Biofilm Formation in Acute and Chronic Infections with Special Emphasis on Common Chronic and Nosocomial Infections

Parama Das Gupta, Upal Das Ghosh and Rini Roy*
Department of Microbiology, Bidhannagar College, Kolkata- 700 064, India
*E-mail: for_rini_roy@yahoo.co.in

ABSTRACT

Biofilm is defined as a community of microorganisms that are adhered to living or non-living solid surfaces and embedded in a common, self-made matrix, comprising of exopolysaccharide material. The role of biofilm in chronic diseases deserves special importance as these extracellular polymeric materials developed with quorum sensing support both the primary criteria of infection development namely adhesion and colonisation. Due to their structural and physiological changes, microorganisms present in the biofilm are difficult to treat or eradicate. The presence of a protective layer of extracellular polymers, changes in metabolic activity or a high rate of mutation make them tolerant or resistant to conventional treatment. The persistence of pathogenic microorganisms mostly renders biofilm to be associated with several acute and chronic infections and various nosocomial or healthcare-related infections. Furthermore, cancer development may also result due to biofilm formation. Biofilm may contribute to inflammation. This study deals with molecular aspects of biofilm formation and its role in different disease formations.

Keywords: Biofilm; Signal transduction; Diseases; Cancer; Inflammation

1. INTRODUCTION

About 40-80 % of bacteria in nature establish a stable and firm attachment for colonisation over a solid surface or tissues that leads to the formation of a microconsortia, scientifically termed as biofilm. Biofilm is defined as a microbial community that is adhered to various living or non-living solid surfaces and embedded in a self-made matrix commonly made up of polysaccharides like alginate, proteins like fibrin and extracellular DNAs. Though the first report of biofilm was published in 1683 by Antonie Von Leeuwenhoek, but it gets its importance in the medical field only after 1970 when Neils Hoiby first observed a relation between the etiological agent of persistent infection and bacterial biofilm in cystic fibrosis patients.

It is well documented that the establishment of biofilms follows a few sequential steps which involve pellicle formation, initial adhesion, and attachment of bacteria, colonisation and maturation of bacteria. Biofilm confers numerous advantages to bacterial species including resistance to antibiotics, combat against host defences etc. During the transformation from the free-floating planktonic stage to the static biofilm stage, several physiological and molecular changes occur in the bacterial cell. The production of extracellular polymeric substances, expression of several genes that encode proteins make bacterial cells resistant to antimicrobials. The expression of several genes (like ndvB in Pseudomonas aeruginosa) that encode proteins make bacterial cells resistant to antimicrobials.

The presence of persistent cells in a biofilm also makes them recalcitrant to antimicrobials and protects them from host defences. This recalcitrant nature of biofilm makes them difficult to remove from infection sites and makes them responsible for the development of several chronic infections. Cystic fibrosis, bacterial vaginosis, inflammatory bowel disease, chronic wound infections etc. are some chronic infections that are associated with the development of bacterial biofilm within the infected tissues.

The role of bacterial biofilm in the development of cancer is a relatively new area of research. Some studies showed that intense microbial interaction established by multi-species biofilm is responsible for extensive penetration and disease progression through evasion of immunologic responses of the host. Biofilms have been identified as pivotal participants in establishing and developing colorectal cancer. The present review specifically focuses on the role of biofilm in several common chronic diseases as well as in cancer development.
It also highlights various signal transduction pathways that are involved in the development of biofilm and various host immune responses that are evoked during biofilm formation.

2. MOLECULAR SIGNAL TRANSDUCTION IN BIOFILM FORMATION

The signal transduction systems for biofilm formation by microorganisms comprise of a receptor histidine kinase (HK) and a response regulator (RR). The environmental factors regulating biofilm formation can be detected by analyzing the input signals of these two-component signal transduction systems (TCSs) involved in the pathway of biofilm formation. The underlying mechanism of biofilm formation can be revealed by a combination of the regulatory pathway of biofilm formation with the regulatory mode of TCS.

In various bacterial species, biofilm formation is regulated by oxygen signals. In rhizobacterium and Bacillus amyloliquefaciens SQR9A, decreased levels of oxidised and reduced Nicotinamide adenine dinucleotide (NAD) is evident due to low oxygen levels sensed by HK ResE. The regulatory activity of RR ResD at the transcriptional level directly controls the expression of the qoxABCD and ctaCDEF operons stimulated by the activation of HK ResE which results in the synthesis of terminal oxidases. The key pathway for biofilm formation is activated by the interaction of these terminal oxidases with KinB9 (Fig. 1(A)).

