REVIEW ARTICLE

Biofilms

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Introduction

Commonly encountered as a layer on slime clogging drainpipes, biofilms appear in our everyday life in more than one form. Bacteria suspended in water or aqueous cutures are "Planktonic" and most studies in microbiology have been conducted using such suspensions. In such aqueous environments when bacteria adhere to surfaces and become "Sessile", secreting a slimy-glue like substance for anchorage, they form a biofilm (1).

A single bacterial species can form a biofilm, but in natural environment often biofilms are formed from various species of bacteria, fungi, algae, protazoa, debris along with corrosion products Adhesion to surfaces provides considerable advantage for the biofilm forming bacteria, such as protection from anti-microbial agents, exchange of nutrients, metabolites or genetic material from close proximity to other micro organisms. Such symbiotic relationships although benefit the participating bacterial growth the physical presence of biofilm either damages surfaces or causes obstruction so that the efficiency of the surface is reduced. This kind of surface damage is collectively teamed as "biofouling", and is usually observed to cause problems as dental decay, metal pipe line corrosion and colonization of various medical implants, product contamination, equipment failure and decreased productivity. Biofilms can vary in thickness from a mono cell layer to 6-8 cms thick, but mostly on an average are of about 100µm thickness.

Formation of Biofilms

Pioneering studies by Cholodny, Henrici and Zo Bell commenced more than 50-60 years ago. Usually the methodology involved was unmersion of glass slides into natural environments and observing the biofilms developed under microscope.

Biofilm formation usually commences with the colorization of a surface by bacteria. The adhesion and attraction of the bacteria to the surface may be brought about by different mechanisms including surface charge, gravity, Brownian motion and chemo attraction, provided the surface has nutrients. After attraction, attachment of the bacteria to the surface occurs by a two-step process comprised of reversible binding (2).

The reversible binding is usually brought about by weak Vander Waal forces to hold he bacterium close to the surface before a stronger attachment can form by a combination of both physical or chemical forces. Production of exogenous polysaccharide containing material exuded by bacteria is one of such chemical substances implicated, also called as the glycocalyx. Bacteria divide and grow freely within this glycocalyx to form microcolonies eventually forming a biofilm.

Factors influencing bacterial attachment to surfaces : Nutrient availability at the surface, nutrient concentration, pH available, temperature, electrolyte concentration, the flux of materials and surface types such as :

a) High surface energy materials: These are negatively charged hydrophilic materials such as glass, metal or minerals.
b) Low surface energy materials : These are either low positively or low negatively charged hydrophobic materials such as plastics made up of organic polymers.

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A higher surface energy denotes higher activity leading to more adsorption of dissolved solutes or nutrients, which in term affects the rate of bacterial colonization of the surface (3,4).

A pre-biofilm homogenous surface turns heterogeneous after bacterial colonization. A probable sketch of sequence of event is as follows :

1) One or more bacterial organisms are attracted to a surface influenced by factors such as those mentioned above. 2) The primary colonizing bacteria attaches to the surface and multiplies, producing an altered microenvironment around the established micro colony by its metabolic activity. 3) Homogenous environment is converted to a heterogeneous one after primary colonization by bacteria. Such an altered heterogeneous environment attracts other bacterial & species. 4) A succession of colonizing bacteria attach on to the surface resulting in the formation of a biofilm until a series of complex communities result. Each bacterial community either helps the other with metabolic by-products or being helped in return by being held together by the glycocalyx.

Limiting of a biofilm is brought about by the removal of sessile cells or sections of the biofilm, as a result of erosion, sloughing or abrasion processes.

- Erosion leads to a continuous loss of cells from biofilm. Thickness of biofilm, fluid shear stress and fluid velocity all affect the rate of erosion.
- Sloughing is observed in cases of bulky biofilms and involves a large and rapid loss of material from the biofilm.
- Abrasion occurs when some object repeatedly collides with the biofilm.

The growth of sessile bacterial micro colonies on an immersed surface is at a striking advantage as compared to planktonic growth. Antimicrobial agents are capable of easily eradicating planktonic population of bacteria as compared to sessile forms even at higher concentrations. Previously the exo-polysaccharide glycocalyx was considered to be the physical barrier, not allowing the antimicrobial agents to reach the micro colonies of sessile bacteria. However analysis by confocal scanning laser microscopy (CSLM) and Fourier transmission infrared spectroscopy (FTIR), have helped to develop a variant concept of biofilms and sessile bacteria. At the university of Calgory a FTIR study undertaken by Jana Jass and colleagues showed that although the antibiotics are able to penetrate the biofilm rapidly and reach the surface below the film, they are incapable to penetrate effectively the sessile cells located in clumps or microcolonies. The antibiotics being effective against the planktonic population and a few sessile cells on the outer edges of micro colonies, the inner cells remain viable after cessation of the antibacterial treatment. Countering the theory of impaired antibiotic access, alternative explanations have been offered by other scientists on basis of possible physiological differences between sessile and planktonic bacteria such as growth rates and adherence-dependent differential gene expression (5).

The concentration of available nutrients in the biofilm directly affects the growing cells with in the film. The polyanionic exo-poly saccharide matrix surrounding the microcolonies of sessile bacteria serves as an ion-exchange column, concentrating nutrients and ions especially cations from the surrounding fluid, leading to an increased availability of nutrients for growth. The presence of concentrated nutrients in the biofilm helps the sessible bacteria to tide over adverse bulk fluid conditions which directly hampers planktonic growth.

