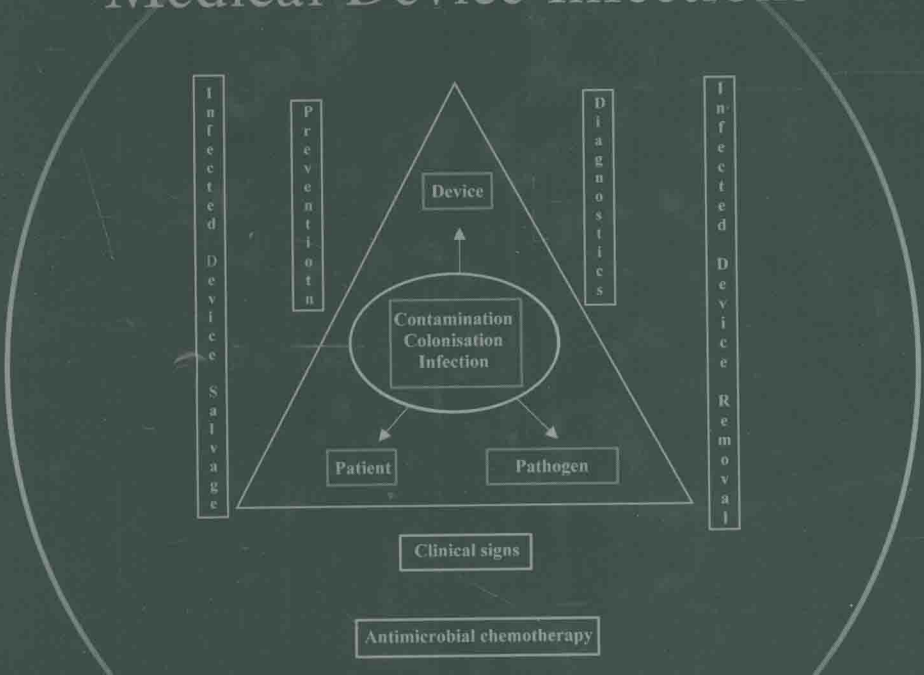
Biofilm Eradication and Prevention

A Pharmaceutical Approach to Medical Device Infections



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A Pharmaceutical Approach to Medical Device Infections

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BIOFILM ERADICATION AND PREVENTION

To my beloved wife, Suriya Prapha, and to my son, Arunachalam. The perseverance and tolerance of my spouse over the years when my eyes were glued on the computer screen, as well as the play-time sacrifice of my son, are highly appreciated.

—Tamilvanan Shunmugaperumal

PREFACE

Microbial biofilms are microcosms attaching irreversibly to abiotic or biotic surfaces and are promulgated as congregates of single or multiple populations. Since there is an increased use of implanted medical devices, the incidence of these biofilm-associated diseases is increasing. Moreover, the nonshedding surfaces of these devices provide ideal substrata for colonization by biofilm-forming microbes. The consequences of this mode of growth are far-reaching. Microbes in biofilms exhibit increased tolerance toward antimicrobial agents and decreased susceptibility to host defense systems. Hence, biofilm-associated diseases are becoming increasingly difficult to treat. Not surprisingly, therefore, interest in biofilms has increased dramatically in recent years. The application of new microscopic and molecular techniques has revolutionized our understanding of biofilm structure, composition, organization, and activities, which result in important advances in the prevention and treatment of biofilm-related diseases.

This book can conveniently be divided into three parts depending on the biofilms' importance in the medical field and the necessity of eradicating them from forming over medical devices. Part I deals with the development and characterization of a biofilm onto the surfaces of implanted or inserted medical devices. Some of the specific answers concerning the reasons why biofilms form over medical device surfaces and what triggers biofilm formation are discussed. Part I consists of five chapters. Chapter 1 is an introduction to the subject matter of this book. A comprehensive overview on the subject matter is provided to the readers so that anyone with little knowledge of medical biofilms can acquire and understand the seriousness of the biofilm formed over the implanted or inserted medical devices. The rationale for biofilm

