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BIOFILM FORMATION BY PATHOGENIC BACTERIA

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Abstract:

A collection of microorganisms that reside in a self-assembled polymeric matrix and come into touch with a range of surfaces is known as a biofilm. These biofilms can have one or more layers, depending on how the component cells and the surface interact. The creation of biofilms gives many bacteria the ability to move from one planktonic form to another. Biofilms are a very normal and common condition for bacteria. Bacteria that generate biofilms are critical to animal health. Animal species seem to be more susceptible to infection than human beings. because of certain variations in the living circumstances and environment of the animal. Numerous bacteria, such as *Pseudomonas aeruginosa, Streptococcus epidermis, Escherichia coli , Staphylococcus aureus, Enterococcus cloacae*, and *Klebsiella pneumonia*, can form biofilms. In bacterial infections, biofilms may both make the organism more pathogenic and protect it against external treatments. Phagocytosis, UV radiation, pH stress, chemicals, dehydration, and antibiotics pose a major danger to the cells protected by biofilms. Tissue culture plates, bioluminescence tests, and Congo red agar are seen to be three essential, useful methods for finding and examining biofilms. Microorganisms development and spread of antibiotic resistance is a serious threat to global health.

Keywords: Biofilm, Pathogenesis, Pathogenic bacteria

INTRODUCTION

Biofilms have just lately been studied, particularly in the food industry, and there are still a lot of unanswered questions. A matrix of extracellular polymeric substances (EPS) encases biofilm microorganisms. Food hygiene experts are particularly interested in the remarkable resistance of biofilm bacteria to antimicrobial treatments. Before the bacteria become embedded in the EPS matrix, within a few hours of the bacteria adhering, the improved resistance will be developed. Therefore, a "microbial community" that sticks to surfaces and is frequently entrenched in a matrix outside of cells can be defined as a biofilm by nutritional hygienists [1,2].

BIOFILM FORMATION

Several genetic and environmental factors regulate how much biofilm is produced. Extracellular polysaccharides, signaling molecules, cell membrane proteins, and bacterial motility all play crucial roles in the formation of biofilms, as genetic research has shown. Fimbriae and flagella are type of protein present on the cell surface that enable bacterial motility [3,4]. Bacteria may float in a liquid media because to their long, spiraling growths known as flagella, while the small, straight growths called fimbriae allow bacteria to move in tiny, twitching motions on the substrate's surface [5,6]. While the creation of microcolonies depends on the movement of bacteria made possible by fimbriae, the interaction between the bacteria and the surface is dependent on the mobility of bacteria by flagella. Certain proteins in cell membranes maintain the initial contact between bacteria and substrate surfaces. Studies on *V. cholera* and *E. coli* have shown that biofilm development does not occur when adhesion activity is blocked. [7,8] The formation of biofilms relies heavily on the extracellular polysaccharide matrix (EPS). In *Staphylococcus epidermis*, studies indicated that the capability to form biofilms diminishes when the genes responsible for EPS matrix synthesis are deactivated. Through interactive communication facilitated by signaling molecules, bacteria can coordinate to create a biofilm functioning as a multicellular organism [9,10].

Environmental factors including pH, temperature, oxygen concentration, and nutrition availability have a big impact on controlling the growth of biofilms. Studies conducted on *Listeria monocytogenes* have demonstrated that the environment's phosphate levels may be altered to reduce the production of biofilms [11]. On the other hand, it has been found that carbohydrates like mannose and trehalose promote the growth of biofilms in *L. monocytogenes*. The

availability of oxygen is a determining factor in the control of biofilm development in *E. coli*. Insufficient oxygen inhibits the production of biofilms because it makes it difficult for the bacteria to cling to substrate surfaces. This emphasizes how important oxygen levels are in controlling when E. coli starts to develop biofilms [12]. Research on how temperature affects *L. monocytogenes* has shown that high temperatures prevent the growth of biofilms. There is no biofilm because the high temperatures prevent germs from adhering to substrate surfaces [13,14]. Studies on *V. cholerae* have indicated that environmental pH is another important factor in the formation of biofilms. 8.2 is the ideal pH for the growth of V. cholerae. When the pH drops below 7, the environment becomes acidic, which hinders the bacteria's ability to form a biofilm. This is explained by the fact that in acidic environments, bacterial cell mobility is lost [15].Bacteria like *S. epidermidis* and *E. coli* show a less strict requirement for an alkaline environment for multiplication than *V. cholerae*[16]. Because of this, they can grow biofilms on urethral catheters even in situations where the pH of the urine is acidic. These bacteria's adaptability demonstrates the variety of environmental conditions that can lead to biofilm formation [17].

