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Bacterial Biofilm and Its Health Impact: A Review

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Abstract: Biofilm is a structured community of bacterial cells enclosed in a self-produced matrix attached to biotic or abiotic surfaces. The formation of a biofilm allows a lifestyle that is entirely different from the planktonic state by protecting cells in the biofilm from physical and mechanical interference. In most biofilms, of the dry mass, the matrix can account for over 90% and the left 10% are bacterial cells. Biofilm forming bacteria have a major impact on both animal and human health, since they can resist antibiotic treatment and host immune responses. The new generation molecular techniques, including polymerase chain reaction, loop-mediated isothermal amplification and confocal laser scanning microscopy have good reproducibility to diagnose a biofilm. Combination therapies of antibiofilm antimicrobials are required for weakening or degradation of the biofilm and for the elimination of biofilm-forming bacteria respectively.

Key words: Bacteria · Biofilm · Extracellular Polymeric Substance · Matrix

INTRODUCTION

Bacteria are able to live either as planktonic cells or as members of organized surfaces attached to microbial communities called biofilms. Biofilms are the most major form of microbial life and are biologically active matrix of cells and extracellular substances in association with a biotic or abiotic surface. The extra cellular polysaccharides are an insoluble and slimy secretion that is released by bacterial cells, encases millions of joining cells in a well-organized and structured matrix. Formation of a bacterial biofilm is a developmental process that begins when a cell attaches to a surface [1].

Biofilm formation is considered a virulence determinant in microorganisms and it strongly contributes to microbial resistance to conventional antimicrobial agents, host protective immune responses, hostile environmental pressures/stresses, predation and shear forces. Dispersed biofilm microbial cells have a greater capability to cause cytotoxicity, virulence and mortality than their planktonic counterparts [2].

Biofilm infections are challenging to diagnose, because biofilm bacteria may not be easily isolated using standard culture swab techniques. Aggregation of bacteria to specific tissue of host often gives negative culture result and false-positive culture result is obtained due to free-floating planktonic microbes. Molecular methods of diagnosis have been shown to have a higher degree of sensitivity.

The objective of this seminar paper is therefore to review the available literatures on the formation of biofilms, to highlight its impact on health, diagnostic and treatment approaches.

Bacterial Biofilm: Bacterial biofilm is a structured community of bacterial cells enclosed in a self-produced matrix (exopolysaccharides, proteins, enzymes and extracellular DNA) attached to biotic or abiotic surfaces [3]. The EPS (extracellular polymeric substance) matrix makes up 90% of the volume of the biofilm and imparts both a physical and chemical robustness to the community by resisting mechanical forces and decreasing the penetration of toxic chemicals (e.g. antibiotics, host defense molecules) [4].

Almost all bacteria can grow as a biofilm and biofilms can be found in every ecosystem including natural, engineered and pathogenic settings. Growth as a biofilm is considered to be a protective mode that allows for

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survival in hostile environments. Many pathogenic bacteria use the biofilm growth mode to persist in the host and avoid clearance by the immune system and antimicrobial chemotherapies leading to the development of persistent infections [5, 6].

Structure of Biofilm: Structure and composition of biofilms depend on the environmental conditions, the surface, the bacterial genome, species, strain and the components embedded in the biofilm matrix. Generally, biofilms have a heterogeneous structure including water, EPS, cells and embedded particles with thicknesses ranging between several µm and several mm. The compositions and morphologies of different biofilms can diverge as even one bacterium can create different types of biofilms when exposed to different conditions [7].

The structure of the extracellular polymeric substance (EPS) matrix of biofilm is composed of one or more of extracellular polysaccharides, DNA and proteins. Additionally, bacteria grown in biofilms can form intricate and complex structures such as channels that allow for water, air and nutrients to get to all parts of the structure [8].

Stages/Processes in the Formation of Biofilms: Biofilm formation can be described in five stages, including: development of a surface conditioning film, movement of microorganisms into close proximity with the surface, adhesion (reversible and irreversible adhesion of microbes to the conditioned surface), growth and micro colony and biofilm formation, biofilm cell detachment /dispersal. Each of these processes will be considered in turn [9].

Development of a Surface Conditioning Film: Surface conditioning film is a complex surface consisting of polysaccharides and glycoproteins. It serves as the platform for surface adherence of microorganism, hence seen as pre requisite to the attachment stage and it provides a concentrated nutrient source and important trace elements. Components of blood, tears, urine, saliva, intravascular fluid and respiratory secretions in an animal can also contribute to conditioning film [10].

