Applied Surface Science 255 (2009) 5806–5811

Contents lists available at ScienceDirect

Applied Surface Science

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

Assessing the antimicrobial activity of zinc oxide thin films using disk diffusion and biofilm reactor

Shaun D. Gittard [a,b], John R. Perfect [c], Nancy A. Monteiro-Riviere [a,b,d], Wei Wei [e], Chunming Jin [e], Roger J. Narayan [a,b,e,*]

[a] Department of Biomedical Engineering, University of North Carolina at Chapel Hill, Chapel Hill, NC, United States
[b] Department of Biomedical Engineering, North Carolina State University, Raleigh, NC, United States
[c] Department of Medicine, Division of Infectious Diseases, Duke University, Durham, NC, United States
[d] Center for Chemical Toxicology Research and Pharmacokinetics, North Carolina State University, Raleigh, NC, United States
[e] Department of Materials Science Engineering, North Carolina State University, Raleigh, NC, United States

ARTICLE INFO

Article history:
Received 4 October 2008
Accepted 7 January 2009
Available online 10 January 2009

Keywords:
Antimicrobial materials
Pulsed laser deposition
Zinc oxide

ABSTRACT

The electronic and chemical properties of semiconductor materials may be useful in preventing growth of microorganisms. In this article, in vitro methods for assessing microbial growth on semiconductor materials will be presented. The structural and biological properties of silicon wafers coated with zinc oxide thin films were evaluated using atomic force microscopy, X-ray photoelectron spectroscopy, and MTT viability assay. The antimicrobial properties of zinc oxide thin films were established using disk diffusion and CDC Biofilm Reactor studies. Our results suggest that zinc oxide and other semiconductor materials may play a leading role in providing antimicrobial functionality to the next-generation medical devices.

1. Introduction

The growth of bacteria and other microorganisms on synthetic materials, especially materials used in medical devices, is a growing area of scientific inquiry. The National Institutes of Health has stated that microbial biofilms are a contributing factor in more than 80% of human infections [1]. Bacteria can be found in two states, either floating in a liquid medium (as planktonic bacteria) or embedded within a matrix (in a microbial biofilm). Bacteria more commonly exist in the sessile form than in free-floating form [2,3]. While much remains unknown about biofilm formation, organization, and function, it is now understood that bacteria within biofilms exhibit different physiological properties than planktonic bacteria. They express different genes, exhibit different growth rates, demonstrate different pathogenic properties, and possess different antimicrobial susceptibility rates [4,5]. Infections caused by biofilms are commonly seen in patients with implanted devices or indwelling foreign objects, including catheters, pacemakers, and endoscopes [6–8].

Biofilms consist of a community of microorganisms held together by a matrix, in which the microorganisms cooperate and interact with one another. This matrix is comprised of an extracellular polymeric substance that is produced by microorganisms in the biofilm and is primarily made up of polysaccharides [5–9]. Microorganisms in a biofilm are found along solid–liquid interfaces and liquid–gas interfaces. Biofilms may contain only one organism or a variety of different microorganisms. The main unit of the biofilm is the microcolony, which contains clusters of microorganisms [3–10]. Microcolonies are located throughout the matrix, and contain channels for the transport of oxygen, nutrients, waste, and other particles [11–13]. In some situations, microscale layers within a biofilm contain cells of the same species that exhibit dissimilar phenotypes [14,15]. Oxygen concentration gradients, pH differences, and other environmental variations are created by these microscale layers.

Biofilm formation begins with adhesion of a microorganism to a surface. The initial interaction between the microbial cells and the surface is tenuous [16–18]. It is during this time that biofilms are the most fragile, with cells frequently attaching and detaching from the biofilm surface [5]. Once the cells have attached, they produce an extracellular polysaccharide matrix, which provides stability to the biofilm by enabling cell–surface and cell–cell interactions [4,16,19]. After stable attachment, the biofilm develops into a more complex environment; additional planktonic cells adhere, microcolonies develop, and complex biofilm

* Corresponding author at: Joint Department of Biomedical Engineering, University of North Carolina and North Carolina State University, 8210 Stonetown Ave, Raleigh, NC 27695-7115, United States. Tel.: +1 919 696 8488; fax: +1 919 513 3814.
E-mail address: roger_narayan@msn.com (R.J. Narayan).

0169-4332/$ – see front matter © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.apsusc.2009.01.009

architecture forms [2,18,19]. Biofilms are a dynamic environment, in which cells from solution are attaching to the biofilm and are being released into the local environment.