TYPES OF BACTERIAL BIOFILM

The classification of biofilms into monolayer and multilayer groups is based on the way the surface interacts with the constituent cells. A single layer of microbial biofilm is formed by a variety of sticky structures. In one case, the presence of flagella or pili facilitates transient surface attachments, which speeds up the formation of monolayer biofilms. In the meanwhile, one step produces microbial adhesions, and the next is the shift to permanent attachment [18].

In the case of a multilayer biofilm, microorganisms adhere not only to the surface but also to each other. Bacterial surface features, in many instances, create repulsion. For instance, the O antigen, typically carrying a negative charge in gram-negative bacteria, defines the chemical traits of the cell wall[19]. To overcome the repulsive force arising from the similar charges among bacteria, essential steps must be taken to establish a multilayer biofilm [20,21].

Steps of Biofilm Formation according to the [22]

1. **Initial or Reversible Attachment**
2. **Irreversible Attachment**
3. **Microcolony Formation**
4. **Biofilm Maturation**
5. **Biofilm Dispersal**

Biofilm Formation by Microorganisms

A.*Escherichia coli* : Rod-shaped, Gram-negative bacteria. Numerous nosocomial and community-acquired illnesses, including as prostatitis and urinary tract infections (UTIs), are caused by it. *E. coli* is capable of producing biofilm and secreting toxins and polysaccharides. It has been reported to generate biofilm in experimental environments. [23,24]. *E. coli* capsules are big molecules with a high molecular weight that attach to the cell's surface. These capsules indirectly contribute to biofilm development by limiting bacterial adhesion to the surface. Several environmental conditions impact *E. coli* capacity to produce biofilm. Exopolymers in *E. coli* biofilms can cause thicknesses of hundreds of microns, making antibiotic treatment difficult [25, 26].

B. *Staphylococcus aureus*: It develops biofilms on chronic wounds and catheters. *S. aureus* uses proteins from its cytoplasm to produce the extracellular matrix during the biofilm-forming process[27]. Because cytoplasmic proteins can function as matrix proteins, *S. aureus* can be more flexible and adaptable while forming biofilms in infectious conditions. Additionally, it could support the growth of mixed-species biofilms in chronic wounds [28,29].

How Biofilms Help Perpetuate Antimicrobial Resistance

Biofilms have a complex relationship with antimicrobial resistance (AMR) and can play a significant role in the emergence of resistance. Antibiotic resistance in bacteria can grow by 10 to 1,000 times when the bacteria are enclosed in a biofilm [30, 31].

Antibiotic resistance in bacteria is caused by a variety of processes, including enzymes, efflux pumps, and point mutations. It is unclear that these processes can contribute to the resistance observed in biofilm organisms, though. Antibiotic efficacy is diminished or eliminated entirely within a biofilm due to a combination of factors that enhance resistance. Recalcitrance is the term for the ability of the organisms inside the biofilm to survive in the presence of high doses of antibiotics due to these processes[32,33]. Three important mechanisms contribute to antibiotic resistance in bacteria within biofilms [32,34]:

1. Resistance at the Biofilm Surface: Antibiotics must get through the slimy, sticky membrane at the biofilm's surface to access the first mechanism. The complex structure of the biofilm, consisting of exopolysaccharides, DNA, and proteins, makes it difficult for antibiotics to penetrate the matrix and reach the bacteria within. Additionally, the slowed diffusion of antibiotics at the surface increases the likelihood of their deactivation before they can effectively diffuse. However, it is important to note that not all biofilms exhibit this mechanism, and its impact on antimicrobial resistance is still not fully understood.