Some other RRs containing enzymatic output domains regulate biofilm formation. These domains are usually taken part in homeostasis with a second messenger, like c-di-GMP. This secondary messenger controls the conversion between planktonic growth and biofilm formation in gram-negative bacteria serving as a core molecule. It has been observed that biofilm formation is involved with high c-di-GMP levels. The conversion from planktonic growth to biofilm formation is governed by specific signals directly and exquisitely, when there is the synthesis of c-di-GMP and RR of TCS, bears the degradation domains.

Bacteria like Legionella pneumophilia, Shewanella oneidensis MR-1, and Vibrio cholerae nitric oxide (NO) can induce biofilm formation. A multi-component signal transduction system is used by NO for regulating biofilm formation. It includes two HKs namely HnoK and HnoS, integrates and three RRs namely HnoC, HnoD, and HnoB (Fig. 1(B)). HnoD which lacks phosphodiesterases (PDE) activity directly interacts with HnoB to subdue its PDE activity. Degradation of intracellular c-di-GMP by HnoB leads to negative regulation in biofilm formation; HnoC has a moderate negative role in mediating the formation of biofilm. HnoK and HnoS (HK family) have inhibitory roles via switching on HnoC and HnoB. PDE is one of the RRs components which is involved not only in signaling transduction systems with intracellular c-di-GMP but also links extracellular-specific signals in the regulation of bacterial biofilm formation.

![Diagram of signal transduction systems](image_url)
In *S. oneidensis* NO stress stimulates the biofilm formation by negatively regulating HnoK and HnoS-dependent multicomponent signal transduction pathway. Signals generated through the activation of HnoK and HnoS act in concert and are trifurcated to elicit the response in a negative fashion through the regulation of HnoB, HnoC, and HnoD in differential way. 

In *S. putrefaciens* CN32 sodium lactate receptor molecule transmits the signal via Periplasmic Binding Protein (PBP) to LrbS where the signal gets bifurcated and carries out phosphorylation of LrbA and the phosphodiesterase activity of LrbS. The latter significantly decreases the c-di-GMP concentration which acts as an inhibitory signal of biofilm formation.
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Figure 2 (A). Cross-regulation schemes and the “control system” with TCS in E.coli.

In uropathogenic E. coli, biofilm formation is controlled by the shifting of equilibrium between the phosphorylated and dephosphorylated QseB. In Fe³⁺ deplete condition, the equilibrium is shifted towards dephosphorylated QseB due to the overactivation of QseC, which cannot bind at the qseBqseC regulator region. Hence despite the binding of PmrA-P, the qseB Coper on gets switched off and biofilm is formed. However, in Feplete condition, the equilibrium shifted towards phosphorylated QseB due to the overactivation of PmrB and planktonic growth continues. Bold arrow represents the over activation.

Figure 2 (B). Cross-regulation schemes and the “control system” with TCS in P. aeruginosa.

Pili-mediated attachment to the solid surface by P. aeruginosa stimulates the PilJ protein to activate membrane-bound ChpA which catalyzes the phosphorylation of PilG. The phosphate group is then transferred to membrane-bound CyaB and the phosphorylated CyaB produces cAMP from ATP. cAMP molecules then get attached to another RR Vfr that directs the cell towards biofilm formation.
A mechanical change of type IV pilus is exerted in *P. aeruginosa* leading to attachment followed by retraction after contacting a solid surface. PilJ protein transfers the signal to the cytoplasm by direct interaction with HK ChpA. The adenylate cyclase CyaB stimulated by TCS ChpA-PilG leads to an increment in cellular cAMP level. This activates Vfr that in turn helps in the promotion of biofilm formation through various pathways (Fig. 2(A) and 2(B)). Thus, TFP behaves as a mechanical sensory “control system” activating TCS ChpA-PilG. The activation of FimS-AlgR signal transduction pathways by TFP connects these pathways and thus integration of these pathways into a regulatory network is also conducted by TFP.

