Microbial Biofilms in Pulmonary and Critical Care Diseases

Andree-Anne Boisvert*[1], Matthew P. Cheng*[2], Don C. Sheppard[3,4], and Dao Nguyen[5]

[1]Department of Pediatrics, and [2]Department of Medicine, McGill University Health Centre, Montreal, Quebec, Canada; [3]Departments of Medicine, Microbiology, and Immunology, McGill University, Montreal, Quebec, Canada, and [4]Infectious Diseases in Global Health Program, Research Institute of the McGill University Health Centre, Montreal, Quebec, Canada; and [5]Meakins-Christie Laboratories, and Translational Research in Respiratory Diseases Program, Research Institute of the McGill University Health Centre, Montreal, Quebec, Canada

Abstract

Microbial biofilms can colonize medical devices and human tissues, and their role in microbial pathogenesis is now well established. Not only are biofilms ubiquitous in natural and human-made environments, but they are also estimated to be associated with approximately two-thirds of nosocomial infections. This multicellular aggregated form of microbial growth confers a remarkable resistance to killing by antimicrobials and host defenses, leading biofilms to cause a wide range of subacute or chronic infections that are difficult to eradicate. We have gained tremendous knowledge on the molecular, genetic, microbiological, and biophysical processes involved in biofilm formation. These insights now shape our understanding, diagnosis, and management of many infectious diseases and direct the development of novel antimicrobial therapies that target biofilms. Bacterial and fungal biofilms play an important role in a range of diseases in pulmonary and critical care medicine, most importantly catheter-associated infections, ventilator-associated pneumonia, chronic *Pseudomonas aeruginosa* infections in cystic fibrosis lung disease, and *Aspergillus fumigatus* pulmonary infections.

Keywords: biofilms; catheter-associated infections; ventilator-acquired pneumonia; cystic fibrosis; chronic pulmonary infections

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*These authors contributed equally to this work.

Correspondence and requests for reprints should be addressed to Dao Nguyen, M.D., Meakins-Christie Laboratories, and Translational Research in Respiratory Diseases Program, McGill University Health Centre Research Institute, 1001 Decarie Blvd, mailstop EM3-2219, Montreal, PQ, H4A 3J1 Canada. E-mail: dao.nguyen@mcgill.ca

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Until more recently, the prevailing view of infectious diseases was based on our understanding of acute infections caused by microorganisms grown in the laboratory as planktonic (free-floating) cells in liquid culture medium. It is only since the early 1980s that the role of biofilms in microbial pathogenesis has become increasingly apparent (1, 2). Bacteria and fungi can grow as biofilms, a multicellular and sessile lifestyle in which cells are aggregated within an extracellular matrix rather than dispersed and free-floating. Most biofilms are surface-associated to biotic (e.g., epithelial or dental surfaces) or abiotic surfaces, whereas others can be untethered microbial aggregates that colonize compromised tissue compartments (e.g., sputum within the lumen of cystic fibrosis airways). Such spatially confined microorganisms typically cause slowly progressive, localized and chronic disease, rather than acute and invasive disease. Biofilm growth on host tissues and medical devices has thus emerged as a key mechanism of virulence for opportunistic pathogens such as *Pseudomonas aeruginosa*, staphylococcal species, and the fungi *Candida albicans* and *Aspergillus fumigatus* (Table 1).

Biofilm cells are physiologically distinct from planktonic cells, with widespread differences in gene and protein expression patterns (3, 4). Most notably, this mode of growth confers phenotypic traits that promote microbial survival in hostile environments and against antimicrobial insults, including drugs and host immunity. A hallmark of biofilm infections is thus their recalcitrance to antimicrobial treatments, leading to difficult-to-treat, relapsing or incurable infections, or the need to physically remove infected tissues or medical devices. Biofilms have been implicated in numerous subacute and chronic infections, such as endocarditis, catheter-associated infections, and chronic bacterial infections in cystic fibrosis lung disease (1, 2). With the increasing use of indwelling catheters and medical devices, it is estimated that up to 60–70% of nosocomial infection are associated with biofilms (5).