2. Resistance Within Biofilm Microenvironments: In the deeper levels of the biofilm, an antibiotic faces a difficult microenvironment if it is able to break through the surface layer. Anaerobic environments are produced in these areas by the accumulation of nutrients, waste, and metabolic wastes as well as possible sharp drops in oxygen levels. Depending on how they are structured and how they work, the interaction of these variables has varying effects on

antibiotics. Low oxygen concentrations, for instance, might lessen the bactericidal properties of antibiotics like ciprofloxacin and tobramycin, while pH variations can adversely affect the activity of aminoglycosides.

3. Resistance of Bacterial "Persister" Cells: There are more ways for the bacteria to avoid antibiotic treatment after they are well into their biofilm. Small subpopulations of bacteria that have managed to avoid being invaded by antibiotics may, in their desperate attempt to survive, become dormant and become immune to harsh environments like chemical treatment or antibiotic action. Persisted cells are the name given to these cells. Antibiotic-resistant cells do not proliferate in the presence of these drugs, and their ability to withstand treatment is not the result of genetic modifications. They resume dividing or are liberated from the biofilm, at which point they revert to their pre-persisted susceptibility profile. Overall, the complex nature of biofilms and the various mechanisms at play contribute to the persistence and resistance of bacteria within these structures, making them a significant challenge in combating antimicrobial resistance.

Bacteria benefit greatly from the biofilm environment due to the close proximity of multiple organisms. This not only enables communication strategies like quorum sensing, but also facilitates the transfer of mobile genetic elements. The biofilm environment promotes plasmid stability and enhances the transmission of resistance information. Additionally, bacteria often transfer transposable DNA elements that encode for factors promoting biofilm formation, which further sustains the biofilm and the infection in the patient[34].

Diseases Related Biofilm

Many diseases have been linked to biofilms, which has substantial implications for public health. These microbes stick to surfaces and create biofilms, which have been connected to a number of disease, including ear, wound, and chronic lung infections. Biofilms have the ability to colonize medical devices such as implants and catheters. Over 80% of bacterial infections are caused by biofilms. They defend against outside therapies and increase the pathogenicity of microorganisms. When it comes to surviving harsh environments including UV rays, pH stress, chemical exposure, phagocytosis, dehydration, and antibiotics, biofilms are very tenacious. Sadly, detection and treatment of biofilm-associated disorders are difficult due of their resistance to both the host immune system and traditional biocides[12,25].

a . Cystic Fibrosis (CF)

A lung disease characterized by the production of thick, sticky mucus that obstructs the airways, leading to breathing difficulties. Approximately 80% of CF patients suffer from long-term infections caused by P. aeruginosa. This bacterium can also be found on medical equipment and devices. However, treating persistent P. aeruginosa infections is challenging as there are limited effective antibiotics available[35]

b. Dental plaque

is a crucial aspect of dentistry, and extensive research has been conducted on biofilms in the oral cavity [35]. Dental plaque consists of approximately 700 species of bacteria and archaea. This plaque is responsible for various oral disorders, including dental caries, periodontitis, and gingivitis. The initial colonization and subsequent formation of biofilms are essential processes in dental plaque development.

In order to adhere to enamel surfaces, bacteria in dental plaque rely on communication with each other. If bacteria are unable to stick to the tooth surface, they are washed away by saliva [36].

c. Wounds

Biofilms are frequently present in chronic wounds. Unlike acute wounds that are typically not associated with biofilms, chronic wounds that are biofilm-related persist and heal slowly. While biofilms typically form on the outer layer of the wound, some biofilms can also be embedded in the deeper layers of the wound [37].

d. Urinary infection

In the case of urinary infections, the presence of biomaterials in the urinary tract, such as catheters, increases the likelihood of bacterial biofilm formation, leading to infection. Bacteria can adhere to the surfaces of synthetic foreign bodies. Almost all urinary catheters are infected by bacterial biofilms, both on the inner and outer surfaces. For instance, biofilms formed by P. mirabilis can become crystalline and block catheters, necessitating their replacement [38].

e. Prosthetic joint infections

Involve gram-positive bacteria, such as staphylococci. Shortly after surgery, bacteria from the blood or lymph can attach to the surface of prosthetic joints and rapidly form biofilms. Biofilm infections on these implants may take some time to show signs like anxiety, in contrast to conventional bacterial infections that give symptoms like fever [39].

f. Cardiac Valve Infection

Prosthetic valve endocarditis is a disorder that can arise from the development of bacterial biofilm on artificial heart valves in the event of cardiac valve infections [38]. Biofilm buildup has the potential to damage or clog the prosthetic heart valve, causing decreased flow, turbulence, or even leaking. Separated biofilm cells can enter the circulation and infect other organs. Blood clots made by biofilm fragments can also obstruct blood flow to vital organs including the kidneys and brain [39].