The TCS QseC-QseB detected in uropathogenic *Escherichia coli*, may respond to quorum sensing, and is associated with pathogenesis and biofilm formation. HK PmrB is excessively activated with the increase in the concentration of ferric iron in the environment, and it phosphorylates QseB more strongly compared to QseC dephosphorylation. Transcription levels of qseBC is increased due to the binding of both RRs PmrAP and QseBP and hence the bacteria continue their planktonic growth.13

The formation of *Enterococcus faecalis* biofilms is controlled by a unienzyme system-mediated signal transduction pathway. gelE, the gene encoding gelatinase, is expressed depending on the fsr genes encoding a bicomponent system. A impairs the ability of *Enterococcus faecalis* V583A strain for in vitro formation of biofilms. Cloning of an active gelE under a constitutive promoter with subsequent expression of the gene in an fsr mutant reinitiates biofilm formation, though the exact role of gelatinase, the product of the gelE gene in biofilm formation still remains unclear.14

The main signalling cascade involved in bacterial biofilm formation is TCS. If more input signals of TCSs can be identified these may provide critical clues for biofilm formation.

### 3. COMMON ACUTE AND CHRONIC INFECTIONS RELATED TO BIOFILM

Acute infections are attributed to a rapid and sudden onset of symptoms which are also resolved quickly either by immune responses (both humoral and cell-mediated) exhibited by the host or by treatment with antibiotics.15 Generally, bacteria present in a planktonic form are responsible for acute infections. They are either controlled by the host immune system or with antibiotics. But planktonic bacteria originating from biofilm, may disperse through the bloodstream or around the source of infection, causing recurrent infections as this subset of bacteria are highly tolerant to antimicrobial therapies. Bacteria that form biofilm are surrounded by a self-made matrix that protect them from host immune system. Due to their changes in cellular physiology, gene expression, bacteria present in a biofilm are generally resistant to antibacterial agents.6 Therefore, infections caused by them are difficult to eradicate, resulting in persistent or chronic infections.

Many chronic diseases are associated with bacterial biofilm. Some of them are briefly discussed below:

**Cystic Fibrosis:** Cystic fibrosis is one of the most common chronic infections caused by bacterial biofilm. Cystic fibrosis disease is caused due to mutation in the cystic fibrosis transmembrane conductance regulator (CFTR) gene16. The disease is characterised by inflammation as well as chronic bacterial infection where bacteria induced inflammatory responses result in permanent damage of pulmonary functions.17,18 Different bacterial genera are involved in this disease like *Hemophilus, Staphylococcus, Burkholderia* with *Pseudomonas aeruginosa* being the major one.19 *P. aeruginosa* colonisation is generally initiated in the paranasal sinuses as a biofilm. It acts as a reservoir for recurrent infections in the lungs that ultimately results in chronic one. In CF patients, *P. aeruginosa* biofilm has been detected in sputum, lung tissue and lung abscess.

A considerable amount of polymorphonuclear leukocytes is accumulated in the affected site, in response to the biofilm, resulting in chronic inflammation with significant tissue damage, impairment of lung function and obstruction of the airways.20 Enhancement of the establishment of drug-resistant biofilm formation by *P. aeruginosa* in the lungs of CF patients is mediated by the excess secretion of a viscous mucus layer in the CF airway that leads to a low oxygen environment and deposition of DNA and actin in CF airway.21,22 All these factors like mucus, DNA, actin facilitate biofilm formation and make them difficult to eradicate by conventional antimicrobial therapy and consequently forms chronic disease.

**Infective Endocarditis:** Infective endocarditis (IE) is an infection of the endocardium, most commonly occurring on valves of the heart or cardiac devices that have been implanted in patients.23 The most common bacterial genera responsible for the disease is *Staphylococcus aureus* and coagulase-negative staphylococci (CoNS), followed by viridans streptococci (i.e., *Streptococcus mutans, S. sanguis, S. sanguinis, S. mitis, S. salivaruis*, and *S. bovis*).24 Presence of vancomycin resistant *Staphylococci* has been established in a 70-year world IE patient in Korea.25

Generally, *S. aureus* colonizes on native heart valves whereas CoNS colonizes on implants. The third most common bacterium is *Enterococcus faecalis* which causes infection on native heart valves in elderly patients. The pathogenesis of IE is characterized by the formation of vegetation on the damaged valve surface which is a mass of bacterial cells encased within host derived molecules like platelets, fibrin and other inflammatory cells.26 The interaction between the biofilm producing microbial species and the cardiac valve endothelium initiates at the damaged site where the bacteria adhere and become embedded within a matrix consisting of platelets, fibrin etc. Then they form micro-colonies and later a stable
biofilm. The persistent nature of biofilm may lead to inflammation that may cause further tissue damage or fragments of biofilm may be disseminated to distant sites resulting in chronic bacteremia and thromboembolic events.\textsuperscript{20,26}

