister population. Finally, our results suggest that a biofilm with a ready supply of nutrient throughout its depth has fewer persister bacteria and hence may be easier to treat than one with less nutrient.

Keywords

biofilm modeling; antimicrobial; silver; nanoparticle; drug delivery

1. Introduction

A biofilm is a community of microorganisms attached to a surface and embedded in a matrix of proteins, nucleic acids, and polysaccharides referred to as extracellular polymeric substance (EPS). Biofilms form readily in both natural and man-made environments [1]. In the latter setting, the occurrence of biofilms often has economic significance. For example, biofilms can foul the hulls of ships, contaminate food processing equipment, or increase the efficiency of waste treatment facilities. In natural environments, of great concern are biofilms responsible for human infections such as chronic sinus infections and infections on medical implants. In these settings, biofilms exhibit heightened antibiotic tolerance. Factors that determine the tolerance of bacteria in a biofilm to antimicrobials include the thickness of the biofilm, the heterogeneity of the structure of the biofilm through its thickness, and variation in the metabolic and reproductive processes of constituent bacteria within the biofilm [2, 3, 4]. This paper is motivated by the growth of biofilms in the lungs of patients with cystic fibrosis (CF).

Cystic fibrosis (CF) is an autosomal recessive disease, afflicting over 30,000 persons in the United States [5]. The disorder is associated with defective ion transport across the lung epithelial layer, resulting in dehydration of the lung mucus and a subsequent breakdown of mucociliary clearance mechanisms. Build up of mucus provides a nutrient rich site for bacterial infection, the leading cause of death in patients with CF [6]. General treatment strategies consist of therapeutics to improve mucus transport, reduce inflammation and remove extracellular DNA in the mucus, and antimicrobials to kill bacteria [7, 8, 9, 10].

An extremely active research agenda seeks to develop new drugs to treat CF, including new antimicrobials, anti-inflammatory agents, mucus modifiers, and ion and water transport modulators. The efficacy of these inhaled therapies hinges upon delivering agents to targeted cells. These targeted cells are often located in the small, terminal airways of the lung, which are difficult to reach through the mucus-plugged, inflamed airways typical of the CF lung. A promising approach to alleviate this critical treatment barrier is the development of engineered nano-size particles to encapsulate and deliver agents.

The work presented here is part of a larger interdisciplinary effort to develop a safe and effective system for delivering antimicrobials to the pulmonary region of the lung. The delivery system is based upon nebulization, the process of aerosolizing therapeutic agents for delivery by inhalation to the respiratory tract. The treatment strategy exploits silver-based antimicrobials, in particular, silver carbene complexes (SCC) [11]. SCC have proved highly effective in treating biofilms [12, 13, 14]. SCC can be mixed in aqueous solution and nebulized. Or SCC can first be embedded in biodegradable polymer nanoparticles, which can then be nebulized. Agents delivered to the biofilm in soluble form enter the biofilm through its surface. Some research suggests that the polymer nanoparticles can enter the biofilm through its cracks and pores, penetrate more deeply into the biofilm, and then dissolve and slowly release the agents over time [15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25].

We present a mathematical model of the growth and treatment of a biofilm. A comprehensive mathematical model of a drug delivery system based on nebulization of SCC should describe (i) the transport of SCC, nebulized either in aqueous solution or in nanoparticles, through the lung airways, (ii) the penetration of nanoparticles into the biofilm, (iii) the erosion of nanoparticles and the subsequent release of agents, (iv) the reactive-diffusive transport of SCC within the biofilm, and (v) the growth or decay of the biofilm in response to antimicrobial treatment. We note that the modeling presented later in this paper and in our other work [26] is an initial effort to address only items (iii)–(v) in an idealized

flow cell setting, rather than a biofilm embedded in mucus. We have partially addressed items (i) and (ii) in the context of biofilms grown in a flow cell setup in [27].

The amount of SCC that must be delivered to the biofilm is the amount necessary to maintain a concentration of antimicrobial above the minimum bactericidal concentration (MBC) for a period of time sufficient to kill the biofilm. (MBC is the minimum concentration of antimicrobial that decreases the population of living bacteria by 99% within one day [28].) The current work estimates the concentration of nanoparticles needed to achieve the MBC.

The model we develop below incorporates the growth and response of the biofilm to treatment and can be used to study treatment strategies based on either aqueous SCC or SCC embedded in nanoparticles. A typical dosing strategy would use nebulization to deliver a number of nanoparticles to the biofilm surface, pause while these particles penetrate into the biofilm, and then deliver additional nanoparticles to the surface. Presently, it is not known whether aqueous antimicrobial, nanoparticle-encapsulated antimicrobial, or some combination of the two is most effective. Determining which combination of these two delivery methods would be most effective by purely empirical means is a difficult task.

Our general model is formulated to examine combined soluble and nanoparticle dosing strategies. In this paper, we examine each antimicrobial strategy in isolation from the other in order to understand basic features of the drug delivery scheme and the response of the biofilm to those features. Dosing strategies based on soluble SCC in aqueous solution are incorporated into the model via a boundary condition at the interface between the top of the biofilm and the interior of the flow cell. We consider this case to be representative of applying a topical treatment to a biofilm [29] or of simulating delivery of antimicrobial in aqueous form to a lung biofilm via nebulization. Dosing strategies based on SCC embedded in nanoparticles lead to an additional source term in the transport equations.