CONCLUSIONS: The creation of biofilms gives many bacteria the ability to move from one planktonic form to another. Biofilms are a very normal and common condition for bacteria. Bacteria that generate biofilms are critical to animal health. Numerous bacteria, can form biofilms. In bacterial infections, biofilms may both make the organism more pathogenic and protect it against external treatments. Phagocytosis, UV radiation, pH stress, chemicals, dehydration, and antibiotics pose a major danger to the cells protected by biofilms.

REFERENCES

1. Brigitte Carpentier, O. Cerf, in Encyclopedia of Food Microbiology, 1999
2. Staudt C, Horn H, Hempel DC, Neu TR (December 2004). "Volumetric measurements of bacterial cells and extracellular polymeric substance glycoconjugates in biofilms". *Biotechnology and Bioengineering*.
3. Sensors (Basel). 2015 Feb; 15(2): 4229-4241. Published online 2015 Feb 12.
4. PRATT L A, KOLTER R 1998 Genetic analysis of *Escherichia coli* biofilm formation-roles of flagella, motility, chemotaxis and type Ipili. Mol Microbiol 30: 285–93
5. O'toole G A, Kolter R 1998 Flagellar and twitching motility are necessary for pseudomonas aeruginosa biofilm development. Mol Microbiol 30: 295–304
6. WATNICK P I, KOLTER R 1999 steps in the development of a *Vibrio cholerae* El Tor biofilm. Mol Mcrobiol 34: 586–95
7. DAVIES D G, GEESEY G G 1995 Regulation of the alginate bio- synthesis gene alg C in Pseudomonas aeruginosa during biofilm de- velopment in continuous culture. Appl Environ Microbiol 61: 860–7
8. HEILMANN C, GERKE C, PERDREAU-REMINGTON F, GOTZ F 1996 Characterization of Tn917 insertion mutants of Staphylococcus epidermidis affected in biofilm formation. Infect Im- mun 64: 277–82
9. KIM K Y, FRANK J F 1995 Effect of nutrients on biofilm formation by Listeria monocytogenes on stainless steel. J Food Protect 58: 24–8
10. LANDINI P, ZEHNDER A J B 2002 The global regulatory hns gene negatively affects adhesion to solid surfaces by anaerobically grown Escherichia coli by modulating expression of flagellar genes and lipopolysaccharide production. J Bacteriol 184: 1522–9
11. GORSKI L, PALUMBO J D, MADRELL R E 2003 Attachment of Listeria monocytogenes to radish tissue is dependent upon tempera- ture and flagellar motility. Appl Environ Microbiol 69: 258–66
12. HOMMAIS F, LAURENT-WINTER C, LABAS V, KRIN E, TEN- DENG C, SOUTOURINA O, DANCHIN A, BERTIN P 2002 Effect of mild acid pH on the functioning of bacterial membranes in Vibrio cholerae. Proteomics 2: 571–9
13. Branda SS, Vik S, Friedman L, Kolter R. Biofilms: The matrix revis- ited. Trends Microbiol. 2005; 13: 20–26.
14. Davey ME, O'Toole GA. Microbial biofilms: From ecology to mo- 32. lecular genetics. Microbiol Mol Biol Rev. 2000; 64: 847–86.
15. Karatan E, Watnick P. Signals, regulatory networks, and mate- rials that build and break bacterial biofilms. Microbiol MolBiol Rev. 2009; 73: 310–347.
16. Feldman MF, Wacker M, Hernandez M, Hitchen PG, Marolda CL, et al. Engineering N-linked protein glycosylation with diverse O antigen lipopolysaccharide structures in Escherichiacoli. Proc 35. Natl Acad Sci USA. 2005; 102: 3016–3021
17. Garrett TR, Bhakoo M, Zhang Z. Bacterial adhesion and biofilms on surfaces. Prog Nat Sci. 2008; 18: 1049–1056
18. Toyofuku MT, Inaba T, Kiyokawa N, Obana Y, Yawata, Nomura N. 59. Environmental factors that shape biofilmformation, Bioscience, Biotechnology, and Biochemistry. 2016; 80: 7–12.
19. Donlan RM, Costerton JW. Biofilms: survival mechanisms of clin- 33. ically relevant microorganisms. Clinical Microbiology Reviews. 2002; 15: 167–193.
20. Sauer K, Camper AK, Ehrlich GD, Costerton JW, Davies DG. Pseudomonas aeruginosa displays multiplephenotypes during development as a biofilm. Journal Of Bacteriology. 2009; 184: 1140–1154
21. Dunne W. Bacterial adhesion: seen any good biofilms lately? 34. Clinical Microbiology Reviews. 2002; 15: 155-166.
22. Zhao X, Zhao F, Wang J, Zhong N. Biofilm formation and control strategiesof foodborne pathogens: food safety perspectives. RSC Advances. 2017; 7: 36670–36683.
23. Petrova OE, Sauer K. Sticky situations: key components that con- trol bacterial surface attachment. Journal of Bacteriology. 2012; 194: 2413–2425.
24. Donlan RM. Biofilms: microbial life on surfaces. Emerging Infec- tious Diseases. 2002; 8: 881–890.
25. Kostakioti M, Hadjifrangiskou M, Hultgren SJ. Bacterial biofilms: development, dispersal, and therapeutic strategies in the dawn of the postantibiotic era. Cold Spring Harbor Perspectives in Medicine. 2013; 13: a010306.
26. Sekhar S, Kumar R, Chakraborti A. Role of biofilm formation in the persistent colonization of Haemophilus influenzae in chil- dren from northern India. J Med Microbiol. 2009; 58:1428-1432
27. Fux CA, Costerton JW, Stewart PS, Stoodley P. Survival strategies of infectious biofilms. Trends Microbiol. 2005; 13: 34-40.
28. Tasneem U, Yasin N, Nisa I, Faisal Shah, Ubaid Rasheed, et al. Biofilm producing bacteria: A serious threat to public health in 58. developing countries. Journal of Food Science and Nutrition.2018; 1: 25-31.
29. Larson EL, Gomez-Duarte C, Lee LV, Latta PD, Kain DJ, et al. Mi- crobial flora of hands of homemakers. American J Infect Control. 2003; 31: 72-79.
30. Novick RP, Geisinger E. Quorum sensing in staphylococci. Annu Rev Genet. 2008; 42: 541-564.
31. Avery SV (2006) Microbial cell individuality and the underlying sources of heterogeneity. Nat Rev Microbiol 4:577–587 *Clinical Orthopaedics and Related Research* 437():p 41-47, August 2005
32. Hall MR, Mc Gillicuddy E, Kaplan LJ. Biofilm: basic principles, pathophysiology, and implications for clinicians. Surg Infect 40. (Larchmt.) 2014; 15: 1–7.

