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International Journal of Current Research
Vol. 6, Issue, 12, pp.10420-10424, December, 2014

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

ISSN: 0975-833X

RESEARCH ARTICLE

BIOFILM: A NATURAL MECHANISM OF BACTERIAL RESISTANCE

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ARTICLE INFO

Article History:
Received 17th September, 2014
Received in revised form
15th October, 2014
Accepted 25th November, 2014
Published online 27th December, 2014

Key words:
Biofilm, Resistance,
Bacteria, Antibiotic,
Mechanism.

ABSTRACT

Biofilms are a population of cells that grown attached to a surface involved in exopolysaccharide matrix which protects them from attack by antibiotics or immune system. Many chronic and persistent infections are caused by bacterial biofilms, which increase significantly the resistance to antimicrobials. Different mechanisms of resistance have been proposed such as: synthesis of extracellular polymers (physical barrier effect), enzymatic modification of antimicrobials, decrease of bacterial growth rate, phenotypic changes in bacterial cells (as a result of the acquisition of resistance genes within the biofilm), and the persistence of a small group of cells in the bacterial community. But all is not bad in life, bacterial biofilms also may have a protective role, for example the normal flora.

INTRODUCTION

Microorganisms are susceptible to environmental factors that around them, for that reason they are not able to generate significant damages to a living organism for themselves (Betancurth *et al*., 2004; Bjarnsholt, 2013; Decho, 2013). In that context, the microorganisms have been associated forming biofilms, which are complex communities of bacteria surrounded by a exopolymer matrix; they form colonies adherent to inert surfaces for example catheters, prosthesis, glasses, or human tissues and organs (Ceri *et al.*, 1999; Donlan, 2011). It has reported that a biofilm consist of bacteria, water, exopolisacaride matrix, proteins, nucleic acids and bacterial lysis products (Decho, 2013). Biofilm has an architecture as a mushroom-shaped tower wrapped in exopolysaccharide matrix and proteins that are produced by resident bacteria. It has been observed that bacterial population in the biofilm shows an extraordinary resistance to biocides, to antimicrobial treatments also and host immune response (Bridier *et al*., 2011; Sauer, 2003).

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The first step in the formation of a biofilm is the coaggregation process formed by the association between bacteria, consisting in cell-cell recognition, allowing bacteria that constitute to recognize and adhere to each other by adhesins. These structures, along with the phenomena of hydrophobicity, electrostatic and Van der Waals forces favor the binding to proteins, glycoprotein and polysaccharide receptors on the surfaces of host (for example: dental plaque, damaged endothelium in native valve) or biomaterials as prosthetic devices (Costerton, 1999; Decho, 2013; Johnjulio *et al*., 2012; Donlan and William, 2002). Therefore, a stress sharp as that produced by a change in the flow direction or flow rate or changes in the concentration of certain substrates, can cause increased erosion of the biofilm and promote cell detachment or the otherwise cause greater aggregation (Matthew and Maestre, 2004).

Bacterial Biofilm

The presence of biofilm gives certain advantages to bacteria as protection from the environment, resistance to the bactericidal action of the antimicrobial, altered host defense mechanisms (hinders macrophage phagocytic activity by interfering with the coating antibodies to block opsonization and phagocytosis) (Kostakioti *et al*.,, 2013; Shiau and Wu, 1998). Bacteria exist in nature in two forms or stages: free-floating planktonic

bacteria and bacteria forming biofilm (Donlan and William, 2002). Since Koch´s times, bacteriologists and clinicians have focused the study of planktonic bacteria. This is due among other things to the fact that the investigation of bacterial biofilms is difficult. Unfortunately, this focus on the development of planktonic cells in laboratory cultures has limited the understanding regarding the interactions between bacteria and host; only a small fraction of bacteria live in planktonic stage (Thomas and Nakaishila, 2006). It is postulated that 99% of all bacterial cells exist as biofilm and only 1% as planktonic stage (Costerton *et al*., 1995; Costerton, 1999; Sanclement *et al.,* 2005). Bacterial biofilms are created when planktonic cells are sensing a surface which they adhere and then produce chemical signals to coordinate differentiation and the formation of structures (including the production of polysaccharide protective cover) (Romanova *et al*., 2011; Scott and Manning, 2003). The life cycle is a dynamic process that can be divided into 3 parts: adhesion, growth and cell separation (Ramadan *et al.,* 2005).