eradication from medical devices is fully explored in Chapter 2. A range of medical devices used in modern day medical practices are categorically shown in order to see the discussed subject matter clearly. Here calculations are also given in terms of expenses to support biofilm prevention and eradication to patients who have been given medical devices to salvage the already lost normal function of organs present in their body. A need-based reason to look for an approach to prevent biofilm formation over medical devices is thus explained *per se*. Chapter 3 contains the stepwise development of biofilms onto the implanted or inserted medical devices. Hence, pathogenesis of device-related nosocomial infections starts with justifications from environmental, biochemical, physiological, and biomechanical points of view, and so on. Some details concerning the consequences of medical biofilms to cause a particular infection are also discussed in this chapter along with case studies. Chapter 4 focuses on how biofilm microbes build up resistance–tolerance against conventional antimicrobial agents when they are treating device-related nosocomial infections. This partially explains the need for alternate strategies to administer antimicrobial agents in order to prevent the development of tolerance by biofilm microbes, but at the same time to demonstrate the ramification of pharmaceutical knowledge on this subject matter. Chapter 5 briefly covers the important topic of studying and investigating medical biofilms using various analytical techniques. Starting from conventional plate counting and continuing through modern day electron microscopic and molecular methods, these are arranged by year of application in analyzing biofilms as they appeared in the reports published by different research groups across the world.

Although medically relevant biofilms develop commonly on inert surfaces, such as medical devices or on dead tissue (sequestra of dead bone), they can also form on living tissues, as in the case of endocarditis. Tissue samples taken from patients with dental caries, periodontitis, otitis media, biliary tract infections, and bacterial prostatitis also show the presence of bacterial microcolonies surrounded by an exopolymeric matrix (i.e., somehow biofilm-related). Therefore, these established infections could be termed as nondevice-related chronic infections. Part II elaborates on these types of biofilm-mediated chronic infections that occurred in various organs. Biofilm-related infections developed in ocular tissues, oral cavity, topical skin regions and lung with cystic fibrosis (CF) are selected to illustrate cases of potential interest. Chapter 6 studies biofilm-related infections occurring in both intra- and extraocular tissues. The usual way of treating these ocular diseases (i.e., topical application of aqueous-based and collyre-type eyedrops containing antimicrobial agents) are not so effective. Thus, it becomes necessary to discuss the potential of oil- and polymer-based nanocarriers (e.g., nanosized emulsions and nanoparticles) for eradicating the ocular infections found in this chapter. Chapter 7 incorporates biofilm-related infections occurring in the oral cavity. This always moist site should provide impetus to the development of chronic infections specifically due to the presence of biofilm-forming microbes in the oral cavity. Here too, treating with conventional antimicrobial agents would not produce a

desired effect. Hence, shifting toward modern pharmaceutical approaches to treat infections of the oral cavity becomes more precocious. Some of these pharmaceutical approaches, although at the research level, are not far from becoming commercialized. Most promising antimicrobial agent-laden novel drug delivery systems are explored as case studies in this chapter. In addition, the formation of a biofilm over dental chair units, as well as dental waterliners in conjunction with currently developed prevention strategies including a centralized, automated dental hospital water quality and biofilm management system, are discussed.

The epidemic increase in obese humans worldwide is followed by a similar increase in diabetes and cardiovascular diseases. Such patients are particularly prone to the development of chronic wounds, which become colonized by a number of bacterial species. Chapter 8 presents a hypothesis aimed at explaining why venous leg ulcers, pressure ulcers, and diabetic foot ulcers develop into a chronic state. The lack of proper wound healing is at least in part caused by inefficient eradication of infecting, opportunistic pathogens, a situation reminiscent of chronic *Pseudomonas aeruginosa* infections found in patients suffering from CF. Implications of biofilm formation in chronic wounds and CF are shown here.

The introduction of novel drug delivery carriers in pharmaceutical sciences helps the physician to achieve the required therapeutic concentration of the drug at the diseased region of the body while minimizing drug exposure to nondiseased normal organs. To extend the pharmaceutical knowledge gained over the decades on novel drug delivery carriers to medical biofilm prevention and eradication, it has become necessary first to explore the already developed strategies for prevention of device-related nosocomial infections. Recommended technological and nontechnological strategies in conjunction with electrical, ultrasound, and photodynamic stimulation to disrupt biofilms by enhancing the efficiency of certain antimicrobial agents are shown. Thus, Chapter 9 begins with already available strategies followed by pharmaceutical approaches like the potential of lipid- and polymer-based drug delivery carriers for eradicating biofilm consortia on device-related nosocomial infections.