Adhesion/Attachment: This is a crucial stage in biofilm formation involving attachment or adhesion of bacteria to a substratum, the process occurs in two steps, reversible and irreversible adhesion Reversible adhesion is an initial event in biofilm development, by interaction between planktonic bacteria and substrate surface in which some bacteria attach to the substrate surface only for a brief period and then detach from it, as a result of a response to the absence of nutrient availability, it lasts a few minutes [11].

Cellular and extracellular appendages play a vital role in microbial surface attachment. The common appendages often used in bacterial attachment to plant and animal tissues surfaces include pili and fimbriae, which consist of multiple different appendages and other structures such as curli, adhesins, intimins and invasins [12]. In the initial attachment, flagella and type IV pili mediated motilities play important roles. Flagella help to attach bacterial cells with surface, whereas Type IV pili mediated twitching motilities enable attached cells to aggregate. Once the attachment becomes irreversible, the biofilm begins to grow and mature [13].

Development and Maturation: Microbial growth, development of microcolonies (35 layers deep community of bacterial cell) and recruitment of additional microorganism occurs after adsorption of macromolecules and attachment of microbial cells to a substratum. Subsequently, after the initial colonization, the biofilm grows through a combination of cell division and recruitment. As the attachment of microorganism occurs, the colonizing bacteria grow with the production and accumulation of extracellular polymers. Mere attachment of microorganism to a surface does not imply the formation of micro colony, hence, coherent cell cell interactions are needed to establish and hold the micro colony together. In a thick biofilm (>100 layers), bacteria are arranged according to their metabolism and aerotolerance, in which anaerobic bacteria prefer to live in deeper part to avoid exposure to oxygen. Once maximum thickness attained, the final stage of biofilm development occurs [14].

Detachment/Dispersal: Detachment of cells from the biofilm colony and their dispersal into the environment marks the final and indeed an essential stage of biofilm life cycle; this contributes to biological dispersal, bacterial survival and disease transmission. It lasts nine to twelve days. Biofilm detachment can also occur as a result of a low nutrient condition indicating. Therefore, detachment is not just important for promoting genetic diversity, but also escaping unfavorable habitat aiding in the development of new niches [15].

Signaling in Biofilm Formation (Quorum Sensing): Quorum sensing is a process by which the microorganisms within a developing biofilm remain in constant communication (Bacteria 'talk' to each other) using chemicals for words including small molecules or small peptides and acyl homoserine lactone for Gram positive and gram negative bacteria respectively. This communication helps to further coordinate the developing biofilm's architecture, microbial growth rates, enzyme production, species interactions, toxin production, antimicrobial resistance and bacterial virulence factor [16].

Layers of the Biofilm: The biofilm comprises three layers; The initial layer consists of the linking or conditioning film which is attached to the surface of the tissue or biomaterial, the second layer is the biofilm base which contains microbes and the surface film acts as an outer layer (third layer) where planktonic organisms are released as free floating organisms and spread to the surrounding compartment [17].

Impact of Bacterial Biofilm on Health

Impact on Animal: Biofilms are recognized as being clinically important in veterinary medicine [18]. Biofilm is implicated in most bacterial infections in the human body and is a cause of persistent chronic infections, from which dental disease and infection are common [19]. A dental biofilm is first established in stagnant areas of the teeth where the bacteria are protected. If left undisturbed by insufficient dental hygiene procedures, the supragingival biofilm may gradually spread along the root of the tooth into the periodontal pocket and a subgingival biofilm is formed [20].

Biofilms on the tooth surfaces may cause dental caries, while supra- and subgingival biofilms along and under the gingival margin may cause periodontal diseases. Dental calculus develops when non mineralized biofilms, extremely rich in oral bacteria, become mineralized with calcium phosphate mineral salts. These mineralized biofilms form both supragingival and subgingivally. Non-mineralized dental biofilm entraps particles from the oral cavity, including large amounts of oral bacteria, human proteins, viruses and food remnants and preserves their DNA [21].

Chronic non healing wounds are the other clinical impact of biofilm formation. The chronic non healing wounds commonly harbor a complex, biofilm predominant microflora originating from multiple sources additionally, wounds of all types are frequently colonized or infected with antibiotic-resistant bacteria, which have been shown to be the most prevalent biofilm formers in chronic infections [22].