**Chronic Inflammatory Bowel Disease:** Chronic inflammatory bowel disease is defined as an inflammation of digestive system, comprising both small and large intestine. Symptoms include abdominal pain, diarrhoea, bloody stool, weight loss. The disease is characterised by the influx of neutrophils and macrophages that produce cytokines, proteolytic enzymes, and free radicals that result in inflammation and ulceration.\textsuperscript{27,28} The cause of IBD is not fully understood but recent studies indicate that several factors including genetic predisposition of the host, environmental factors and dysbiosis of gut microflora, abnormalities in immune responses may lead to the disease.\textsuperscript{29} In healthy individuals, the mucosal layer provides a protective covering to the intestinal epithelium and any breaches in this layer increase the chance of invading pathogens to constitute biofilms. IBD may be associated with biofilms present in the intestinal mucosa and dysbiosis of the gut microbiota which collectively results in an exaggerated immune response that leads to colonic inflammation. Bacteria present in biofilms associated with IBD patients are mainly Bacteroides fragilis and members of *Enterobacteriaceae*.\textsuperscript{20} Though no such direct case report against the involvement of biofilm in chronic inflammatory bowel disease has been reported till date.

**Chronic Wounds:** Wounds refer to injuries on living tissues due to any trauma like cuts, burns, surgery, or because of some diseases like diabetes.\textsuperscript{20} A wound can be acute where normal healing occurs within a specific period of time. But it can be considered as chronic where normal healing and timely repair does not occur due to several internal and external factors.\textsuperscript{29} Factors like the production of pro-inflammatory cytokines, increased production of metalloproteases and phenotypic alterations of fibroblasts and keratinocytes lead to delayed wound healing, resulting in chronic wounds. All these factors stimulate different bacteria to proliferate and generate recalcitrant biofilms. Microbial products produced by bacterial cells in biofilms play a significant role in inflammation thus inhibiting wounds to heal. Several studies indicate that about 90% of chronic wounds are associated with biofilms.\textsuperscript{30} Both aerobic and anaerobic bacteria contribute to chronic wounds. Aerobic bacteria like *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Streptococcus epidermidis* are present at the surface of the wounds whereas anaerobic bacteria like *Bacteroides*, *Fusobacterium*, *Clostridium* present at deeper tissues and associated with chronic wounds.\textsuperscript{20}

**Bacterial Vaginosis:** The most prevalent vaginal infection that affects women of reproductive age globally is bacterial vaginosis.\textsuperscript{31} Though the majority of affected women don’t have any symptoms or complications but sometimes it is associated with premature delivery, causes pelvic inflammatory disease which may lead to infertility and makes women more susceptible to several sexually transmitted diseases like HIV, gonorrhoea, herpes simplex virus infection etc.\textsuperscript{32} Symptoms may include irritation, vaginal discharge with a foul fishy odour which may be more prominent after sexual intercourse and during menstruation. This disease is characterised by a considerable lowering in the number of beneficial Lactobacillus sp. and an increase in various anaerobic bacteria like *Gardnerella*, *Atopobium*, *Mobiluncus*, *Prevotella*, *Streptococcus*, *Ureaplasma*, *Megasphaera* etc. in the vagina.\textsuperscript{33}

Initially, it was assumed that *Gardnerella* vaginosis is the only causative agent of bacterial vaginosis but later several studies indicate that it is a polymicrobial infection. Several microbial species form a stable biofilm with *Gardnerella* being the major one. It initially adheres to the epithelium of the vagina and provides a site for other microorganisms to attach and produce a firm and stable biofilm.\textsuperscript{34} High rate of relapses and recurrence of bacterial vaginosis also indicative that it is a biofilm related infection.\textsuperscript{34} Although the exact role of biofilm in bacterial vaginosis is not fully understood but in vitro studies demonstrated that *G. vaginalis* biofilm exhibits high resistance to hydrogen peroxide and lactic acid produced by vaginal lactobacilli\textsuperscript{35} and also tolerance against antibiotics.\textsuperscript{36} Recent transcriptomic analysis showed that *G. vaginalis* exhibit a specific gene expression pattern according to its phenotype which may make it to resist host defences and allow colonisation to vaginal epithelium.\textsuperscript{37} Clinical evidence regarding the association of *Chlamydia trachomatis* and *Nesisseria gonorrohae* in a 26-year-old male to female transexual with recurring bacterial vaginosis has been reported.\textsuperscript{38}