Biofilm biology focuses on the life cycle and interactions with the environment. Bacteria can create conditions to form biofilms almost any liquid environment. The solid-liquid interface between a surface and an aqueous medium such as water, blood and so on provides an ideal environment for the attachment and growth of microorganisms (Sanderson *et al.,* 2006). Thus, biofilms are ubiquitous in nature and are worldwide (Chole and Faddis, 2003). Bacterial biofilms represent an ancient prokaryotic survival strategy. This is because bacteria acquire significant advantages because they provide protection against environmental fluctuations, humidity, temperature, pH, also concentrating nutrients and facilitating waste disposal. Fossil´s registration shows that prokaryotes have been living in biofilms for more than 3 billion years (Bridier *et al*., 2011; Flores-Encarnación *et al*., 2009; Merle *et al.,* 2002). The ability to form biofilm seems not restricted to any specific group of microorganisms, know a days it is considered that under proper environmental conditions the vast majority of bacteria, regardless of the species, may exist within biofilms adhering to surfaces at an solid-liquid interface or liquid-gas interfase, including microorganisms that are causative agents of numerous infectious diseases (Anderl *et al.,* 2000; Bjarnsholt, 2013).

It has been reported that the formation of bacterial biofilm may be generated in response to extreme changes in the environment, for example temperature, limitation of nutrients, oxygen availability, extreme pH (O'Toole *et al.,* 2000). For years bacterial biofilms have caused multiple infectious diseases in human and they have been cause of contamination of orthopedic and medical devices (Costerton, 1999; Cha *et al*., 2013). List includes Gram positive and negative bacteria, aerobic and anaerobic bacteria, some yeasts and actinomycetes. Among bacterial genera may be mentioned: *Streptococcus spp* causing bacterial endocarditis, dental caries, necrotizing fasciitis; *Staphylococcus spp* causing musculoskeletal infections, colonization of sutures, catheters, arteriovenous pathways, heart valves, prostheses; *Pseudomonas spp* causing contamination of contact glasses; *Haemophilus influenzae* causing chronic otitis; *Escherichia coli* causing prostatitis, colonization of urinary catheters (Costerton *et al*., 1999; Ferrières *et al*., 2007; Flores-Encarnación *et al*., 2014; Post, 2001). Human normal flora of the digestive tract, skin and other anatomical sites such as vagina, are efficient barriers to the establishment, growth and development of pathogenic bacteria (Costerton *et al*., 1995; Domingue *et al.,* 1991; Leccese-Terraf *et al*., 2014; Marsh, 2004; Romanova *et al*., 2011).

Quorum sensing is a regulatory mechanism dependent accumulation of signal molecules (autoinducers) in the environment, which allows the bacteria sense the existing population density (Costerton, 1999). Myxobacteria were the first microorganisms which quorum sensing was observed. However, the best known example is the regulation of light production in *Vibrio fischeri,* a bioluminescent bacterium that lives by symbiont in organ generator of light of hawaiian squid. When *V. fischeri* is growing in planktonic stage the concentration of autoinducers is low and it not produce bioluminescence (Donland, 2002). It has been shown that biofilm formation in *P. aeruginosa* has two different systems of cell-cell signaling: *lasR-lasI* and *rhlR-rhlI* systems. Once obtained a sufficient density of population, cell-cell signaling reaches the concentrations required to activate genes involved in biofilm differentiation; mutants unable to produce both signals produce notoriously a thinner biofilm and without its typical architecture. They can be removed more easily from surfaces by surfactants. Adding acyl-homoserine lactone to the culture medium containing mutant biofilms gives rise to wild-type bacterial phenotype (Bassler, 2002; Steindler *et al.,* 2009; Whiteley *et al.,* 2001).