2. Governing Equations

2.1. Overview of the Model

We describe a one-dimensional continuum model of a portion of a biofilm growing in a flow cell. The basis of our model is the multispecies biofilm model presented in [30, 31]. The biofilm is attached to a substratum that represents the cell wall. Our one-dimensional model describes a typical line within the biofilm perpendicular to the substratum. Consistent with the one-dimensional approximation, the biofilm has lateral extent L that is large compared to its thickness H . As has been previously recognized for some biofilm configurations, one-dimensional approximations may provide reasonable insight into biofilm behavior because of the small rates of change in planes parallel to the substratum compared to the direction perpendicular to this surface [32, 33].

We introduce coordinates so that $z = 0$ corresponds to the substratum. We let $z = H(t)$ represent the height of the biofilm at time t . Hence at time t the biofilm occupies the region $\{z: 0 \leq z \leq H(t)\}$. The space above the biofilm is occupied by the liquid media of the flow cell. This space and its constituents (source of the soluble nutrient and antimicrobial components) is only accounted for in the model through boundary conditions at $z = H(t)$.

The model consists of a set of reaction-advection equations for four particulate components in the biofilm coupled to a set of reaction-diffusion-advection equations for two soluble components in the biofilm. These equations are coupled to an equation for the advective velocity within the biofilm and an evolution equation for the height of the biofilm.

The four particulate components are living bacteria, inert bacteria, persister bacteria, and EPS (the extracellular polymers). Living bacteria reproduce, die, are killed by antimicrobial, and convert to and from persister bacteria. Inert bacteria are bacteria that have died but, because they are embedded in EPS, remain in the biofilm. In our model, inert bacteria increase by the death of living bacteria. Persister bacteria are living bacteria that, because they express certain genes, have switched into a low-metabolizing state. As a consequence, persisters are highly tolerant of antimicrobial, which many believe explains the high level of tolerance to antimicrobial displayed by biofilms [34, 35, 36]. There is some evidence that the rate at which living cells convert to persisters depends on the availability of nutrient and on the presence of antimicrobial [37, 38]. In our model, the rate at which living bacteria convert to persisters depends on the nutrient level—the rate of conversion is higher as less nutrient is available. Persisters switch back to living bacteria at a constant rate and constitute a small portion of the bacteria population. For example, in planktonic cultures of *P. aeruginosa*, persister cells make up approximately 0.1% of the population. This proportion increases to 1% upon quorum sensing activation [39]. Our treatment of persister bacteria is similar to that in [40]. We assume EPS is secreted by living bacteria at a rate proportional to a Monod expression times the concentration of living bacteria. The role of EPS in biofilms was modeled in [41] and has been described in other models such as [42, 43, 44, 45, 46].

The particulate components are transported within the biofilm by advection. The advective velocity is generated by the growth and decay of the particulate components.

Any region within the biofilm not occupied by the four particulate components is described as void. We refer to the volume fraction of void space as the porosity of the biofilm. Thus in our model the sum of the volume fractions of the particulate components and the porosity is 1 at every point in the biofilm.

The model tracks two soluble components, nutrient and antimicrobial. Nutrient is necessary for the production of living bacteria and EPS. Also, the concentration of nutrient decreases as it is consumed by living bacteria. Our treatment of nutrient as a soluble component that diffuses from the surface through the void is similar to that in [43]. The antimicrobial in our model describes the action of SCC. As mentioned above, our model allows for the addition of antimicrobial in free or nanoparticle-encapsulated form. Free antimicrobial has been included in several previous models, including [4, 40, 47, 48, 49]. Antimicrobial embedded in nanoparticles may be released either by slowly diffusing from within the nanoparticle or by the degradation of the nanoparticle itself. Our model assumes the latter and we apply an empirical model due to Hopfenberg for drug release by surface erosion of the polymer [50, 51]. To our knowledge, our biofilm model is the first to describe the release of antimicrobial embedded in nanoparticles.

The evolution of the height of the biofilm is determined by setting its velocity equal to the advective velocity in the biofilm evaluated at the top of the biofilm plus any detachment or attachment of particulate components [30]. The mechanism of detachment, which occurs when cells break free from the biofilm, appears to regulate the height. This height depends upon the kind of bacteria found in the biofilm. Biofilms composed of bacteria associated with CF typically grow in vitro to a height of somewhere between 100 μm and 300 μm [24, 52]. We assume that detachment occurs at a rate proportional to the square of the height of the biofilm, an approach proposed in [53]. This detachment term takes effect only after the biofilm has reached a critical height, similar to [54].

In the next several subsections, we present our model. Table 1 lists the variables used in this model and Table 2 lists the parameters used. In cases where measured values for model parameters are not available, we assumed values. For example we assumed nutrient

concentration and mass diffusivity values to be on the same order of magnitude as the corresponding measured values for antimicrobial. Some parameters, specifically the natural death rate of bacteria and parameters determining the rate of biofilm detachment, were tuned so that acceptable results were achieved.

