BIOFILM AND DEVICE ASSOCIATED INFECTIONS

Thesis

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ABSTRACT

Medical devices associated infections continue to be a significant source of morbidity and mortality in patients requiring devices and increase medical expenses by prolonging hospitalization. These infections are most commonly caused by biofilm producing organisms. **Objectives**: to study microbial biofilms in different medical devices, bacteriologically and morphologically, and their association with diseases and antimicrobial resistance. **Methods:** 103 patients with different indwelling medical devices were studied to isolate and identify the organism present on the device surfaces and to test for biofilm production using tube and plate adherence methods as well as spectrophotometric methods.Also the organisms were studied for antimicrobial resistance using Calgary's devices. Scanning Electron microscopy was used on some of the devices to confirm the presence of the biofilm. **Results:** The results revealed isolation of different species from 65.1% of the studied devices. Associated infections detected by blood and urine cultures were 48 % and 61% respectively. Biofilm production by the isolates showed that 11.9% were weak, 17.9% were strong, and 70.2% were non producers. *Staphylococcus spp*. represented 40% of organisms producing biofilm. Antimicrobial resistance showed statistical significant difference between planktonic and biofilm cells as measured by MIC and MBEC ($p < 0.001$). **In conclusion:** This study indicated that there were a significant percentage of organisms able to grow within biofilm produced on indwelling medical device surfaces and considered as a source of infections. Plate adherence and spectrophotometric methods both are reliable tests, by which we can use any of them to diagnose biofilm formation. The choice depends on the availability of different reagents and equipment. Spectrophotometric method is the most reliable test to differentiate between weak and strong biofilm producers compared with the tube method. Biofilm producing isolates were highly resistant to the antimicrobials in comparison to their planktonic counterparts.

Keywords: Biofilm, Indwelling medical devices, Tube adherence, Plate adherence, Spectrophotometry, Calgary device, Scanning Electron microscopy, *Staphylococcus spp*

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INTRODUCTION

Biofilms can be defined as communities of microorganisms enclosed in a self-produced polymeric matrix and attached to an inert or living surface. Microorganisms undergo profound changes during their transition from planktonic (free swimming) organisms to cells that are a part of complex surface attached community ***(O'Toole et al., 2000 & Dunne, 2002).***

Though it appears as slime to the naked eye, biofilm is actually composed of several hills and valleys of varying heights and depths. These structural differences allow for nutrients to make their way to all bacteria within the biofilm community ***(Wood, 2008).*** Biofilm can become hundreds of micrometers in depth and can display complex structural and functional architecture ***(Lawrence et al., 1991; Costerton et al., 1994 & Prigent-Combart et al., 2000)***.

Its development is a complex process **(*Kolter and Losik, 1998; Costerton et al., 1999 & Davey and O'Toole, 2000)***. It is initiated by cell attachment and formation of "microcolonies" on the surface. A variety of surface factors facilitate attachment and microcolony formation ***(Pratt and Kolter, 1998 & Danese et al., 2000)***. Differentiating microcolonies produce a matrix that encloses the biofilm and typically contains polysaccharides as its major components. Ultimately, planktonic cells are released, can complete the development cycle and colonize elsewhere ***(Sutherland, 2001)***.

Biofilms play important roles in interactions of both nonpathogenic and pathogenic bacteria with eukaryotic hosts. Nonpathogenic biofilms in the mammalian gut and on the roots of plants provide barriers to invading

pathogens ***(O'Toole and Kolter, 1998 & Reid and Habash, 2001).*** It protects pathogens from attack by the immune system, complicates chronic infections that are difficult to eliminate with antibiotic therapy, and is involved in prostatitis, biliary tract infection and urinary catheter cystitis ***(Costerton et al., 1999 & Donlan and Costerton, 2002)***.

Cells in biofilm are resistant to antimicrobial agents. Consequently, biofilm-related infections are inherently challenging to treat and difficult to fully eradicate with normal treatment regimens ***(Soto et al., 2007)***. For this reason biofilm infections typically show recurring symptoms after cycles of antibiotic therapy until the sessile population is surgically removed from the body ***(Aparna, 2008)***.

Free bacterial cells release antigens and stimulate the production of antibodies, but the antibodies are not effective in killing bacteria within biofilms and may cause immune complex damage to surrounding tissues. Even in individuals with excellent cellular and humoral immune reactions, biofilm infections are rarely resolved by the host defence mechanisms ***(Aparna, 2008).***

AIM OF THE WORK

The objectives of the present work are to study microbial biofilms in different medical devices, bacteriologically and morphologically. Also, we aim to study their association with diseases and antimicrobial resistance.