33. Jones AM, Govan JR, Doherty CJ, Dodd ME, Isalska BJ, et al. Spread of a multiresistant strain of Pseudomonas aeruginosa in an adult cystic fibrosis clinic. Lancet. 2001; 358: 557–558.
34. Marsh PD. Dental plaque as a microbial biofilm. Caries Res. 2004; 38: 204–211.
35. Sbordone L, Bortolaia C. Oral microbial biofilms and plaque-re-lated diseases: microbial communities and their role in the shift 57. from oral health to disease. Clin Oral Investig. 2003; 7: 181–188.
36. James GA, Swogger E, Wolcott R, Pulcini EL, Secor P, et al. Bio- films in chronic wounds. Wound Repair Regen. 2008; 16: 37–44.
37. Jacobsen SM, Stickler DJ, Mobley HL, Shirtliff ME. Complicated catheter-associated urinary tract infections due to Escherichia coli and Proteus mirabilis. Clin Microbiol Rev. 2008; 21: 26–59.
38. Trampuz A, Zimmerli W. Prosthetic joint infections: update in diagnosis and treatment. Swiss Med Wkly. 2005; 135: 243–251.
39. Costerton JW, Lewandowski Z, Caldwell DE, Korber DR, Lappin- 30. Scott HM. Microbial Biofilms. Annu Rev Microbiol.