In summary, biofilm growth and its treatment through antimicrobial in both aqueous solution as well as embedded in biodegradable nanoparticles are modelled under the following assumptions:

- Living bacteria, inert bacteria, persister bacteria, and EPS are tracked.
- Diffusion of antimicrobial is fast compared to its release from nanoparticles so that concentrations are spatially uniform through the thickness of the biofilm.
- Detachment rate of the biofilm is proportional to the height squared of the biofilm.

Under these assumptions we estimate the amount of antimicrobial necessary to achieve MBC. We assume no loss of the antimicrobial to formation of salts or leakage to the surrounding environment.

2.2. Equations for Soluble Components

We let S denote the concentration of nutrient in the void space. We assume only diffusive transport through and reactions in the biofilm. The governing equation is

$$\phi S_t = (\phi D_S S_z)_z - \mu_S \frac{S}{K_S + S} B. \quad (1)$$

Here ϕ denotes the biofilm porosity, D_S is the diffusivity of the nutrient, K_S is the Monod saturation constant for the nutrient, μ_S is a constant representing the rate at which the bacteria consumes the nutrient, and B is the density of living bacteria.

An impermeable boundary condition for S is used at $z = 0$, the substratum defining the bottom of the biofilm,

$$S_z = 0. \quad (2)$$

At $z = H(t)$, the top of the biofilm, we have

$$-\phi D_S S_z = k_{HS} (S - S_{\text{source}}). \quad (3)$$

In (3), the constant k_{HS} is a mass transfer coefficient and S_{source} is the prescribed concentration of nutrient. A boundary condition similar to (3) could be applied at the substratum, to describe the permeability of the lung wall. However, we choose the simpler impermeable condition, which is consistent with a flow cell. We note that in the lung environment there are multiple organic substrate sources in addition to oxygen. For simplicity we have chosen a single limiting substrate, say oxygen, that is delivered through the medium above the biofilm.

We let C denote the concentration of antimicrobial in the void space. Like the nutrient, antimicrobial is transported in the biofilm by diffusion. We assume that the concentration of antimicrobial is not significantly decreased by its interaction with the bacteria or any other ions, such as chloride anions. The transport and reactions of the antimicrobial are governed by

$$\phi C_t = (\phi D_C C_z)_z + \mathcal{R}. \quad (4)$$

For the second term on the right-hand side of (4), which describes the release of antimicrobial from the nanoparticles, we define

$$\mathcal{R}(z, t) = \begin{cases} 4\pi R^2 k \rho_{\text{nano}} \left[1 - \frac{kt}{C_0 R}\right]^2, & 0 \leq t \leq \frac{C_0 R}{k}, \\ 0, & t > \frac{C_0 R}{k}. \end{cases} \quad (5)$$

We assume that the number density of nanoparticles is the constant ρ_{nano} . Each nanoparticle is spherical with initial radius R and with a uniform initial concentration C_0 of antimicrobial.

Hence, the total mass of antimicrobial released by one nanoparticle is $M_{\text{inf}} = \frac{4}{3}\pi C_0 R^3$. The constant k describes erosion. Our expression for the rate of release of the antimicrobial from a nanoparticle is based on Hopfenberg [50]. The experimental work in [14] supports our use of this expression and the choice of numerical values for R , C_0 , and k . More sophisticated expressions for the release rate M have been developed [55].

The numerical value we use for ρ_{nano} is based on the experimental work in [27], which shows that densities of 10^9 nanoparticles per mL can be attained. Note that we do not model the transport of nanoparticles through the biofilm. (See, however, [27].) Rather, we assume that after delivery, the nanoparticles stick to and penetrate the biofilm interface, and immediately begin releasing antimicrobial. The released antimicrobial quickly diffuses uniformly through the volume of the biofilm. Further, the nanoparticles themselves distribute uniformly throughout the biofilm. Experiments suggest that nanoparticles become uniformly distributed through the biofilm within a few hours after delivery to the top of the biofilm [27]. On the other hand, the total degradation of the nanoparticles and the complete release of SCC take place over days.

The boundary condition for C at $z = 0$ is

$$C_z = 0. \quad (6)$$

The boundary condition for C at $z = H$ is

$$C = C_{\text{source}} \quad (7)$$

for aqueous delivery of antimicrobial or

$$C_z = 0 \quad (8)$$

for antimicrobial delivery through release from the nanoparticles. In (7), the constant C_{source} represents the concentration of antimicrobial in aqueous solution delivered to the surface of the biofilm. For simplicity we assume that antimicrobial readily penetrates the biofilm surface. Note that $\rho_{\text{nano}} = 0$ in (5) and $C_{\text{source}} = 0$ in (7) represents continuous delivery of aqueous antimicrobial to the biofilm surface, while $\rho_{\text{nano}} = 0$ and the no flux condition (8) represents delivery solely by nanoparticles. This manuscript only considers these two cases in order to identify features of each in isolation from the other. These two cases represent the extreme cases of no flux at all boundaries (for nanoparticles), or a constant supply of aqueous antimicrobial. We note that these conditions at the top and bottom of the biofilm describe a ‘best case’ scenario for the treatment strategy, because all aqueous antimicrobial or antimicrobial released within the biofilm from the nanoparticles remains in the biofilm. In [26] we examine a variety of potential dosing strategies combining each form of delivery and considering ‘completely leaky’ boundaries. In addition that work considers a two-dimensional continuum model for a biofilm with a spatially varying thickness, $H(x, t)$, of the biofilm. We can thereby use these models to predict lower and upper bounds on the amount

of antimicrobial that must be delivered to the biofilm to keep concentrations within the biofilm at or above the MBC for a given period of time.