Resistance to Immune System: Bacterial resistance to leukocytes in biofilms was at first explained by a lack of penetration of leukocytes into biofilms and a decreased ability of phagocytes to kill biofilm encased bacteria. A mature biofilm has a dense polymeric matrix that is difficult to engulf by macrophages result in impairing biofilm clearance therefore, the infection becomes chronic [23].

Antibiotic Resistance: Biofilm has been described as a privileged environment for the horizontal spread of antibiotic resistance genes and virulence factors and conjugation between cells within biofilm has been reported as being more efficient than among free-living (planktonic) bacterial cells. Bacteria in a biofilm are more resistant to antibiotics or the reactive molecules that are produced by the host immune systems than planktonic bacteria [24].

Plasmid borne antibiotic resistance could also be possible in single species or multi species biofilm by horizontal gene transfer. In biofilms, the frequencies of horizontal plasmid transfer are much higher than between planktonic cells Multi species biofilm is more resistant to antimicrobial agents, although some genetically heterozygous single species biofilms are highly resistant to antimicrobial therapy as well as the host immune response [25].

Biofilm and Zoonosis: Biofilm forming microorganisms are frequently cause infection in human and animal and can be transmitted from each other. Biofilm forming bacteria in oral cavity of dogs can transmit infection to human through bites [26]. Similarly, biofilms formed in water tank, drinker and farm equipment can be transmitted easily to the farm animal as well as animal handlers and workers. *P. aeruginosa*, *Acinetobactorbaumannii*, *Staphylococcus aureus* are the notable examples, which can cause wound infection and implanted device related infection in man and animal [27]. *Mycobacterium avium* and *M. intracellulare* are also potentially zoonotic and can form biofilm in drinking water system [28].

Diagnostic Approaches of Biofilm Infection: Biofilm infections are challenging to diagnose, because biofilm bacteria may not be easily isolated using standard culture swab techniques. Aggregation of bacteria (biofilm) to

specific tissue of host often give negative culture result and false positive culture result is obtained due to free floating planktonic microbes [29].

Molecular methods such as polymerase chain reactions, loop-mediated isothermal amplification (LAMP) and confocal laser scanning microscopy (CLSM) have been shown to have a higher degree of sensitivity than culture. Other diagnostic criteria like, positive culture identification of microbes, microscopic evidence of microbial aggregates and biofilm structure known to be associated with biofilm formation. History of a condition that predisposes to the development of biofilm formation, recurrence of infection despite appropriate antimicrobial therapy and evidence of antibiotic failure despite adequate antimicrobial therapy can readily be applied to raise the suspicion of the presence of a biofilm infection [30].

Therapeutic Approaches of Biofilm Infection: As the biofilm-producing bacteria are badly resistant to antimicrobial agent, incorporation of antibiofilm agents with conventional antibiotics are the need of the hour for effective control of biofilm-associated infection and it provides several benefits: increasing bacterial susceptibility and enhancing effectiveness of antibiotics and antiseptics, reducing the propensity for antibiotic resistance associated horizontal gene transfer and reducing the pathogenic potential in polymicrobial chronic infections [31].

The exogenous enzymatic treatment facilitates the detachment of cells from the biofilm colony and their planktonic release into the environment. Various classes of enzymes that used for the dispersal of biofilms include; proteases, deoxyribonucleases and glycoside hydrolases. By using these enzymes at elevated concentrations result in dispersion of cells, making the microbes more susceptible to the host immune system and antibiotics/antimicrobials. Inhibition of biofilm by DNase enzyme is approach supported by a number of studies, by using exogenous DNase enzymes can inhibit/weaken the formation of biofilms, or can disperse preformed biofilms. Thus, DNase treatment of biofilms has been shown to increase sensitivity to antimicrobials [32].

Biofilms may also be inhibited by controlling the formation of amyloid-like fibers with the two molecules of AA-861 and parthenolide, which were screened from among a collection of hundreds of known bioactive molecules, are a group of small molecules that could potentially attenuate bacterial virulence or inhibit biofilm formation. AA 861 prevented the TasA protein from forming functional amyloid-like fibers. Chemical counter measures such as, inhibition of signaling molecule in quorum sensing also help to inhabit bacterial communication, result in susceptibility to therapeutic agents. Anti adhesion surface of implanted devices are helps to reduce the attachment of pathogenic bacteria leading to decreased in biofilm formation.Mechanical removal of biofilms can be employed to treat biofilm, these include regular brushing of teeth, sharp or surgical and ultrasonic. Debridement forms an important part of the management of all wounds [33].