**Chronic Rhinosinusitis:** Rhinosinusitis is defined as an inflammation of the nose and paranasal sinuses, attributed to nasal blockage, congestion, nasal discharge, loss of smell, facial pain or pressure. It may be acute or chronic depending upon the duration of the disease. If the symptoms last for more than 12 weeks, it is considered as chronic, otherwise, the disease is referred to as an acute infection. Generally, viruses like rhinoviruses, coronaviruses, influenza viruses may account for majority of the acute rhinosinusitis. During acute rhinosinusitis host immune responses eventually remove the invading pathogens but the dead host cells, immune cells generated during the process makes an environment suitable for secondary bacterial infections which may lead to chronic rhinosinusitis.\textsuperscript{20}

Chronic rhinosinusitis is presently considered as a multifactorial disease, depending upon several extrinsic factors like allergy, asthma, aspirin intolerance, bacterial biofilm, superantigens, etc., and intrinsic factors like immunodeficiency, anatomical variations, ciliary dysfunction, presence of other diseases like cystic fibrosis.\textsuperscript{39} Several studies have demonstrated that patients with chronic rhinosinusitis develop *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Hemophilus influenzae*, *Moraxella catarrhalis*, *Streptococcus pneumoniae* biofilm in their paranasal sinuses.\textsuperscript{40}
Several studies have indicated that biofilm formation in sinonasal mucosa results in the dysfunction of both innate and adaptive immunity of the host. Establishment of biofilm resulting in the decreased level of formation of antimicrobial peptides like lactoferrin, MUC7 and down-regulating Toll like receptors that are effective against Gram-positive bacteria. Biofilm also destructs the mucociliary layer of nasal mucosa, reduces the frequency of the rhythmic beating of cilia or may result in the complete absence of the cilia. All these factors may contribute to bacterial persistence. Besides innate immunity biofilm also affects adaptive immunity. Several studies have indicated that the presence of biofilm in the sinonasal mucosa stimulates both Th1 and Th2 responses. In presence of biofilm level of inflammatory cytokines increase rapidly which may result in mucosal inflammation.

**Periodontitis:** Periodontitis is a polymicrobial inflammatory disease that leads to the destruction of the tissue supporting the tooth. Bacterial biofilm is considered as the main etiological agent of periodontitis. The oral cavity is a habitat of a complex microbial community, the majority of which provides beneficial effects to their human host. But an imbalance in the oral microbiome causes dysbiosis and results in various oral diseases like periodontitis. Three Gram-negative oral pathogenic bacteria, *Porphyromonas gingivalis*, *Tannerella forsythia* and *Treponema denticola* are the major causative agent of periodontitis. Recent studies have indicated the presence of Gram-positive *Peptostreptococcus* and *Filibactor alocis* are also present in the diseased site. Although the etiology of periodontitis is bacteria but the pathogenesis is related to the host response. The pathogenicity involves the alteration in the production of inflammatory mediators due to dysbiosis of the oral microbial community. This leads to the production of toxic compounds resulting in severe tissue damage.

**Gastric Cancer:** The very first information about the formation of biofilm by Helicobacter pylori, the potential bug behind gastric cancer was first reported by Stark, *et al.* in 1999 through in vitro abiotic model. The first report of biofilm progression from a clinical sample in an abiotic model was claimed by Cole, *et al.* in 2004. Endogenous quorum sensing molecule Autoinducer-2 (AI2) acts as a chemotactic repellent of *Helicobacter pylori* and facilitates biofilm formation over Madin Darby Canine Kidney (MDCK) epithelial cell surface. SEM analysis of endoscopic biopsy specimens of infected individuals show clear presence of biofilm compared to non-infected individual. Biofilms are also evidenced on the gastric mucosa surface of 97.3 % of peptic ulcer patients. Besides *Helicobacter pylori*, two normal pathogens of the GI tract, *Lactobacillus fermentum* and *Lactobacillus casei* were also positively evidenced to form biofilms over gastrointestinal cell lines.