We next describe a set of assumptions that will permit the use of straightforward analytical solutions of equation (4), by which we can then explore the two basic treatment strategies—one based on aqueous SCC and one based on SCC embedded in nanoparticles.

SCC Delivered in Aqueous Solution—To simulate the treatment of the biofilm by SCC delivered only in aqueous solution, we set $\rho_{nano} = 0$, which appears in $\mathcal{R}$ on the right-hand side of (4). This eliminates the source term describing the release of SCC from nanoparticles. Hence, we have the constant solution $C = C_{Source}$, so that there is a uniform concentration of antimicrobial within the biofilm that equals the concentration delivered to the surface of the biofilm.

SCC Delivered in Nanoparticles—We set $C_{source} = 0$ and assume fast diffusion compared to antimicrobial release from the nanoparticles, so that the released antimicrobial spreads uniformly through the volume of the biofilm. The experimental results presented in [27] show that within a flow cell environment, nanoparticles readily deposit onto the surface of a biofilm and diffuse to a spatially uniform distribution within the biofilm in a matter of hours. Under these assumptions we find the solution to (4) is

$$C(z, t) = \frac{M(t)}{\phi}, \quad (9)$$

where

$$M(t) = \begin{cases} \frac{4}{3}\pi R^3 C_0 \rho_{nano} \left[1 - \left(1 - \frac{kt}{C_0 R} \right)^3 \right], & 0 \leq t \leq \frac{C_0 R}{k}, \\ \frac{4}{3}\pi R^3 C_0 \rho_{nano}, & t > \frac{C_0 R}{k}. \end{cases} \quad (10)$$

2.3. Equations for Particulate Components

We let B , B_i , B_p , and E denote the densities of living bacteria, inert bacteria, persister bacteria, and EPS. Note that these densities are mass of bacteria or EPS per unit volume of the biofilm. We let ϵ_B denote the volume fraction of the living bacteria and ρ_B denote the density of a typical living bacteria cell. We have $B = \epsilon_B \rho_B$. Defining ϵ_{B_i} , ϵ_{B_p} , ϵ_E , ρ_{B_i} , ρ_{B_p} , and ρ_E analogously, we have $B_i = \epsilon_{B_i} \rho_{B_i}$, $B_p = \epsilon_{B_p} \rho_{B_p}$, and $E = \epsilon_E \rho_E$.

The governing equation for B is

$$B_t + (WB)_z = \kappa_g \mu_s \frac{S}{K_s + S} B - \kappa_\mu C \frac{S}{K_s + S} B - bB - k_f(S_{source} - S)B + k_r B_p. \quad (11)$$

Here W denotes the advective velocity generated by bacteria growth and decay. Its governing equation will be defined below. The first term on the right-hand side of equation

(11), $\kappa_g \mu_s \frac{S}{K_s + S} B$, represents the growth of bacteria. The growth constant κ_g describes the efficiency with which the bacteria convert nutrient consumption into growth. The constant μ_s describes the rate at which nutrient transfers into the bacteria. The term $\kappa_\mu C \frac{S}{K_s + S} B$ represents the killing of bacteria by antimicrobial; κ is the efficiency with which the antimicrobial kills the bacteria. In this model we have chosen to simulate antimicrobial activity linked to the metabolic rate of the bacteria. In [26] antimicrobial activity in the

absence of nutrient is also included. The term bB represents the natural death of the bacteria, where b is the natural death rate. Finally, $k_f(S_{source} - S)B$ and $k_r B_p$ represent the conversion of living bacteria to and from the persister state. We allow the rate of conversion of living bacteria to persisters to be proportional to $S_{source} - S$, where S_{source} is the nutrient at the top of the biofilm and S is the nutrient distribution through the thickness of the biofilm.

The densities B_i of inert bacteria, B_p of persister bacteria, and E of EPS are governed by

$$B_i + (WB_i)_z = \kappa \mu_s C \frac{S}{K_s + S} B + bB, \quad (12)$$

$$B_{pi} + (WB_p)_z = k_f(S_{source} - S)B - k_r B_p, \quad (13)$$

$$E_i + (WE)_z = \kappa_{EPS} \mu_s \frac{S}{K_s + S} B. \quad (14)$$

Note that in our model persister bacteria cannot die without first transforming into living bacteria, which reflects that persister cells are in a non-respiring dormant state that is

unresponsive to antimicrobial and nutrient. In (14), $\kappa_{EPS} \mu_s \frac{S}{K_s + S} B$ describes the production of EPS by living bacteria, where κ_{EPS} describes the efficiency with which bacteria produces EPS. Finally, no flux of bacteria is prescribed at the substratum.