CONCLUSION

Biofilm forming bacteria produce exopolysaccharide as a major component of their matrix which provides protection to the bacterial community. As the biofilm matures, increased EPS accumulation, result in reduced entry and activity of antimicrobial agents. This makes biofilm-forming pathogens progressively more resistant to antibiotic and hosts immune systems. The currently used antimicrobial agents in the clinical area were developed mostly to act on planktonic cells, due to a shortage of anti-biofilm and lack of awareness toward the impact of biofilm formation, result in an increased risk of the problem. Due to microbial infections related to biofilms are usually treated with high or combined doses of antimicrobials for a prolonged period of time, it results in other consequences for the patient. Generally, it is shown that strategies to control biofilm are insufficient.

Therefore, detailed understandings, up to molecular level of the structure and development of biofilm, minimizing use of antimicrobial agents that only act on planktonic cells by maximizing production of anti-biofilm agents and aware of the society toward the control of biofilm may helpful to improve their control strategy. To reject consequences on patients, due to the administration of a higher or combined dose of antimicrobials during treatment of biofilm infection, must be considered for future invention.

REFERENCES

1. Felipe, V., M.L. Breser, L.P. Bohl, R. Elizabete, M. Andrea, C. Graciela and C. Porporatto, 2019. Chitosan disrupts biofilm formation and promotes biofilm eradication in Staphylococcus species isolated from bovine mastitis. Int. J. Biol. Macromol., 126: 60-67.