Biofilm and Resistance to Antibiotics

In the last 15 years, biofilms have been progressively recognized as important factors in the pathogenesis of many human persistent infections, including periodontal infection, dental plaque, pneumonia, chronic cystitis, bacterial endocarditis, osteomyelitis and chronic prostatitis (Diemond and Miranda, 2007; Donlan, 2011; Krom and Oskam, 2014).
One of the feats of modern Medicine has been the progress in the diagnosis and treatment of infectious diseases with antibiotics, making it possible to effectively control acute infections. However there are two exceptions to the rule: bacteria are innately resistant to drugs and bacteria that reside within biofilm have a greater resistance to antimicrobials than those living in planktonic stage (Hall-Stoodley *et al.,* 2008).
Clinical importance of bacteria forming biofilm is that they are more resistant to antibiotics; they can to survive against higher concentrations of antibiotics compared to planktonic stage bacteria (Stewart and Costerton, 2001). *P. aeruginosa* and *Streptococcus spp* biofilms are the best studied bacterial and they represent a public health serious problem. Biofilms produce physical barriers that are important for antimicrobial therapy. It has been found that biofilms cause an increasing antibiotic resistance in a factor reaches hundreds or thousands of times (Greenberg, 2003; Stewart and Costerton, 2001). *P. aeruginosa* biofilms have been difficult to treatment and eradicate in patients presenting acute respiratory diseases (O'Toole *et al.,* 2000). Another example is *S. mutans*, a bacterium causing caries and gingivitis in oral cavity (Kolenblander, 2000; Matsumura *et al.,* 2003). Also it is

speculated that the biofilm acts as a niche for the generation of resistant organisms given the ability of certain bacteria to exchange genetic material by conjugation and contribute to the transmission of possible factors of resistance to antibiotics or factors involved in adhesion and biofilm development (Guigo, 2001). Some recent studies argue that bacteria within biofilm are so sensible to antibiotics as planktonic bacteria, however persistence of cells depend by invulnerability that the exopolysaccharide matrix confers them to immune system. This would explain the presence of a subpopulation of persistent cells causing the chronicity of infection (Lewis, 2001). Different mechanisms have been proposed to try to explain the resistance of the biofilm to antibiotics: preventing or retarding the penetration of antimicrobial agent through the biofilm matrix; altereding growth rates of microorganisms in the biofilm due to physiological changes of biofilm stage (Donlan and William, 2002).

Antimicrobial molecules must diffuse through the biofilm matrix in an organized manner to inactivate the biofilm cells. Extracellular polymeric substances constituting the matrix act as a diffusion barrier for these molecules, influencing the rate of transport of the molecule within the biofilm or in the interaction of antimicrobial substance with biofilm matrix (Donlan and William, 2002). Biofilm gives the microcolonies a resistance mechanism different than those commonly described, being extremely effective and conferring resistance to bacteria by a factor of about 500 times than usual (Bruce *et al.,* 2007). Biofilms also provide an ideal niche for the exchange of extrachromosomal DNA (plasmids). Conjugation occurs between high rates of both planktonic and biofilm cells. It has been suggests that some strains of bacteria containing conjugative plasmids develop more easily biofilm (Guigo, 2001). Using DNA microarrays it compared gene expression in *E. coli* cells forming biofilm and planktonic stage cells. Comparison was a change in more than 600 genes, with 9% of the total as activated genes and 4.5% as inactive genes in the biofilm. When the profile of biofilm cells was compared with the exponential cell growth, there was a different expression pattern and only 230 genes expressed differentially.