2.4. Equations for Porosity, Advective Velocity, and Height of the Biofilm

One checks that for constant ϕ , dividing (11)–(14) by ρ_B , ρ_{B_i} , ρ_{B_p} , and ρ_E , respectively, then summing the resulting equations, and lastly using

$$\phi + \frac{B}{\rho_B} + \frac{B_i}{\rho_{B_i}} + \frac{B_p}{\rho_{B_p}} + \frac{E}{\rho_E} = 1 \quad (15)$$

yields for W the equation

$$W_z = \frac{1}{1 - \phi} \left(\frac{RHS_B}{\rho_B} + \frac{RHS_{B_i}}{\rho_{B_i}} + \frac{RHS_{B_p}}{\rho_{B_p}} + \frac{RHS_E}{\rho_E} \right), \quad (16)$$

where RHS_B denotes the right-hand side of (11), etc. We assume that $W(0, t) = 0$ because there is no flux of particulate components across the substratum.

The height H of the film satisfies the kinematic condition

$$\frac{dH}{dt} = W(H, t) - \frac{D(H)H^2}{E}. \quad (17)$$

The second term on the right-hand side of (17) describes detachment. The function $D(H)$ is defined by

$$D(H) = \frac{1}{2} r_{det} [\tanh(a_{det} H) + 1], \quad (18)$$

where r_{det} and a_{det} are positive constants. The values of these parameters are chosen so that the untreated growth of the biofilm is simulated realistically, and so that the steady-state

height of the biofilm is about 100 μ m [24, 52]. We note that detachment increases with distance from the substratum and increases as the EPS concentration decreases. Furthermore, all particulate components are lost due to detachment as the biofilm height decreases because, as the biofilm volume decreases, the component number densities also decrease.

3. Solution Procedure

Our assumptions reduce the governing equations presented in Section 2 to a coupled system of partial differential equations for the nutrient and particulate components, analytical expressions for the antimicrobial concentration, and an evolution equation for the height of the flat air-biofilm interface. The processes described by our model occur on different time scales: diffusion of nutrient and antimicrobial (on the order of 10s for transport through a 100 μ m thick biofilm per the diffusivities listed in Table 2), growth and decay of bacteria and EPS (on the order of 10⁴s per the bacterial growth parameters listed in Table 2), and degradation of nanoparticles (on the order of one day for release of half the embedded antimicrobial [14]). Our assumption that the growth of the particulate components occurs on a different time scale than the diffusion of the soluble components is similar to [41, 43, 56].

The governing partial differential equations are solved numerically using FiPy, a package that is based on the finite volume method and is implemented in the Python programming language [57]. The code is structured to exploit the disparity in the time scale for transport within the biofilm and the time scale for growth of the biofilm. Hence the transport equations for particulate and soluble components are solved over several time steps holding the height of the biofilm constant. The advective velocity is computed based on the values of the particulate components, after which the height of the biofilm is updated. The transport equations are then solved on the new spatial domain, and we repeat the process.

4. Results and Discussion

In this section we study the effectiveness of the two basic treatment strategies. All our treatment simulations start from conditions generated by simulating two days of untreated biofilm growth. The simulations of untreated growth start with a biofilm of height $H = 1 \times 10^{-3}$ cm, with spatially constant initial volume fractions $B/\rho_B = .01$ and $E/\rho_E = .09$ of living bacteria and EPS, and with initial volume fractions of 0 for inert and persister bacteria. A typical growth simulation predicts that the biofilm has reached a steady state at the end of 2 days. This steady state provides the starting conditions for our treatment simulations. We ran additional untreated growth simulations starting at the same height $H = 1 \times 10^{-3}$ cm but varying the initial volume fractions of the particulate components. These simulations always predicted approximately the same steady state for the biofilm after 2 days.

We simulate two strategies for applying the antimicrobial. Strategy I corresponds to the delivery of aqueous SCC. Here we assume a constant supply of antimicrobial is delivered to the surface of the biofilm, so that the concentration throughout the biofilm remains constant in space and time. In strategy II, the antimicrobial is delivered to the interior of the biofilm

in nanoparticles. We use (9), $C = \frac{M}{\phi}$, with k chosen so that a typical nanoparticle degrades completely over four days.

4.1. Strategy I, Aqueous SCC Delivered to the Biofilm Surface

For these results, the antimicrobial concentration is uniform throughout the biofilm, $C = C_{source}$. The values for κ_g , the growth efficiency factor, and κ , the antimicrobial-induced

death factor, are estimates chosen to give results that are consistent with the observations of Panzner et al. in [12] for the action of antimicrobial SCC1, a particular silver carbene complex, against the growth of planktonic *Bacillus anthracis* after four days. The value for

κ_g was chosen to be 0.45 and the value for κ was chosen to be $8 \times 10^3 \frac{\text{cm}^3}{\text{g}}$.

In the simulations discussed below, the biofilm grows without treatment for two days, after which the antimicrobial is applied. In our model, the growth of the biofilm is limited by detachment (18) to a height of approximately $H = 100\mu\text{m}$, which is consistent with [24, 52].

Figure 1 shows the mass of living bacteria in the biofilm over time as predicted for a set of C_{source} uniform concentrations of antimicrobial within the biofilm. We note that for the first 2 days, the growth is the same in all simulations, prior to the application of antimicrobial.