2. André, L., C. Anna, P. Thaís, D. Lívia, H. Marta, M. Ana, C. José and R. Maryam, 2018. What are the advantages of living in a community? A microbial biofilm perspective. Mem Inst Oswaldo Cruz, Rio de Janeiro, 113(9).
3. Beebout, C.J., A.R. Eberly, S.H. Werby, S.A. Reasoner, J.R. Brannon, S. De, M.J. Fitzgerald, M.M. Huggins, D.B. Clayton, L. Cegelski and M. Hadjifrangiskou, 2019. Respiratory heterogeneity shapes biofilm formation and host colonization in uropathogenic *E. coli*. mBio., 10(2).
4. Lieleg, O, M. Caldara, R. Baumgartel and K. Ribbeck, 2011. Mechanical robustness of Pseudomonas aeruginosa biofilms. Soft Matter, 7: 3307-3314.
5. Thurlow, L.R., M.L. Hanke, T. Fritz, A. Angle, A. Aldrich, S.H. Williams, I.L. Engebretsen, K.W. Bayles, A.R. Horswill and T. Kielian, 2011. Staphylococcusaureus biofilms prevent macrophage phagocytosis and attenuate inflammation *in vivo*. J Immunol., 186: 6585-6596.
6. Mathe, L. and P. Van Dijck, 2013. Recent insights into Candida albicans biofilm resistance mechanisms. Curr. Genet., 59: 251-264.
7. Marchand, S., J. Block, V. Jonghe, A. Coorevits, X. Heyndrickx and L. Herman, 2012. Biofilm Formation in Milk Production and Processing Environments; Influence on Milk Quality and Safety. In Compr. Rev. Food Sc. Food Safety, 11(2): 133-147.
8. Jacques, M., A. Virginia and D. Yannick, 2010. Biofilm formation in bacterial pathogens of veterinary importance. Anim. Heal Res. Rev., 11(2): 97-121
9. Banerjee P., M. Madhulika and S. Varsha, 2015. Biofilm Formation: A Comprehensive Review. Int. J. Phar. Res. Health Sci., 3(2): 556-560.
10. Takahashi, H., T. Suda, Y. Tanaka and B. Kimura, 2010. Cellular hydrophobicit of Listariamonocytogenes involves initial attachment and biofilm formation on the surface of polyvinyl chloride. Lett. Appl. Microbiol., 50(6): 618-625.
11. Alebachew, T., H. Sibehat, T. Ayichew and H. Tsehaye, 2016. Review on Biofilm and Microbial Adhesion. Int. J. Microbiol. Res., 7(3): 63-73.
12. Spurbeck, R., A. Stapleton, J.R. Johnson, S.T. Walk, T.M. Hooton and H.L. Mobley, 2011. Fimbrial profiles predict virulence of uropathogenic Escherichia colistrains: Contribution of ygi and yad fimbriae. Infect Immun., 79(12): 4753-4763.
13. Rabin, N., Y. Zheng, T. Opoku, E. Bonsu and H. Sintim, 2015. Biofilm formation mechanisms and targets for developing antibiofilm agents. Fut Med. Chem., 7(4): 493-512.
14. Abdullahi, U.F., E. Igwenagu, A. Mu'azu, S. Aliyu and M.I. Umar, 2016. Intrigues of biofilm: A perspective in veterinary medicine. J. Vet. World, 9(1): 12-18.
15. Kaplan, J.A., 2010. Biofilm dispersal: mechanisms, clinical implications and potential therapeutic uses. J. Dent Res., 89(3): 205-218.
16. Galié, S., G.C. García, E.M. Miguélez, C.J. Villar and F. Lombo, 2018. Biofilms in the Food Industry: Health Aspects and Control Methods. Front Microbiol., 9: 898.
17. Veerachamy, S., Y. Tejasri, M. Geetha and K.D.V. Prasad, 2014. Bacterial adherence and biofilm formation on medical implants: A review. J. Eng. in Med., 228(10): 1083-1099.
18. Gardner, A.J., S.L. Percival, D.C. Knottenbelt and C.A. Cochrane, 2011. Biofilms and Veterinary Medicine: Berlin Heidelberg: Springer, pp: 11-28.
19. Cunha, E., T. Tiago, P. Ana, N. Telmo, S. Raquel, M.S. Jorge, S. Berta, T. Luís, S. Ana and O. Manuela, 2018. Potential of two delivery systems for nisin topical application to dental plaque biofilms in dogs. BMC Vet. Res., 14(1): 375.
20. Akcali, A. and N.P. Lang, 2017. Dental calculus: the calcified biofilm and its role in disease development. Periodontol., 76(1): 109-115.
21. Larsen, T. and N.E. Fiehn, 2017. Dental biofilm infections. APMIS, 125(4): 376-384.
22. Hurlow, J., E. Blanz and J.A. Gaddy, 2016. Clinical investigation of biofilm in nonhealing wounds by high resolution microscopy techniques. J. Wound Care, 25(9): 11-22.
23. Arciola, C., D. Campoccia and L. Montanaro, 2018. Implant infections: adhesion, biofilm formation and immune evasion. Nat. Rev. Microbiol., 16(7): 397-409.
24. Bravo, M.L., N.C. Silva, M.X. Rodrigues, G.O.E. Silva, E. Porto and G.L. Sturion, 2019. Phenotypic and genotypic characterization of *Staphylococcus* spp. Isolated from mastitis milk and cheese processing: Study of adherence and biofilm formation. Food Res. Int. doi:10.1016/j.foodres.2019.04.017.
25. Savage, V., I. Chopra and A.J. O'Neill, 2013. Staphylococcus aureus biofilms promote horizontal transfer of antibiotic resistance. Ant Agents. Chemother, 57(4): 1968-1970.
26. Zambori, C., C. Cumpanasoiu, B. Mladin and E. Tirziu, 2013. Biofilms in oral cavity of dogs and implication in zoonotic infections. Sci. Pap. Anim. Sci. Biotech., 46(1): 155-158.

27. Abrahamian, F. and E. Goldstein, 2011. Microbiology of animal bite wound infections. ClinMicrobiol. Rev., 24(2): 231-246.
28. Percival, S., S. Malic, H. Cruz and D. Williams, 2011. Introduction to biofilms: Biofilms and Veterinary Medicine. Springer, Berlin, Heidelberg, pp: 41-68.
29. Abdullahi, U.F., R. Naim, S.A. Liyu, A. Mu'azu, W.R. Wan Taib, A. Saleh and A.A. Baig, 2015. Loop mediated isothermal amplification, an innovation in gene amplification: Bridging the gap in molecular diagnostics; a review. Indian J. Sci. Technol., 9(12): 57-67.
30. Rowson, C. and R. Townsend, 2016. Biofilms: prevention and treatment. Br J. Hosp Med Lond., 77(12): 699-703.
31. Bowler, P.G. , 2018. Antibiotic resistance and biofilm tolerance: a combined threat in the treatment of chronic infections. J. Wound Care, 27(5): 273-277.
32. Jakubovics, N., R. Shields, N. Rajarajan and J. Burgess, 2013. Life after death: the critical role of eDNA in microbial biofilms. Lett. App. Microb., 57(6): 467-475.
33. Chen, M., Q. Yu and H. Sun, 2013. Novel strategies for the prevention and treatment of biofilm related infections. Int. J. Mol. Sci., 14(9): 18488-18501.