Microbial biofilms possess several unique attributes that convey resistance to antimicrobial agents. For example, the dosage of an antimicrobial that would eradicate a bacterium in planktonic form may have a less significant effect on a bacterium within a microbial biofilm. In fact, doses of antimicrobial agents that are thousands of times stronger than the minimum amount required to kill planktonic microorganisms may not be sufficient to eradicate microorganisms within a biofilm [6]. In addition, antibacterial metal ions such as copper and silver are highly effective against planktonic bacteria but are significantly less effective against bacteria in the biofilm state [6]. The same matrix that holds the biofilm together may also act as a diffusion barrier that slows the rate at which antimicrobials reach the microorganisms [20]. It is thought that cells are able to transfer genetic information, allowing resistance properties of one cell to spread to other cells within the biofilm [6]. One theory is that a small percentage of cells in a biofilm, called persister cells, adapt a phenotype that is highly resistant to antibiotics and is similar to that of fungal spores. Little is known about this phenomenon, but it is currently being investigated by several research groups [21,22]. The environmental variations within a biofilm may allow certain regions of a microbial biofilm to be susceptible to a particular antibiotic, while other regions remain impervious. Layering within a microbial biofilm, cells can exist in a variety of metabolic states [7,23]. Cells can be found growing both aerobically and anaerobically in the same biofilm; as a result, antimicrobial agents that only act on aerobic bacteria may not completely eradicate the biofilm [12]. Cells may also have different growth rates depending on their position within the biofilm. For example, β-lactam antimicrobials that only act upon dividing cells (e.g., penicillin) may only kill a portion of the bacteria within a layered biofilm [4–20]. Local regions within microbial biofilms may possess low pH values due to hindered acidic cell waste removal; as a result, the structure of antimicrobial agents may be altered and rendered harmless [7–24]. Several studies have examined the mechanics and kinetics of initial cell–surface adhesion [17,18]. One unique function of microbial biofilms that makes them unique from their planktonic counterparts is their information-sharing capability. Studies have shown that not only do cells in a biofilm communicate with each other, but also cell–cell communication is necessary for normal biofilm development. Cells in a biofilm secrete chemical signals that are not produced by planktonic bacteria; without these signals, microorganisms within biofilms are more susceptible to antimicrobial agents [24]. Quorum sensing is another microbial communication phenomenon that has received much attention by researchers. It is believed that cells in a biofilm can obtain a census of surrounding cells using chemical signaling [14–24]. Genetic studies of biofilms have shown that microbial cells within biofilms express different genes, with some genes being up regulated and other genes being down regulated [15]. Finally, it has been shown that fluid shear forces have significant effects on biofilm formation. Increased hydrodynamic forces have been correlated to higher density, higher stability, lower thickness, and increased extracellular polysaccharide production [16].

Developing effective methods to reduce biofilm formation is a growing area of biomaterials research. Several approaches have been taken to combat biofilm infections. One strategy is to disrupt the extracellular polysaccharide component of the microbial biofilm, thereby returning the microorganisms to their antibiotic-susceptible planktonic state [6]. Halogenated furanones are a set of compounds that inhibit quorum sensing. Researchers theorize that by disrupting quorum sensing they can eliminate some of the resistant behavior of microbial biofilms [25]. Another anti-biofilm technique involves the development of materials with antimicrobial properties that destroy the microorganisms on contact before they can develop into an organized biofilm [25]. Other groups are focusing on developing mechanisms to prevent cell adhesion processes that are necessary for microbial biofilm formation [26,27].

Standard methods to quantify antimicrobial efficacy are necessary in order to develop novel approaches for eliminating biofilms. One of the most simple and commonly administered studies is the Kirby-Bauer disk diffusion test [26]. The principle of this method is that when a biomaterial is placed on an inoculated agar plate, its antimicrobial activity will diffuse into the surrounding agar and produce a "zone of inhibition" in which microbial growth does not occur. The shake test is another method to examine antimicrobial susceptibility [28–30]. In this method, the material of interest is placed in a bacterial suspension. The diffusion of antimicrobial agents from the material into the suspension is examined by measuring the cell density before and after addition of an antimicrobial agent.

Bioreactors have become a frequently used tool for studying microbial biofilms. The common element of these devices is that they provide a controlled environment, which contains both (a) a growth medium and (b) an organism that is to be cultured. A variety of bioreactors have been developed, in which variety of experimental parameters, including temperature, volume, flow rate, hydrodynamic forces, and cell density, may be controlled. One of the first bioreactors used to study biofilm formation is the Robbins device. This device consists of a flow chamber with holes for removable round plugs on which the microbial biofilm grows [31]. Approximately 20 years ago, researchers began creating modifications of the Robbins device, which have been modified to meet various experimental needs [32,33]. Some types of modifications include alterations in the material used, plug type, number of plugs, plug orientation, and flow rate (Tyler Research, Edmonton, AB) [34,35]. Another bioreactor that was developed in the early years of biofilm research was the perfused biofilm fermenter [20–36]. The advantage of this device is that by regulating the flow rate of media, the growth rate of the biofilm can be controlled. The perfused biofilm fermenter was later modified to provide more efficient examination of antimicrobial susceptibility; for example, a system was developed that enabled multiple biofilms to be exposed to different concentrations of an antimicrobial agent. A scaled-down model of the original perfused biofilm fermenter has been fabricated using Swinnex filter units (Millipore, Billerica, MA), which allows microbial biofilm studies to be conducted with low quantities of material [37].

Biosurface Technologies (Bozeman, MT) carries several types of bioreactors that may be used to examine microbial biofilms. The annular reactor is a vessel with a rotating cylinder for creating fluid shear and 20 removable coupons for carrying test materials [38,39]. This bioreactor is intended for industrial research of water systems, electronics, and chemical processes. The drip flow reactor consists of channels, which contain surfaces on which the microbial biofilm is grown. Media is dripped onto these channels to create an environment for studying biofilms under low shear stress conditions [23]. For microscopic studies, Biosurface Technologies has prepared a device with five lines of flow cells, which are compatible with fluorescent and confocal imaging under a variety of flow conditions [40]. The rotating disc reactor consists of a continuous flow media vessel, which contains six removable coupons on a rotating disk that can be used to expose microbial biofilms to surface shear stress [41,42]. This device is meant for antimicrobial efficacy studies. Similar to the rotating disc reactor is the CDC Biofilm Reactor. The bioreactor consists of 24 stationary removable coupons in a continuous flow media vessel, in which shear stress is provided by a rotating baffle. This reactor has been