Antimicrobial is added at day 2. As expected, increasing the concentration of antimicrobial decreases the mass of bacteria. The concentration of $64 \mu\text{g/mL}$ reduces the mass of living

bacteria by 99% in about $3\frac{1}{2}$ days. Hence we could take this value as a lower bound for the minimum bactericidal concentration (MBC), which is the minimum concentration of antimicrobial that decreases the population of living bacteria by 99% within one day.

Under constant concentration, antimicrobial treatment demonstrates a biphasic killing phenomenon, as illustrated by the $128 \mu\text{g/mL}$ results in Figure 1. The active bacterial population is killed quickly depending upon the concentration. The persister population, however, must revert to active bacteria before they can be killed. The rate of reversion k_r , assumed to be constant here, is slower than the death rate of the active bacteria for large concentrations of SCC. This results in an apparent change in the rate of population decrease once the active population has been reduced to near zero.

Figure 2 shows the height of the biofilm over time as predicted for the simulations in Figure 1. For the two highest concentrations of antimicrobial, a sufficient number of bacteria are killed to cause the height of the biofilm decrease to the initial height in 5 days.

4.2. Strategy II, SCC Delivered in Nanoparticles

Next, we present simulation results for strategy II, in which antimicrobial is delivered to the interior of the biofilm through nanoparticles. Figure 3 shows the mass of living bacteria in the biofilm over time as predicted by a set of simulations for different nanoparticle densities in the biofilm. We note that the simulation results for days 0 – 2, which is prior to antimicrobial treatment, are identical to the results depicted in Figure 1. We mention that the number of nanoparticles used in these simulations corresponds to a number density on the order of 10^9 nanoparticles per milliliter of biofilm. Such a number density is achievable according to the experimental results described in [27].

In Figure 3, we see that increasing the number of nanoparticles increases the concentration of antimicrobial and hence increases the rate at which the living bacteria are killed. We consider the dose of 5×10^9 nanoparticles/mL. Multiplying this dose by the amount of antimicrobial loaded into each nanoparticle ($3.0 \times 10^{-14}\text{g}$) gives $150\mu\text{g/mL}$, which is comparable to the $128\mu\text{g/mL}$ case shown in Figure 1. We consider a representative section of the biofilm with lateral dimensions of one cm by one cm. We assume the height of the biofilm is 0.01cm. For this volume, this dose of nanoparticles contains an amount of total antimicrobial similar to that for the aqueous case. However, this concentration level is not attained immediately because of the slow release of the antimicrobial from each

nanoparticle. Hence, one sees far faster killing for 128 μ g/mL aqueous in Figure 1 than for 5×10^9 nanoparticles/mL in Figure 3.

For 10^{10} nanoparticles/mL and higher we see sufficient killing of viable bacteria to illustrate the biphasic killing as the effect of persisters becomes relevant. Notice the biphasic effect appears at roughly the same level of viable bacteria. Further, the reduced killing rate is independent of SCC concentration as delivered by the different number of nanoparticles.

Figure 4 shows the height of the biofilm over time as predicted for the simulations in Figure 3. Here we see predictions similar to those in the simulations of the surface application of antimicrobial.

Figure 5 depicts the concentration of antimicrobial in the biofilm over time as determined by equation (9) for different numbers of nanoparticles embedded in the biofilm. From the figure, we see that for the three highest densities of nanoparticles/mL the concentration exceeds the MBC value of 64 μ g/mL only after a half day or longer due to the release rate.

In summary, two estimates for antimicrobial interaction with biofilms are discussed in this work (all estimates assume no loss of antimicrobial). The first estimate is that of antimicrobial being delivered in aqueous form. Here, the concentration of antimicrobial is held constant, and the antimicrobial is assumed to quickly diffuse through the biofilm so that all bacteria within the biofilm are exposed. As a result, this estimate showed the largest living bacteria death rates as seen in Figure 1.

For the case of nanoparticle delivery with fast diffusion, as seen in Figure 3, living bacteria are not killed as readily as with constant antimicrobial estimates. This is a result of the slow release of antimicrobial resulting from the degradation of the nanoparticle, and thus limited exposure to higher antimicrobial concentrations for bacteria in the biofilm.

All of these estimates consider no-flux boundary conditions and thus estimates considering only some flux (some leakage of antimicrobial) or complete flux (complete leakage of antimicrobial) from the biofilm boundary would be more conservative estimates. One would anticipate that with leaky boundary conditions, as considered in [26], a higher amount of antimicrobial would need to be administered.

4.3. Effect of Nutrient Level and Persister Population on Biofilm Growth

Due to fast diffusion the concentration of nutrient is nearly uniform from top to bottom through the biofilm thickness. We explore how the biofilm responds to treatment under various, nearly spatially uniform, levels of nutrient within the biofilm. We achieve different nutrient levels within the biofilm by adjusting the nutrient source S_{Source} in (3).

In previous work we modeled the growth of biofilms where the formation and conversion of persisters was independent of the concentration of nutrient which caused living and persister bacteria to be uniformly distributed throughout the depth of the biofilm [58]. In this paper we have allowed the rate of conversion of persister bacteria to be proportional to $(S_{Source} - S(z))$, where S_{Source} is the nutrient at the top of the biofilm and $S(z)$ represents nutrient distribution through the biofilm thickness. Hence, the persister population should decrease in locations that have more nutrient because the living bacteria are metabolizing at a normal rate, and thus do not convert to a persister state. On the other hand, under our assumptions the persister population should increase when there is less nutrient.

