Commun Nonlinear Sci Numer Simulat 54 (2018) 70–83

Contents lists available at ScienceDirect

Commun Nonlinear Sci Numer Simulat

journal homepage: www.elsevier.com/locate/cnsns

Research paper

Dynamic energy budget approach to evaluate antibiotic effects on biofilms

Bjorn Birnir[a], Ana Carpio[b,*], Elena Cebrián[c], Perfecto Vidal[b]

[a] Department of Mathematics, University of California, Santa Barbara, CA 93106, USA
[b] Departamento de Matemática Aplicada, Universidad Complutense, 28040 Madrid, Spain
[c] Departamento de Matemáticas y Computación, Universidad de Burgos, 09001 Burgos, Spain

ARTICLE INFO

Article history:
Received 29 December 2016
Revised 29 April 2017
Accepted 19 May 2017
Available online 20 May 2017

Keywords:
Dynamic energy budget
Bacterial biofilm
Antibiotic
Numerical simulation

ABSTRACT

Quantifying the action of antibiotics on biofilms is essential to devise therapies against chronic infections. Biofilms are bacterial communities attached to moist surfaces, sheltered from external aggressions by a polymeric matrix. Coupling a dynamic energy budget based description of cell metabolism to surrounding concentration fields, we are able to approximate survival curves measured for different antibiotics. We reproduce numerically stratified distributions of cell types within the biofilm and introduce ways to incorporate different resistance mechanisms. Qualitative predictions follow that are in agreement with experimental observations, such as higher survival rates of cells close to the substratum when employing antibiotics targeting active cells or enhanced polymer production when antibiotics are administered. The current computational model enables validation and hypothesis testing when developing therapies.

1. Introduction

Biofilms are bacterial aggregates that grow on moist surfaces, encased in a self-produced polymeric matrix, see Fig. 1. The matrix creates a favorable environment for their development, facilitating nutrient, oxygen and waste transport [28]. It also acts as a shield against external aggressions by flows, disinfectants and antibiotics. The minimal bactericidal concentration (MBC) and minimal inhibitory concentration (MIC) of antibiotics on bacteria in their biofilm habitat may be up to 100–1000 fold higher compared with planktonic bacteria [1,15].

Implant associated infections typically involve biofilm growth on the surface of the implant [38]. They form on medical equipment and prostheses, such as pacemakers and endotracheal tubes, central lines, intravenous catheters, stents and artificial joints. Bloodstream infections, and many other hospital-acquired infections, may be caused by them. Biofilms may also spread on body surfaces such as heart valves (endocarditis), teeth, the lungs of cystic fibrosis patients (pneumonia), the middle ear and nose (otitis, rhinosinusitis), bones (osteomyelitis) or in chronic wounds [15]. The biofilm matrix hinders phagocytosis and other actions of the inmune system. Bacteria surviving standard antimicrobial therapies are able to reproduce, originating chronic infections [36]. To tackle this problem, we must be able to understand how antibiotics act and how resistance to antibiotics develops.

* Corresponding author.
E-mail addresses: birnir@math.ucsb.edu (B. Birnir), carpio@mat.ucm.es, ana_carpio@mat.ucm.es (A. Carpio), elenac@ubu.es (E. Cebrián), pervidal@ucm.es (P. Vidal).

http://dx.doi.org/10.1016/j.cnsns.2017.05.016
1007-5704/

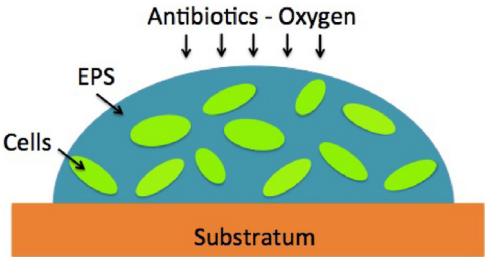

Fig. 1. Schematic representation of a biofilm. The film, formed by bacterial cells encased in a polymeric matrix (EPS), adheres to a substratum and receives nutrients, oxygen and antibiotics from the surrounding flow.