Expression of 79 genes representing 1.84% of the genome of *E. coli* was significantly altered during formation of the biofilm compared with planktonic stage (Prigent *et al.,* 2001). Among the genes that increased the expression in biofilm were found those related with adhesion and auto aggregation, so as coding for structural proteins, for example: OmpC, OmpF and OmpT. Also *lpxC*, a gene coding for a protein associated with lipid synthesis, and *slp a* gene coding for outer membrane lipoprotein were expressed. *slp* and *ompC* genes have recently been associated in the early stages of biofilm formation on inert surfaces (Schembri *et al.,* 2003). In the analysis of DNA microarray of *Ps. aeruginosa* was observed that only 1% of expressed genes are different when bacteria is forming biofilm *vs* planktonic stage. The study revealed that the average expression of genes in cells forming biofilm is similar to planktonic cells, growning under similar environmental conditions (Whiteley *et al.,* 2001). A important protein by bacterial biofilm formation is RpoS, a subunit σs of RNA polymerase. RpoS control the expression of genes induced during the stationary phase of growth and it is considered as master protein, responsible for regulating of stress in *E. coli*. Schembri *et al.,* (2003) reported that 46% of the genes that were found differentially during biofilm growth were regulated by RpoS (Schembri *et al.,* 2003). RpoS of *P. aeruginosa* seems to have an opposite role to RpoS of *E. coli*. *rpoS* gene of *P. aeruginosa* was found suppressed in biofilm. A mutant of *P. aeruginosa* without *rpoS* formed more biofilm and it was also more resistant to antibiotic treatment.These findings are related with previous reports in which *rpoS* mutants of *P. aeruginosa* were the most virulent in mouse models. Biofilm formation and its characteristic resistance to antimicrobial agents are the cause of persistent and chronic infections. The biofilm has shown the ability to colonize a wide variety of medical devices and it is associated with important human diseases such as endocarditis, urinary tract infections, cystic fibrosis, otitis media, lacerations (Sauer, 2003).

Conclusion

The microorganisms throughout history have been able to adapt to adverse environmental conditions. Unfortunately, pathogens have developed various functional strategies that have enabled them to show resistance to antibiotics. The biofilm is a form of organization that has enabled them to contend against antibiotics. It is necessary to raise awareness about the use of antibiotics and prevent misuse of them. According to the data presented, the bacteria are able to adapt to antibiotics and resist them in relatively short time frames, which disputed that in the future can fight infectious diseases under the current schemes antimicrobial therapy. Currently, it is are seeking new strategies to eliminate pathogenic microorganisms and especially to those who are able to form biofilm.

Acknowledgements

Thank to PRODEP and VIEP-BUAP for the facilities provied for the development of this work. Thank to Isis Neferatri Gómez-Bravo and Daniel Huelgas-Méndez by Biomedicina-BUAP for their valuable technical support to carry out this work.

REFERENCES

Anderl, J.N., Franklin, M.J. and Stewart, P.S. 2000. Role of antibiotic penetration limitation in *Klebsiella pneumoniae* biofilm resistance to ampicillin and ciprofloxacin. *Antimicrob. Agents Chemother*., 44: 1818-1824.

Bassler, B.L. 2002. Small talk: cell-to-cell communication in bacteria. *Cell*., 109: 421-424.

Betancurth, M., Botero, J.E. and Rivera, S. 2004. Biopelículas: una comunidad microscópica en desarrollo. *Colomb Med*., 35: 34-39.

Bjarnsholt, T. 2013. The role of bacterial biofilms in chronic infections. *APMIS*., 121: 1 -51.

Bridier, A., Briandet, R., Thomas. V., Dubois-Brissonnet, F. 2011. Resistance of bacterial biofilms to disinfectants: a review. *Biofouling*., 27: 1017-1032.

Bruce, D., Diamond, J.B.H. and Miranda, N.G. 2007. Biofilm: Latent threat or protective factor? State of art. *Enf Inf Microbiol*., 27: 22-28.

Ceri, H., Olson, M.E., Stemick, C., Read, R.R., Mork D. and Buret, A. 1999. The Calgary biofilm device: new technology for rapid determination of antibiotic susceptibilities of bacterial biofilms. *J. Clin. Microbiol*., 37: 1771-1776.

Cha, J.O., Yoo, J.I., Yoo, J.S., Cheng, H.S., Park, S.H., Kim, H.S., Lee, Y.S. and Cheng, G.T. 2013. Investigation of biofilm formation and its association with the molecular and clinical characteristics of methicillin-resistant *Staphylococcus aureus*. *Osong Public. Health Res. Perspect*., 4: 225-232.