This change in the number of persisters has a very significant effect on the biofilm's resistance to antimicrobial as shown in Figure 6 under the influence of 5×10^{10} nanoparticles/mL. During the first two days (before antimicrobial is added) the biofilm

grows fastest in the highest nutrient case and slowest in the lowest nutrient case. This is partly explained by the faster metabolic rate in the highest nutrient case. Another possible explanation is that the higher persister population in the lowest nutrient case keeps the living bacteria population from growing as high as it did for the lower persister population. Thus, the living bacteria with higher persister populations will grow at a slower rate than those living bacteria with lower persister populations. Physically, this represents that a biofilm that has a greater proportion of its population in the dormant persister state would not be as likely to grow when subject to the constraint that ϕ is constant. We note that living bacteria with higher persister populations have been observed to grow at a slower rate than those living bacteria with lower persister populations [39].

Once the antimicrobial is added, the biofilm grown with the highest nutrient dies more rapidly than the biofilm with less nutrient. This is because the biofilm with less nutrient contains many more persisters, which gives it a degree of protection from antimicrobial that is lacking in the highest nutrient case. This model may explain experimental results in which feeding the cells with arginine has been shown to increase their susceptibility to antimicrobial [59] by reducing the persister population.

Furthermore, Figure 6 illustrates differences in the biphasic killing behavior for different levels of nutrient. While the bacteria in the biofilm were being threatened by antimicrobial, the ability of the living bacteria to form persister bacteria acted as a mechanism of resistance in the lowest nutrient case. The persister population is able to convert to living bacteria and repopulate the biofilm. Hence, for day 3 and beyond when the killing is controlled by the persister population, there is a larger mass of viable bacteria in the lowest nutrient case. For a treatment option to be effective, not only must a certain concentration of antimicrobial be present in the biofilm, but the concentration must also be present for sufficient time to eradicate the living bacteria [60]. This result further demonstrates the difficulty of successfully eradicating infections caused by biofilms since the biphasic killing rate decreases significantly in the presence of persisters. Hence, the persisters may stave off the threat of the antimicrobial until it is no longer present, and then repopulate the biofilm.

Since biofilm growth is dependent upon the rate of persister formation and conversion, it is dependent on the values of k_r and k_f . Figure 7 shows the effect of changing k_r , the rate at which persister bacteria convert to living bacteria, and the effect of changing k_f , the rate at which living bacteria convert to persister bacteria, in the presence of 5×10^{10} nanoparticles/mL.

Notice that when $k_r = 0$, the biphasic killing is not present because persisters cannot convert back to living bacteria. Increasing k_r eventually leads to a larger percentage of persisters repopulating the biofilm. Hence, the biphasic killing appears at higher population levels of living bacteria. However, once k_r reaches a sufficiently high value ($10^{-4} s^{-1}$), the persisters convert so quickly to the living state that their protective effect is no longer present. Finally, as expected, increasing k_f leads to a larger persister population and earlier appearance of the biphasic effect.

5. Conclusions

The goal of our research is to develop an effective drug delivery system and treatment strategy to kill bacteria growing in biofilms in the lung. The preliminary modeling effort presented in this work describes the response of a biofilm to treatment based on SCC delivered either in aqueous solution or embedded in nanoparticles. The model describes the transport and biological reactions of particulate components in the biofilm, the diffusion and biochemical reactions of soluble components including the erosion of the nanoparticles and

the subsequent release of agents, and the growth or decay of the biofilm in response to antimicrobial treatment.

Key results from our numerical simulations are

- Minimum bactericidal concentration (MBC) levels of antimicrobial in both free and nanoparticle-encapsulated forms are estimated. Under ideal conditions of no loss of antimicrobial to the environment, we predict the extent of time the antimicrobial must stay above the MBC to ensure the bacteria are killed. Compared to the aqueous solution, higher concentrations of nanoparticles are required to reach the MBC because the nanoparticles release antimicrobial slowly.
- Persister bacteria act as a mechanism for biofilm tolerance to antimicrobials. Antimicrobial treatment demonstrates a biphasic killing phenomenon, where the active bacterial population is killed quickly followed by a slower killing rate, which indicates the presence of a persister population.
- A biofilm with a ready supply of nutrient throughout its depth has fewer persister bacteria and hence may be easier to treat than one with less nutrient. This observation is supported by the experimental work of Borriello et. al, who showed that supplying a biofilm with arginine can increase the susceptibility of the biofilm to some antimicrobial [59].

In other work we address relaxing the various simplifying assumptions (such as no leakage of antimicrobial from the biofilm) made here [26]. Also, we simulate treatment strategies that combine SCC delivered both in aqueous solution and embedded in nanoparticles. The results in [60] suggest that such combined strategies may be more effective.

In our model the terms that govern switching between the states of living bacteria and persister bacteria do not depend on the concentration of antimicrobial. Recent research suggests that in some bacteria there are mechanisms that can downregulate the cell to a persister state in direct response to certain antimicrobials [61, 62]. We note the work in [47], which addresses this issue. In addition we have not included downregulation that may be dependent upon the presence of quorum sensing molecules.