**Colorectal Cancer:** Biofilms have been identified as a pivotal participant in establishing and developing colorectal cancer, preferentially in the right colon of humans. In sporadic colorectal cancer, five species have been identified so far as the causative agents, which include *Fusobacterium nucleatum*, *Bacteroides fragilis*, *Gemella morbilliform*, *Parvinonas micra* and *Peptostreptococcus stomatis*. A study on microbial carcinogenesis has revealed the role of *Fusobacterium nucleatum*, a periodontal pathogen in inducing local inflammation and increasing the expression of cytokines such as IL 6, IL-8, IL-12, TGF-β and TNF-α during colorectal cancer. The presence of enterotoxic *Bacteroides fragilis*, however, triggered the disease progression by passively activating transcription activators of STAT 3 dependent pathway which ultimately results in suppression of T cell-mediated tumor immune surveillance.

A tussle between the host and the pathogenic biofilm members in search of nutrients has been evidenced in deciphering the role of such pathogenic biofilm in initiating the disease. The pathogens under the biofilm microenvironment collect their micronutrients and other nutritional benefits to destroy and damage the host.

Dejea, *et al.* in 2014 have shown that biopsy samples of colon of colorectal cancer patients have a more prevalent biofilm structure than the normal individual. A comparative study between the biofilm community isolated from diseased and healthy individual have shown significant diversity with respect to community members as well as their activities. In mouse Apc model scientists have established the carcinogenicity of a biofilm-positive colon/mucosal tissue from CRC individual. Metatranscriptomics analysis has revealed the role of specific bacteria associated with the biofilm to induce tumorigenesis. These biofilms act as a potential source of dehydrogenation and deconjugation of several bile acids which in turn expose the tissues associated with it to be exposed to secondary bile acids. In addition, these biofilms also serve as a pool of hydrogen sulfide and nitrosamine that results in DNA damage and genomic instability which ultimately leads to carcinogenesis.

**4. ROLE OF BIOFILM IN CANCER**

The role of single bacterial species in cancer development is well established for years but the association of multispecies biofilms in the development of cancer is a comparatively new area of research. However, very recently in various separate research, it has been found that it is not a single species, but a salient microbe-microbe interaction established by multi-species biofilm is responsible for extensive penetration and disease progression through evasion of immunologic responses of the host.

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Molecular level studies have revealed that the biofilm formation over the gastric mucosa has been initiated upon induction of a signalling cascade which in turn activates the bacterial quorum sensing molecules like Hp, or AHL, or DSF pathways. These genes attract other biofilm community members close together through chemotactic movements and the aggression is finally
completed with the help of extracellular matrixes, outer membrane vehicles and adhesins.\textsuperscript{49}

**Other Cancers:** Carcinogenic effects of biofilm formed by different pathogens in other organs have not been studied extensively so far. Very limited reports are there in support of biofilm formation in lungs, liver, urinary bladder and other organs. In a very recent report, the role of Mycobacterial biofilm in virulence and drug resistance of the pathogen has been published. Biofilm produced by *Pseudomonas aeruginosa* in chronic lung infection is well documented and can be inhibited upon treatment with L-methionine.\textsuperscript{50} Chronic typhoid patients carry biofilm of *Salmonella typhi* which may lead to hepatobiliary cancer. Biofilms formed by enteropathogenic *E. coli*, a suspected potential pathogen associated with colorectal cancer specimens and the parasite *Schistosoma mansoni*, are involved in the development of squamous carcinoma of urinary bladder.

5. **HOST DEFENCE RELATED TO BIOFILM**

The establishment of disease by pathogenic bacteria consists of several steps. The first step involves the adhesion of the pathogenic bacteria to host epithelial cells through receptor-ligand interaction, formation of extracellular polymeric substances like capsule or attachment with appendages like pili, and fimbriae. The next step involves invasion where the pathogen penetrates the epithelial cells. Some pathogens are non-invasive in nature, they attach to epithelial cells and carry out colonisation. Colonisation refers to the multiplication of bacterial cells. After colonisation pathogens produce several virulence factors like several enzymes (Coagulase, Hyaluronidase, etc.), siderophores, and metabolic by-products like ammonia. $\text{H}_2\text{O}_2$, toxins etc. responsible for tissue damage.\textsuperscript{51}

Bacteria living as biofilms are responsible for persistent and destructive inflammatory responses. In many bacteria species, biofilm formations can be considered as an efficient mechanism regulated by genetics. In contrast, the host response to bacterial biofilms is less investigated, and it is assumed that bacteria in biofilms develop methods to evade recognition or the immune clearance system. The host response to bacterial biofilms is mediated by neutrophils which due to their phagocytic nature can reach the site of bacterial infection and possess a variety of antibacterial and toxicity-producing materials.\textsuperscript{52}