LIST OF ABBREVIATIONS

AAP	Accumulation associated protein
A. baumannii	*Acinetobacter baumannii*
AHLs	Acylated homoserine lactones
AI-1	Auto-inducer-1
AI-2	Auto-inducer-2
CABSI	Catheter associated blood stream infection
C. albicans	*Candida albicans*
CAMHB	Cation adjusted Muller Hinton Broth
CBD	Calgary biofilm device
CF	Cystic fibrosis
C. freundii	*Citrobacter freundii*
CLSI	Clinical and Laboratory Standards Institute
CLSM	Confocal Laser Scanning Microscope
CoNS	Coagulase negative staphylococci
CVC	Central venous catheter
DNA	Deoxyribonucleic acid
E. aerogenes	*Enterobacter aerogenes*
E. coli	*Escherichia coli*
E. faecalis	*Enterococcus faecalis*
ELISA	Enzyme linked immunosorbent assay
EPS	Extracellular polymeric matrix
ICU	Intensive care unite
IUDs	Intra uterine devices
IV	Intravenous
K. pneumoniae	*Klebsiella pneumoniae*
MBEC	Minimum biofilm eradication concentration
MDR	Multi drug resistance
MIC	Minimum inhibitory concentration
M. morganii	*Morganella morganii*

MRD	Modified Robbins device
NVE	Native valve endocarditis
ODs	Optical densities
OM	Otitis media
PBS	Phosphate buffered saline
PIA	Polysaccharide intercellular adhesion material
Pr. mirabilis	*Proteus mirabilis*
Ps. aeruginosae	*Pseudomonas aeruginosae*
RBCs	Red blood cells
RNA	Ribonucleic acid
S. aureus	*Staphylococcus aureus*
SD	Standard deviation
SEM	Scanning electron microscope
S. epidermidis	*Staphylococcus epidermidis*
S. viridans	*Streptoccoccus viridans*
TBRI	Theodor Bilharz Research Institute
TSB	Trypticase soy broth
UTI	Urinary tract infection
UV	Ultraviolet
Vol	Volume

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BIOFILM

Historical Basis

As early as 1973, microbial slimes in industrial water systems were studied and showed that they were not only very tenacious but also highly resistant to disinfectants such as chlorine ***(Characklis, 1973).***

Biofilm was first discovered as a cause of human disease during microscopic examination of scrapings from a dental plaque in the seventeenth century ***(Costerton et al., 1978).***

Zobell (1943) observed that the number of bacteria on surfaces was dramatically higher than in the surrounding medium**.** However, a detailed examination of biofilms would await the electron microscope, which allowed high resolution photomicroscopy at much higher magnifications than did the light microscope.

Based on observations of dental plaque and sessile communities in mountain streams, ***Costerton et al. (1978)*** put the theory of biofilm that explained the mechanisms whereby microorganisms adhere to living and nonliving materials and the benefits accrued by this ecologic niche. Since that time, studies of biofilms in industrial, ecologic settings and environments have basically paralleled each other ***(Donlan and Costerton, 2002).***

Structure

The basic building block or structural unit of the biofilm is the microcolony. Cells of microcolonies are arranged in the form of towers or

mushrooms like structure and are embedded in extracellular polymeric matrix, which is considered the primary matrix material of the biofilm. Each colony is separated from other microcolonies by interstitial voids that form water channels and may be considered as primary circulation **(Fig. 1).** Well developed water channels conduct water in convective flow and deliver nutrients to most parts of the community ***(Donlan and Costerton, 2002)***.

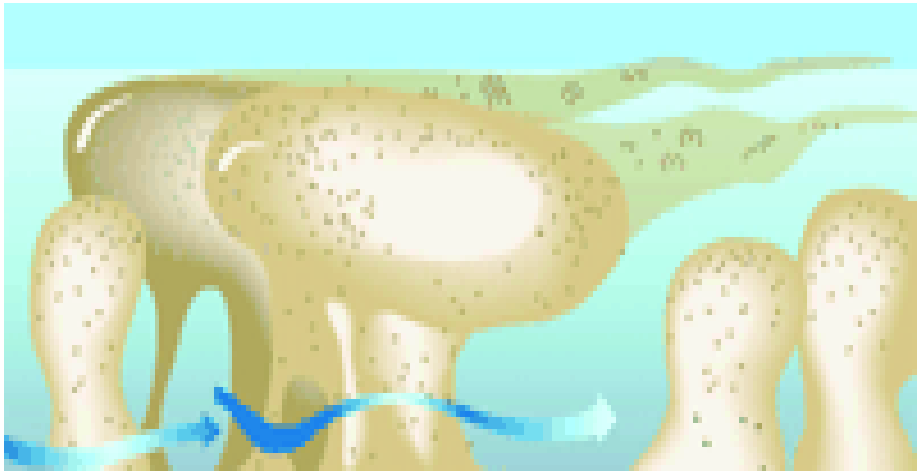

Fig. 1: structural elements of biofilm: mushroom-like microcolonies ***(Donlan and Costerton, 2002)*.**