Chole, R.A. and Faddis, B.T. 2003. Anatomical evidence of microbial biofilms in tonsillar tissues: A possible mechanism to explain chronicity. *Arch. Otolaryngol. Head Neck Surg*., 129: 634-636.

Costerton, J.W, Lewandoski, D.E., Cadwell, D.R., Corber, H.M. and Lappin-Scott, H. 1995. Microbial biofilms. *Annu. Rev. Microbiol*., 49: 711-745.

Costerton, J.W. 1999. Introduction to biofilms. *Int J Antimicrob Agents.,* 11: 217-221.

Decho, A.W. 2013. The EPS matrix as an adaptive bastion for biofilms: Introduction to special issue. *Int. J. Mol. Sci*., 14: 23297-23300.

Diemond, J. and Miranda, G. 2007. Biofilm: ¿amenaza latente o factor de protección?. *Enf. Inf. Microbiol*., 27: 22-28.

Domingue, P.A., Sadhu, K., Costerton, J.W., Bartlett, K. and Chow, A.W. 1991. The human vagina: normal flora considered as an in situ tissue-associated, adherent biofilm. *Genitourin. Med*., 67: 226-231.

Donland, R.M. 2002. Biofilms: Microbial Life on Surfaces. *Emerg. Infect. Diseases*., 8: 881-890.

Donlan, R.M. 2011. Biofilm elimination on intravascular catheters: important considerations for the infectious disease practitioner. *Clin. Infect. Dis.,* 52:1038-1045.

Ferrières, L., Hancock, V. and Klemm, P. 2007. Biofilm exclusion of uropathogenic bacteria by selected asymptomatic bacteriuria *Escherichia coli* strains. *Microbiol*., 153: 1711-1719.

Flores-Encarnación, M., Cortés-Maldonado, L., Guzmán-Flores, J.E., García-Nito, F., Márquez-Rojas, C.P. El papel de las biopelículas bacterianas en el mecanismo de resistencia a los antibióticos en infecciones crónicas. En: Lozano PZ, Rocha RCG, Martínez YL, editores. Antibióticos: uso, mecanismos de acción, de resistencia y antibioticoterapia. 1ª Edición. México. *Benemérita Universidad Autónoma de Puebla*., 2009. p. 271-285.

Flores-Encarnación, M., González-Gutiérrez, J.Y., Meza-de la Rosa, J.L., Cabrera-Maldonado, C., Carreño-López, R., Nava-Nolazco, R.M., García-García, S. and León-Tello, G. 2014. The bacterial biofilm and importance to human health. *Basic Res. J. Med. and Clin. Sci*., 3: 28-32.

Greenberg, E.P. 2003. Bacterial communication: tiny teamwork. *Nature*., 424: 134.

Guigo, J.M. 2001 Natural conjugative plasmids induce bacterial biofilm development. *Nature*., 412: 442-445.

Hall-Stoodley, L., Costerton, J.W. and Stoodley, P. 2008. Bacterial biofilms: from the natural environmental to infectious diseases. *Nat. Rev. Microbiol*., 2: 95-108.

Johnjulio, W., Fuge, L.H., Kad, M., Post, C. 2012. Introduction to biofilms in family medicine. *South Med J.,* 105: 24-29.

Kolenblander, P.E. 2000. Oral microbial communities: biofilms, interactions and genetic system. *Annu. Rev. Microbiol*., 54: 413-437.

Kostakioti, M., Hadjifrangiskou, M. and Hultgren, S.J. 2013. Bacterial biofilms: development, dispersal, and therapeutic strategies in the dawn of the postantibiotic Era. *Cold Spring Harb. Perspect. Med*., 3:1-23.

Krom, B.P. and Oskam, J. 2014. Microbial biofilms and wound healing: an ecological hypothesis. *Phlebology*., 19:168-173.

Leccese-Terraf, M.C., Juarez-Tomás, M.S., Nader-Macías, L., and Silva, C. 2014. Screening of biofilm formation by beneficial vaginal lactobacilli and influence of culture media components. *J. Appl. Microbiol*., 113: 1517-1529.