Presence of highly hydrated glycocalyx which binds water molecules protects the sessile bacterial cells within from the effects of desiccation. The same safe-guard is unavailable to the planktonic bacteria which are directly dependent on the availability of in the immediate surrounding.

A consortium of bacterial species within the biofilm interact with each other in ways such as removal of toxins produced by one species, degradation of complex substrates or compounds such as cellulose to be utilized as an energy or carbon sources, recycling of substances produced on lysis or death of cells. Close proximity of different microbial colonies can bring about complex and rapid microbial degradation requiring combined metabolic capabilities leading to enhancement of

previously mentioned advantages of a sessile mode of growth.

Environmental changes can cause phenotypic variations in bacteria. In vitro-tests have demonstrated that under nutrient rich conditions mucoid strains of some bacteria become non-mucoid or a pilli producing strain, stops producing it as a result of change in metabolism or alteration in composition of their outer cell membrane. Biofilm formation is partially controlled by quorum sensing, an interbacterial communication mechanism dependent on population density (6).

It was demonstrated by M. Fletcher at University of Maryland that bacteria attached to a surface are more metabolically active than planktonic bacteria. Close proximity of cells also warrants transfer of plasmid DNA from one to another, conferring beneficial capabilities to the recipient. Altered gene expression and increased opportunities for gene transfer were recognized as consequences of the association of microbes with surfaces (7).

Biofilms in Medical Systems

Recent advancements in technology has brought to use a plethora of implants or devices made of inert metals, plastics and other synthetic products such as:

Orthopedic implants (Screws, pins, plates, and other prosthetic devices) : Ocular lenses, heart values, vascular grafts, intra uterine devices, temporary indwelling catheters, intra-venous catheters ports/caths and reverse osmosis membrane filters (8).

The material used for manufacturing these devices range from : Vitallium (Cobalt-chromemolybdenum), titanium, stainless steel, polyethylene, polyethylene terepthalate (dacron), polymethyl methacrylate, silicone rubber, polytetra fluoroethylene (teflon) and poryvinylchloride (PVC)

Although the rate for such implants associated infections is low (0.5-1%), the resultant infections lead to formation of extensive biofilms on the surface of such devices. The infection in such cases does not resolve and is treatable only on removal of infected implants, causing major morbidity or occasional mortality.

Body surfaces especially skin have a wide range of rich normal host microbial flora being dominated by Staphylococcous epidermidis. Such bacteria quickly invade the implants on coming in contact and form extensive biofilms on the surfaces Occasionally the infection may reach grevious proportions and cause severe complications such as Staphylococcus aureus infection of I/V catheter leading to heart wall colonization and endocarditis.

Prophylactic use of antibiotics at the time of insertion of such implants is indicated to reduce the rate of around infection and limit the secondary infection of the prosthesis or eliminate any contaminating bacteria at the time of insertional surgery. Early usage of anti-microbial agents is helpful incriminating a few number of bacteria, before a biofilm is fully established.

Biofilms in Medical Appratuses

In a medical set-up a range of apparatuses are in-use which are in contact with water in one way or another.

Common examples can be water pipelines (hot or cold), water filter systems used in dialysis units or else where, dental units (9), storage tanks or vessels, section apparatuses, artificial ventilator pipes, gas tubes and the like.

The usual spectrum of problems which bacterial biofilms present in the above mentioned systems include insulation against heat exchange, reduction of fluid/water flow, corrosion (Biofouling) of metal surfaces (10,11), biofouling of computer chips and harboring water borne potential pathogenic microorganisms.

Such processes are seen to occur in most of the following systems where the ambient physical conditions favour growth of micro-organisms. Such sessile biofilm organisms are very difficult to eradicate and biocidal substances, which kill biofilm organisms, are used at 50-600 times the planktonic minimal bactericidal concentrations (MBC).

Biocide efficacy is most of the times wrongly judged as being "successful" at MBC concentrations by the assessment of live planktonic bacteria.

An additional problem of medical biomaterials in the urinary tract environment is the development of encrustation and consecutive obstruction. Modification of the biomaterial surface seems to be the most promising prevention strategy for bacterial biofilms (12).

In these hardy microbial communities, pathogens like nontuberculous mycobacteria, Pseudomonas aeruginosa, Legionella pneumophila, and other bacteria not only survive but proliferate and lie in wait for susceptible hosts (13).

Candida species are the most common causative agents of these infections, and biofilms formed by these fungal organisms are associated with drastically enhanced resistance against most antimicrobial agents. This enhanced resistance contributes to the persistence of this fungus despite antifungal therapy. Recent studies showed that Candida biofilms exhibit antifungal resistance against most antifungal agents with the exception of echinocandins and lipid formulations of amphotericin B (14,15).

Biological corrosion "cells" develop on metallic surfaces in-between discrete microcolonies within biofilms. Each microcolony develops its own microenvironment depending on the kinds of metabolites or biopolymers produced eg. acidic if acid byproducts are formed or rich in metal cations if surrounding glycocalyces of the individual cells selectively attract or concentrate them (16).

Large ionic gradients are produced between adjacent regions on the surface, becoming effectively anodes and cathodes depending upon local differences in pH, Eh, ionic and metabolite concentrations. Metal losses occur at anodes causing heavy corrosion and subsequent damage to the metallic surfaces.

In the end it can be summarized that if effective control measures are devised to control the growth of biofilms it can result in an enormous saving of finances, drugs, manpower and finally life itself.

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