The mathematical modeling will need to incorporate dosing strategies that may involve combinations of nanoparticles with different erosion constants k . We model a small set of materials for which we can empirically quantify release rates k in different liquids. In addition the model may be modified to account for additional therapeutics such as efflux inhibitors, anti-quorum sensing drugs, and enzymes that degrade the (EPS) and thereby break down the structure of the biofilm. Ultimately, a higher-dimensional model including detachment promoting agents and inhomogeneities in directions other than the vertical axis should be considered.

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Highlights

- Preliminary efforts to integrate modeling with in vitro and in vivo studies of silver-based antimicrobial
- Predicts MBC values of silver carbene complexes using aqueous solution and embedded nanoparticles
- Higher concentrations of nanoparticles are needed to reach MBC values due to slower release of antimicrobial
- A biofilm with a uniformly high concentration of nutrient may be easier to eradicate

Figure 1.
Effect of different concentrations of surface antimicrobial (C_{source}) on the mass of living bacteria.

Figure 2.
Effect of different concentrations of surface antimicrobial (C_{source}) on the height of the biofilm.

Figure 3.
The mass of living bacteria as a function of time predicted by simulations for different numbers of nanoparticles/mL.

Figure 4.
Effect of different amounts of antimicrobial-loaded nanoparticles/mL on the height of the biofilm.

Figure 5. Concentration of antimicrobial present in biofilm vs time due to antimicrobial release from nanoparticles/mL.

Figure 6. Mass of living bacteria over time for different source values of nutrient in the presence of 5×10^{10} antimicrobial-loaded nanoparticles/mL.

Figure 7.
Effect of changing k_r and k_f on the mass of living bacteria in the presence of 5×10^{10} nanoparticles/mL.

Table 1

Variables

Variable	Description	Units
S	concentration of nutrient	$\frac{\text{g}}{\text{cm}^3}$
C	concentration of antimicrobial	$\frac{\text{g}}{\text{cm}^3}$
B	concentration of living bacteria	$\frac{\text{g}}{\text{cm}^3}$
B_i	concentration of inert bacteria	$\frac{\text{g}}{\text{cm}^3}$
B_p	concentration of persister bacteria	$\frac{\text{g}}{\text{cm}^3}$
E	concentration of EPS	$\frac{\text{g}}{\text{cm}^3}$
ϕ	volume fraction of void	unitless
H	height of biofilm	cm
W	advective velocity	$\frac{\text{cm}}{\text{s}}$

Table 2

Parameters

Constant	Description	Value	Units	Source
ρ_B	density of the living bacteria	0.2	$\frac{g}{cm^3}$	[63]
ρ_{B_i}	density of the inert bacteria	0.2	$\frac{g}{cm^3}$	[63]
ρ_{B_p}	density of the persister bacteria	0.2	$\frac{g}{cm^3}$	[63]
ρ_E	density of the extracellular polymeric substance (EPS)	0.033	$\frac{g}{cm^3}$	[63]
ϕ	void volume fraction	0.9	unitless	assumed
D_S	diffusivity coefficient of the nutrient	6.6×10^{-5}	$\frac{cm^2}{s}$	assumed
K_S	saturation level of nutrient	2.55×10^{-6}	$\frac{g}{cm^3}$	assumed
k_{HS}	mass transfer coefficient of nutrient through the top of the biofilm	1×10^{-3}	$\frac{cm}{s}$	assumed
S_{source}	concentration of nutrient at biofilm surface	1×10^{-5}	$\frac{g}{cm^3}$	assumed
D_C	diffusivity coefficient of the antimicrobial	1×10^{-5}	$\frac{cm^2}{s}$	[64]
C_{source}	concentration of antimicrobial at biofilm surface	various	$\frac{g}{cm^3}$	[12]
C_0	concentration of antimicrobial inside nanoparticle	3.55×10^{-2}	$\frac{g}{cm^3}$	[14]
k	rate at which nanoparticle degrades	5.36×10^{-12}	$\frac{g}{cm^2 \cdot sec}$	estimated [14]
R	radius of nanoparticle	5×10^{-5}	cm	[14]
b	natural death rate of bacteria	1.16×10^{-7}	s^{-1}	assumed
k_f	coefficient for changing living bacteria into persister bacteria	5.83×10^{-7}	s^{-1}	estimated [65]
k_r	rate at which persister bacteria change into living bacteria	1.16×10^{-5}	s^{-1}	assumed
κ	efficiency with which antimicrobial is converted into bacterial death	8×10^3	$\frac{cm^3}{g}$	estimated [12, 64]
κ_g	efficiency with which bacteria convert nutrient into growth	0.45	none	estimated [12]
μ_S	rate of nutrient transfer into bacteria	1.93×10^{-4}	s^{-1}	[4]
H_C	critical biofilm height	100	μm	estimated [24, 52]

Constant	Description	Value	Units	Source
κ_{EPS}	efficiency with which bacteria create EPS	1.4	none	[47]
r_{det}	detachment coefficient	2×10^{-5}	$\frac{g}{s \cdot cm^4}$	assumed
a_{det}	detachment coefficient	1×10^4	$\frac{1}{cm}$	assumed