Stages of Biofilm Formation

The biofilm may be formed on a wide variety of surfaces, including living tissues, indwelling medical devices, industrial or potable water system piping, or natural aquatic systems ***(Donlan and Costerton, 2002).*** The following are major stages involved in the process of biofilm formation (**Fig. 2)** ***(Houdt and Michiels, 2005).***

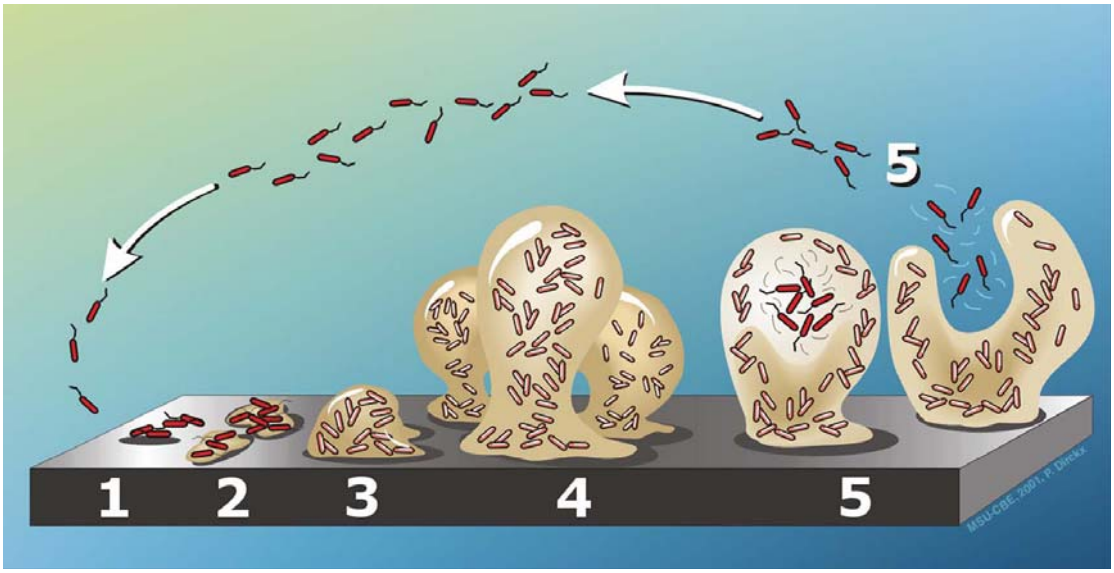

Fig. 2: Developmental stages of biofilm formation ***(Houdt and Michiels, 2005).***

1. Reversible attachment:

A primary reversible attachment of planktonic bacteria that approach the solid surface by fluid stream or through motility and that has overcome the repulsive forces between the cell and the surface is formed. The solid surface generally is a conditioned surface which is modified by adsorption of various solutes and has altered properties compared to the unconditioned surface (negative-charged, hydrophilic and smooth surfaces) ***(Houdt and Michiels, 2005).*** A solid-liquid interface between a surface and an aqueous medium (e.g., water or blood) provides an ideal environment for the attachment and growth of microorganisms ***(Donlan, 2002).***

This stage is controlled by a number of physical and chemical variables. First, the organism must be brought into close approximation to a surface. Once the organism reaches critical proximity to a surface (usually <1 nm), the final determination of adhesion depends on temperature and a number of forces

including electrostatic, hydrophobic, Van Der Waals and hydrodynamic forces ***(Jucker et al., 1996)***.

2. Irreversible attachment:

Transition from reversible to irreversible attachment results from production of extracellular polymeric matrix by the bacteria and/or by specific adhesins located on pili and fimbriae ***(Donlan and Costerton, 2002)***. This extracellular matrix is composed of mixture of materials, such as polysaccharides, proteins, nucleic acids and other substances. These materials are essential in cementing bacterial cells together in the biofilm structure, in helping to trap and retain nutrients for biofilm growth and in protecting cells from dehydration and from the effects of antimicrobial agents ***(Boyd and Chakrabarty, 1994 & Davies and Geesey, 1995).***

3. Maturation:

Once irreversibly attached to a surface, bacterial cell undergo phenotypic changes and the process of biofilm maturation begins. Bacteria start to form microcolonies either by aggregation of already attached cells, clonal growth (cell division) or cell recruitment of planktonic cells or cell flocks from the bulk liquid ***(Lawrence et al., 1991).***

Extracellular polymeric substances, that serves as an adhesive matrix and which trap nutrients from the environment, continue to be produced. Complex architectures with pedestal-like structures, water channels and pores are formed. Bacteria develop specific patterns of growth and a different physiology and metabolism from that of planktonic cells ***(Donlan and Costerton, 2002)***.