Lewis, K. 2001. Riddle of biofilm resistance. *Antimicrob Agents Chemother*., 45: 999-1007.

Marsh, P.D. 2004. Dental plaque as a microbial biofilm. *Caries Res*., 38: 204-211.

Matsumura, M., Izumi T., Matsumoto, M., Tsuji, M., Fujiwara, T. and Ooshima, T. 2003. The role of glucan-binding proteins in the cariogenicity of *Streptococcus mutants*. *Microbiol. Inmunol*., 47: 213-215.

Matthew, M.M. and Maestre J.R.V. 2004. Biofilm: modelo de comunicación bacteriana y resistencia a los antimicrobianos. *Rev. Esp. Quimioterap*., 15: 26-28.

Merle, E.O, Howard, C., Douglas, W., Morck, A.G. and Ronald, R.R. 2002. Biofilm bacteria: formation and comparative susceptibility to antibiotics. *The Can. J. of Vet. Research*., 66: 86-92.

O'Toole, G., Kaplan, H.B. and Kolter, R. 2000. Biofilm formation as microbial development. *Ann. Rev. Microbiol*., 54: 49-79.

Post, J.C. 2001. Direct evidence of bacterial biofilms in otitis media. *Laryngoscope*, 111: 2083- 2094.

Prigent, C.P., Brombacher, E., Vidal, O., Arnaud, A., Lejeune, P., Landini, P. and Dorel, C. 2001. Complex regulatory network controls initial adhesion and biofilm formation in *Escherichia coli* via regulation of the *csgD*. *Gene.*, 24: 7213-7223.

Ramadan, H.H., Sanclement, J.A. and Thomas, J.G. 2005. Chronic rhinosinusitis and biofilms. *Otolayngol. Head Neck Surg*., 132: 414-417.

Romanova, I.M., Gintsburg, A.L. 2011. Bacterial biofilms as a natural form of existence of bacteria in the environment and host organism. *Zh Mikrobiol Epidemiol Immunobiol*., 3: 99-109.

Sanclement, J.A., Webster, P.L., Thomas, J. and Ramadan, H.H. 2005. Bacterial biofilms in surgical specimens of patients with chronic rhinosinusitis. *Laryngoscope*., 115: 578-582.

Sanderson, A.R., Leid, J.G. and Hunsaker, D. 2006. Bacterial biofilms on the sinus mucosa of human subjects with chronic rhinosinusitis. *Laryngoscope*., 116: 1121-1126.

Sauer, K. 2003. The genomics and proteomics of biofilm formation. *Genome Biol*., 4: 219.1-219.5.

Schembri, M.A., Kjaergaard, K. and Klemm, P. 2003. Global gene expression in *Escherichia coli* biofilms. *Mol Microbiol.,* 48: 253-257.

Scott, C. and Manning, C.S. 2003. Basics of biofilm in clinical otolaryngology. *Ear. Nose Throat. J*., 82: 18-20.

Shiau, A.L. and Wu, C.L. 1998. The inhibitory effect of *Staphylococcus epidermidis* slime on the phagocitosis of murine peritoneal macrophages is interferon-independent. *Microbiol. Inmunol*., 42: 33-40.

Steindler, L., Bertani, I., De Sordi, L., Schwager, S., Ebert, L. and Verturi, V. 2009. LasI/R and RhIl/R quorum sensing in a plant benecial strain of *Pseudomonas aeruginosa*. *Appl. Environ. Microbiol*., 75: 5131-5140.

Stewart, P.S. and Costerton, J.W. 2001. Antibiotic resistence of bacteria in biofilms. *Lancet*., 358: 135-138.

Thomas, J. and Nakaishila, G. 2006. Managing the complexity of a dynamic biofilm. *J. Am. Dent. Assoc*., 137: 10S-15S.

Whiteley, M., Bangera, M.G., Bumgarner, R.E., Parsek, M.R., Teitzel, G.M., Lory, S. and Greenberg, E.P. 2001. Gene expression in *Pseudomonas aeruginosa* biofilms. *Nature*., 413: 860-864.

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