Treatment of biofilm infection is a serious and important problem to the medical practitioners. Invasive procedures for eradication cause the recurrence of biofilm. It has been shown that proinflammatory immune responses are suppressed by biofilm-derived products, which is observed by the accumulation of myeloid-derived suppressor cells and migration of macrophages creating an anti-inflammatory state. Recent research has revealed that changes in leukocyte metabolism modulate their inflammatory phenotype and

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Figure 3. Schematic representation of the immune response to a bacterial biofilm. Different organisms appear to modulate T-cell response favourable for establishing chronic infection (Th1 or Th2). Though the initial activation of CD4+ T cell is common for all types of infection within the host, however, the Th1-Th2 balance gets shifted towards the more favourable pathogenicity. The main channelisation of the immune response is decided by the type of cytokines produced by the initially activated CD4+ cells in presence of different bacterial biofilms. Here, the differential immunological modulation led by *S. aureus, P. aeruginosa, B. pertussis* has been shown. However, the immune response against *Salmonella* is still speculative.\textsuperscript{52}
function. For example, oxidative phosphorylation and aerobic glycolysis are mediated by anti-inflammatory macrophages and proinflammatory macrophages respectively. The planktonic, and biofilm bacterial infections exhibit immune responses based on the metabolic properties of macrophages and neutrophils related to bacterial growth conditions in both cases.

Investigations on the recognition of *S. pneumoniae* by the complement system and its interactions with human neutrophils showed that biofilm formation distracts the activation of alternative complement pathway by a PspC-mediated mechanism and disturbs phagocytosis of pneumococcal biofilms. This study ensures that biofilm formation in *S. pneumoniae* evades both the classical and the PspC-dependent alternative complement pathways of the host immune system.53

In some cases, the biofilms skew T-cell response toward a balance that allows a progression between the host and the pathogen, in which the infection can become persistent54 (Fig. 3). A study was conducted among chronically vs. nonchronically infected Cystic Fibrosis patients (n = 14). The levels of the Th2 marker interleukin 4 (IL-4) and levels of IFNγ were significantly higher in chronically infected patients and nonchronic patients respectively. It was concluded that chronic patients have a high Th2-type immune response.55

Th1 response is observed in Gingivitis lesions while a Th2 response is shown in chronic periodontitis. In medical implants associated biofilms, the commensal organism *Enterococcus faecalis* can cause opportunistic infections. Dislodged biofilms cause reduced levels of the proinflammatory cytokines TNF-α and IL6 in macrophages. Furthermore, biofilms induce Th1 and Th2 due to increased expression of CD 80 and CD 86 respectively, indicating a possible skew in T-cell response.

The prospects for targeting DNABII family members can act as a potential universal strategy for treating biofilm diseases.

The results from a separate study showed that a common core of secreted proteins is contained in both types of exoproteomes in the biofilm matrix independent of the nature of the biofilm matrix. Administration of an exoproteome extract of an exopolysaccharide-dependent biofilm intradermally induces a humoral immune response and this enhanced the production of interleukin 10 (IL-10) and IL-17 in mice. The promotion of opsonophagocytosis and killing of *S. aureus* was done by antibodies raised against such an extract. The potential of biofilm matrix exoproteins as a promising candidate multivalent vaccine against *S. aureus* biofilm-associated infections has been elucidated by these data.

Biofilms develop their resistance mechanisms by under different physical, physiological, and gene-related factors since antibiotic resistance associated with biofilm formation make medication difficult. By understanding the requirement for growth and the means to diminish biofilm production we can develop methods to control them.

Biofilm induces innate as well as adaptive immune responses revealed in recent in vivo and in vitro studies. In synergy, these both arms of the immune response cause collateral tissue damage. Focusing on this aspect, treatment for biofilm infections could be designed.

6. DISCUSSION

Biofilm formation by bacteria is considered as an important mechanism for the colonisation of the pathogens and the establishment of diseases, by successfully evading the host defence system. The extracellular polymers produced by certain bacteria help to form a microconsortia where a stretch of local two-component signal transduction pathway is involved to have communication between the members. Biofilm formation by different bacteria has been evidenced to be governed by several environmental factors like oxygen or nitric oxide stress that switch on separate signalling cascade for the response. The external environmental stimulators have been sensed by HK receptors which in turn activate different RR molecules that ultimately activate concerned transcription factors. Thus, host tissue/organ-specific microenvironment may be treated as the positive/negative regulator of biofilm formation by the pathogens.