Liposomes loaded with antimicrobial agents could effectively be applied as an antibiofilm coating to reduce microbial adhesion–colonization onto medical devices, and as drug delivery carriers to biofilm interfaces in intracellular infection. All these applications are discussed in detail in Chapter 10.

Many polymer-based carrier systems also have been proposed, including those based on biodegradable polymers [e.g., poly(lactide-*co*-glycolide)], as well as fibrous scaffolds and thermoreversible hydrogels and surface (properties) modified polymeric catheter materials (e.g., antimicrobial, antiseptic, or metallic substances-coated polymeric materials). Their contribution to the prevention–resolution of infection is reviewed in Chapter 11. Additionally, the Chapter 12 explores an interesting topic of novel small molecules (e.g., iron and its complexes) to control medical biofilm formation.

Through these three parts of the book we intend to cover recent advances in pharmaceutical approachs to prevent medical device- and nondevice-related infections caused by biofilm-forming microorganisms. Many other approaches either within the pharmaceutical sciences or other allied disciplines are still in their rudimentary research stages. On the other hand, biofilm structural elucidation observed through different advanced analytical techniques is constantly progressing as usual. An intriguingly combined research based on knowledge derived both from the pharmaceutical approach and biofilm structural elucidation should contribute to a more efficacious way for biofilm prevention and eradication in the future. Nevertheless, further studies are warranted to translate knowledge on the mechanisms of biofilm formation into applicable therapeutic and preventive strategies.

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CONTENTS

PART I

DEVELOPMENT AND CHARACTERIZATION OF BIOFILMS

CHAPTER 1

INTRODUCTION AND OVERVIEW OF BIOFILM

1.1. INTRODUCTION

Any surface, whether synthetic or biomaterials, is primarily coated initially with local environmental constituents (e.g., water, electrolytes, and then organic substances). This conditioning film often exists before the arrival of any microorganisms onto the material surfaces. Indeed, the presence of water, electrolytes, and organic substances could give impetus for microbial growth and its further colonization onto the material surfaces *in vitro*. Subsequently, the presence of surface-bound microorganisms can provide a profound effect on the materials performance. If the material is meant for assisting in any course of medical treatment, then, it is essential that the biomaterial should be free from harmful microorganisms (e.g., bacteria, fungi, and protozoa).

The idea that bacteria grow preferentially on surfaces has come to the fore at regular intervals, for >150 years [1]. Steadily, throughout the history of microbiology, a very small proportion of microbiologists, by performing direct microscopic examinations, have found, however, that these free-floating or "planktonic" bacteria grow differently after they adhere to a surface and initiate biofilm formation. Moreover, in microbiology, knowledge has traditionally been gained from studies of suspensions of cells grown from a single cell in laboratory culture plates. These planktonic cells, for example, have been used in studies of how well antibiotics can kill bacteria. However, microbes can also aggregate themselves termed as biofilms (i.e., organized layers of cells attached

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to a surface). Therefore, over the past century, the study of microbial adhesion has generated a language all its own. Since many of these terms are still in use, a brief discussion of their meaning with reference to biofilm development would be apropos. The terms sessile and planktonic have evolved to describe surface-bound and free-floating microorganisms, respectively. The surface of interest to which sessile organisms are attached can be either abiotic (inert materials) or biotic (living tissue or cells) [2].