Antibiotics affect cells in diverse ways [17]. β-lactams (penicillins, cepha-losporins, carbapenems) and glycopeptides (vancomycin) inhibit cell-wall synthesis. Aminoglycosides (streptomycin, gentamycin, tobramycin) inhibit protein synthesis. Quinolones (ciprofloxacin, ofloxacin) inhibit DNA replication. Tetracyclines inhibit translation. Polymyxins such as colistin disrupt charge distributions in the outer cell membrane. Eventually, the damage caused to the cell produces its death. To exert their antibacterial action, antibiotics undergo a certain process. They need to penetrate the cells, remain stable and accumulate to reach inhibitory concentrations. Sometimes they have to take an active form. After detecting their target, they interact with it to exert their action. Interferences in either of these processes may result in cell resistance to the antibiotic. Resistance typically proceeds through efflux systems (the antibiotic is pumped out of the cell), chemical alterations of the antibiotic (cellular enzymes degrade it), mutations in antibiotic target molecules, and non-heritable resistance caused by environmental conditions [9,15,17,34,35]. The main resistance mechanisms for different types of antibiotics are summarized in [17].

The biofilm environment enhances bacterial resistance in a number of ways. Biofilm development is influenced by quorum sensing [8]. Through quorum sensing mechanisms, bacteria sense when a critical number of them are present in the environment. They respond by activating genes that produce exopolysaccharides [7]. The polysaccharide matrix surrounding the bacterial community delays diffusion of antibiotics inside the biofilm. Nevertheless, direct measurements suggest that some antibiotics equilibrate within the biofilm [6] after a waiting time. *Pseudomonas aeruginosa* tends to be the main source of gram-negative infections in intensive care units in developed countries [30]. *P. aeruginosa* and other bacteria express β-lactamase, an enzyme that attacks β-lactams. An enzyme breaking the antibiotic at a rate at which it crosses the cell membrane combined with delayed diffusion might explain resistance to penicillins, but not to other antibiotics [6].

As mentioned above, as we penetrate from the outer biofilm surface towards the interface with the substratum, gradients of oxygen and nutrients develop. Oxygen depletes [5,40]. These gradients result in increased doubling times for cell division and reduced bacterial metabolic activity. The intensity of metabolic processes is stratified: high activity in the outer layers and slow growth or no growth in the inner core. These dormant cells are partially responsible for tolerance to antibiotics. Popular monotherapies with β-lactams are only active against dividing cells [3], forcing combinations with antibiotics that are active against nondividing cells, such as colistin [15]. Oxygen limitation and metabolic rates are also important factors enhancing the tolerance of biofilms to ciprofloxacin and aminoglycosides [39].

In the biofilm, bacteria are exposed to oxidative stress, that causes hipermutability. Enhanced production of reactive oxygen species (ROS), either released in response to the infection or produced by the alterations in the DNA repair system of the bacteria, leads to an environment with low oxygen tension filled with oxygen radicals [15,27]. Augmented β-lactamase synthesis, overexpression of efflux-pumps and increased EPS production follow [4,15,24]. Studies with toxicants have also shown increased EPS production and ability to adapt to the toxicant, repairing damage to the cell [12]. Quorum sensing inhibitors [14], efflux inhibitors [25,26], antioxidants reducing the oxidative stress and mutations [27], together with enzymes able to dissolve the biofilm matrix [2], may provide strategies to overcome resistance mechanisms.

Mathematical modeling can assist in the design of therapies and the interpretation of experimental data. Early models were able to reproduce elementary qualitative behavior. Reference [33] uses coupled reaction-diffusion equations for the concentration of oxygen, antibiotics and the volume fractions of live and dead cells to predict survival profiles inside thick biofilms due to slow growth. Reference [31] predicts that the biofilm matrix can not prevent diffusion of β-lactam antibiotics into the bacteria provided the amount of chromosomal β-lactamase is low. The diversity of the mechanisms involved in biofilms resistance to antibiotics suggests the opportunity of adapting dynamic energy budget (DEB) frameworks to describe the effect of antibiotics on them. DEB models have already been exploited to describe the effects of toxicant exposure on populations of floating bacteria [19]. Like antibiotics, toxicants interfere with the metabolism of cells and increase the energy required for cell maintenance. The cell requires additional energy to expel the toxicant and repair damage caused by toxicant activity (DNA, RNA, protein repair).

Dynamic energy budget models relate biomolecular processes to individual physiology and population dynamics [21]. They have been successfully used to study scaling behaviors of all sorts of living beings, from plants and animals to cells [13]. Basic DEB models describe acquisition of biomass and energy, as well as energy allocation for cell maintenance, growth and division. A standard description of aging and death processes is also available [21]. Surplus reactive oxygen species (ROS)