A number of chronic and acute infections have already been reported and discussed here, in short, considering their establishment and progression related to biofilm formation. The vital organs involved the lungs, heart, and GI tract. In most of the cases, *Staphylococcus* and *Pseudomonas* are the principal biofilm maker. A stringent quorum sensing mechanism is involved in all the cases to get the threshold number of bacteria for the establishment of biofilm. Once a biofilm has been formed the organisms get protected against the host immune system. Biofilm components can successfully suppress proinflammatory signals and thereby inhibits the recruitment of anti-inflammatory macrophages. However, T cell response has been elicited by the increased expression of CD 80 and CD 86 molecules by biofilms. Biofilms can also inhibit the activation of the classical complement system by preventing C3b deposition and impairing C1q activity.

Bacterial biofilms support the pathogens involved in disease establishment and progression in evading host immune attack and also render them resistant to antibiotics. Though current research has established different MIC values for certain antibiotics when treated against biofilm than planktonic cell.56 Modern clinical manifestations that target the biofilm include quorum sensing inhibitors, C-di-GMP modifiers, disruption of bacterial amyloids and bacteriophage therapies.57

Bacterial biofilms are also found to play a dual role if cancer progression is concerned. Mainly colorectal cancer and gastric cancer, in a further extent lung cancer has been evidenced to be associated with the development of bacterial biofilm in the target organ. However, on the other hand, in multiple cases, biofilms
have also been successfully administered in controlling the establishment and progression of cancer.

Thus, as a whole, in modern medical microbiology, biofilms are being considered as an important consideration both if disease progression and disease prevention is considered.

7. CONCLUSIONS

The formation of biofilms by bacteria is an intrinsic property in which they secrete some exopolysaccharides to form a microconsortium that facilitates the disease progression. The presence of a discrete two-component signal transduction mechanism is well evidenced in the formation of biofilms under given environmental conditions. A number of diseases are well documented that are geared up by the formation of biofilm which on the other hand helps to evade the host immune response. Days ago, colonisation of the pathogenic agents within the host system was the primary area of research for the scientists when the disease establishment and progression were concerned, but now a days, the formation of biofilm have changed the overall scenario and got its importance in the field of medical microbiology. Lots of more detailed research are going on worldwide to decipher the contribution of biofilms in disease progression and how to evade the defence exerted by these biofilms against the host immune system and even against drugs administered.

8. FUTURE PROSPECTS

As biofilm formation has been established to play a pivotal role in escaping host defence by pathogens and as it has been already proved that biofilm-based pathogens are more resistant towards different antibiotics than floating ones, most of the medical-related research should find out a new way to either disintegrate the biofilms within the system or new drugs should be discovered that can penetrate the biofilm to successfully target the causal organisms of different diseases.

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CONTRIBUTORS

Mr Mr Parama Das Gupta received her Post Graduate degree from University of Calcutta in 2004 in Microbiology. She is a Graduate in Microbiology from Dinabandhu Andrews College, Kolkata, University of Calcutta, in 2002. She has been involved in teaching general microbiology as Assistant professor in the Department of microbiology, Bidhannagar College since 2016. She has well expertise in dealing with variety of microbes and their characteristics.

She has contribution in the formulation of abstract, introduction,
common acute and chronic infections related to biofilm for present work.

**Dr Upal Das Ghosh** received his PhD in Biotechnology from University of Calcutta in 2015. He is a Post Graduate of Microbiology and Microbial Technology from University of Kalyani in 2008. He is working now as Assistant Professor in Department of Microbiology in Bidhannagar College, Kolkata since January 2016. He has five years research experience on Plant –metal- microbe interaction study. He is currently working on the field of environmental, medical microbiology and bioinformatics. He has 5 publications in reputed peer-reviewed journals. He has contribution in abstract, molecular signal transduction in Biofilm formation, role of Biofilm in cancer, discussion and conclusion.

**Dr Rini Roy** received her PhD from Lady Brabourne College, Kolkata under University of Calcutta in 2008. She is a Post Graduate of Biochemistry from University of Kalyani in 2001. She is now serving as Assistant Professor in Department of Microbiology in Bidhannagar College. Her research interest includes Biochemistry, neuro-biochemistry and microbiology (medical microbiology, bioremediation and nanobiotechnology). She has nearly 14 years of research experience. She has more than 10 publications in peer-reviewed journals. She has given her inputs in abstract, host defence related to biofilm and references and supervised the overall execution of the work.