The definition of a biofilm has evolved over the last 35 years. In 1976, Marshall [3] noted the involvement of "very fine extracellular polymer fibrils" that anchored bacteria to surfaces. Costerton et al. [4] observed that communities of attached bacteria in aquatic systems were found to be encased in a "glycocalyx" matrix that was found to be polysaccharide in nature, and this matrix material was shown to mediate adhesion. In 1987, Costerton et al. [5] stated that a biofilm consists of single cells and micro colonies, all embedded in a highly hydrated, predominantly anionic exo-polymer matrix. Characklis and Marshall in 1990 [6] went on to describe other defining aspects of biofilms (e.g., the characteristics of spatial and temporal heterogeneity and involvement of inorganic or abiotic substances held together in the biofilm matrix). Again Costerton et al. in 1995 [7] emphasized that biofilms could adhere to surfaces and interfaces and to each other, including in the definition microbial aggregates and floccules and adherent populations within pore spaces of porous media. Costerton and Lappin-Scott [8] at the same time stated that adhesion triggered expression of genes controlling production of bacterial components necessary for adhesion and biofilm formation, emphasizing that the process of biofilm formation was regulated by specific genes transcribed during initial cell attachment. For example, in studies of *Pseudomonas aeruginosa*, Davies and Geesey [9] have shown that the gene (*algC*) controlling phosphomannomutase, involved in alginate (exopolysaccharide) synthesis, is upregulated within minutes of adhesion to a solid surface. Recent studies have shown that *algD*, *algU*, *rpoS*, and the genes controlling polyphosphokinase synthesis are all upregulated in biofilm formation and that as many as 45 genes differ in expression between sessile cells and their planktonic counterparts. Costerton et al. [10] defined a biofilm as "a structured community of bacterial cells enclosed in a self-produced polymeric matrix and adherent to an inert or living surface", whereas Carpentier and Cerf [11] simplify the concept as "a community of microbes embedded in an organic polymer matrix, adhering to a surface". Tamilvanan et al. [12] defined microbial biofilms as microcosm attaching irreversibly to abiotic or biotic surfaces and promulgated as congregates of single or multiple populations. Underlying each of these definitions are the three basic ingredients of a biofilm: microbes, glycocalyx, and surface. If one of these components is removed from the mix, a biofilm does not develop. A glycocalyx is the glue that holds the biofilm fast to the colonized surface and is a complex of exopolysaccharides of bacterial origin and trapped exogenous substances found in the local environment, including nucleic acids, proteins, minerals, nutrients, cell wall material, and so

on [5]. Slime was a term used in 1940 [13] to describe a bacterial biofilm layer and resurrected in 1982 [14] to designate the glycocalyx produced by highly adherent strains of *Staphylococcus epidermidis* recovered from infected biomedical implants.

A current new definition for a biofilm must therefore take into consideration not only readily observable characteristics [i.e., cells irreversibly attached to a surface or interface, embedded in a matrix of extracellular polymeric substances (EPS) that these cells have produced, and including the noncellular or abiotic components], but also other physiological attributes of these organisms, including such characteristics as altered growth rate and the fact that biofilm organisms transcribe genes that planktonic organisms do not. The new definition of a biofilm is a microbially derived sessile community characterized by cells that are irreversibly attached to a substratum or interface or to each other, are embedded in a matrix of extracellular polymeric substances that they have produced, and exhibit an altered phenotype with respect to growth rate and gene transcription. This definition will be useful, because some bacterial populations that fulfilled the earlier criteria of a biofilm, which involved matrix formation and growth at a surface, did not actually assume the biofilm phenotype. These "nonbiofilm" populations, which include colonies of bacteria growing on the surface of agar, behave like planktonic cells "stranded" on a surface and exhibit none of the inherent resistance–tolerance characteristics of true biofilms. We can now speak of biofilm cells within matrix enclosed fragments that have broken off from a biofilm on a colonized medical device and now circulate in body fluids with all the resistance–tolerance characteristics of the parent community.

In nature, probably 99% or more of all bacteria exist in biofilms. For example, in an alpine stream there is typically only 10 bacteria mL^{-1}, whereas bacteria living in slimy biofilms on nearby rocks can occur in numbers like 5×10^8 cm^{-2}. Biofilms can form on various surfaces, including biotic surfaces (e.g., mucosal membranes, teeth), medical devices, and household surfaces. When a bacterium attaches to a hard surface in a moist environment, gene expression is adapted to the new environment. Some genes are upregulated, whereas others are depressed or turned off. Consequently, microbial biofilm systems are studied by many scientific disciplines (microbiology, ecology, immunology, biotechnology, engineering, medical microbiology) and across diverse research fields (environment, industry, medicine). Biofilms can be beneficial when they break down contaminants in soil and water as used in wastewater treatment, but can also cause severe problems in industrial settings, corroding everything from pipes in heating systems to computer chips or causing problems on the hulls of ships.

In the human host, biofilms exist as a community of sessile bacteria embedded in a matrix of EPS they have produced, which adhere to a foreign body or a mucosal surface with impaired host defense [15,16] or ample roughness [17]. Biofilm formation represents a protected mode of growth that allows microbes to survive in hostile environments and also disperse to colonize new