The Sheltered Life of Biofilms

The formation of a self-produced matrix, also referred to as extracellular polymeric

Table 1. Major pathogens causing biofilm infections

Major Organisms	Biofilm-Associated Infection
Staphylococcus aureus and coagulase-negative *Staphylococcus* species	Catheter-associated infections
Pseudomonas aeruginosa	Pulmonary disease
Candida species	Catheter-associated infections
Aspergillus fumigatus	Pulmonary disease

substances (EPSs), is a defining feature of biofilms. It surrounds biofilm cells, provides structural integrity to the microbial community, promotes surface adhesion and cell aggregation, and contributes to antimicrobial and host resistance (6). The matrix is typically composed of exopolysaccharides, extracellular DNA, and proteins, although its composition varies significantly according to growth conditions and among microbial species (Table 2).

Exopolysaccharides are the major EPS components in many microbial species, and function as "molecular glue" to provide adhesive and structural stability to the matrix. These polymers are composed of various polysaccharides, from sucrose-derived glucans and fructans, cellulose, often with mixtures of neutral and charged polysaccharides. For example, *P. aeruginosa* produces three exopolysaccharides: Pel, a cationic polymer of partially deacetylated *N*-acetylgalactosamine and *N*-acetylglucosamine; Psl, a pentamer of mannose, glucose, and rhamnose; and alginate, an anionic polymer of uronic acids. Pel and Psl are key to *P. aeruginosa* biofilm formation by promoting adherence to surfaces and other cells, and interacting with extracellular DNA and host molecules (7–9). Although alginate is overproduced by mucoid *P. aeruginosa* strains frequently isolated from chronic cystic fibrosis lung infections, this EPS has no aggregative properties.

Many *Staphylococcus* species produce deacetylated poly-*N*-acetylglucosamine (PNAG) polysaccharide (also known as polysaccharide intercellular adhesin), which mediates cell-to-cell adherence and is involved in *in vitro* and *in vivo* biofilm formation (10–12). Similarly, *C. albicans* biofilms contain numerous exopolysaccharides, including β-1,3-glucans, α-mannans, and β-1,6-glucans, which confer structural integrity to the cell wall and biofilm matrix (13, 14). *In vitro* studies suggest that these molecules exist in a mannan–glucan complex, with different structural features than their cell wall counterparts (14). Exopolysaccharides found within biofilms of the mold *A. fumigatus* include galactomannan, galactosaminogalactan (GAG, a heteropolysaccharide composed of α-1,4-linked galactose, *N*-acetylgalactosamine, and galactosamine) and α-1,3-glucan (15–17). GAG mediates hyphal adhesion to biotic and abiotic surfaces, maintains matrix integrity, and is critical for *A. fumigatus* biofilm formation (18–20). As with PNAG and Pel, deacetylation of hexosamine sugars is required for the adhesive function of GAG (21).

Table 2. Key characteristics of biofilms

Key steps in surface-attached biofilm formation:	• Surface attachment to biotic and/or abiotic surface • Production of matrix including exopolysaccharide
Mechanisms to increase resistance to host defenses:	• Concealment or down-regulation of pathogen-associated molecular patterns or antigens • Resistance to phagocytic activity, host antimicrobial defenses, and NET killing
Mechanisms to increase resistance to antimicrobial drugs:	• Physiologic heterogeneity in biofilms, leading to subpopulations that are metabolically quiescent, slow growing, or that have induced stress responses • Limited diffusion or sequestration of antimicrobials by biofilm matrix • Increased expression of antimicrobial efflux pumps

Definition of abbreviation: NET = neutrophil extracellular trap.

A hallmark of biofilms is their remarkable resistance to killing, or tolerance to a wide range of antimicrobials, host defenses, and environmental stress conditions, allowing them to persist in hostile natural and host environments. Biofilm cells can survive 100 to 1,000 higher concentrations of antimicrobials and biocides than planktonic cells (22). Notably, this tolerance is phenotypic and reversible when biofilm cells are dispersed and resume a planktonic state (23). Multiple mechanisms contribute to the antimicrobial tolerance of bacterial and fungal biofilms (24–26). Exopolysaccharides within the biofilm matrix can bind or repel antimicrobials through charge and hydrophobic interactions, and limit their intracellular penetration. For example, β-1,3-glucans in *C. albicans* biofilms sequester azoles (27, 28), while diffusion of positively charged aminoglycosides can be limited by *P. aeruginosa* exopolysaccharides (8, 29).

The altered physiological states of biofilm cells also have profound effects in mitigating antimicrobial activity (30). Biofilm growth generates microenvironments that create physiologically heterogeneous cell populations, with different growth and metabolic states, or cells under nutrient or oxidative stress (31, 32). Metabolically quiescent cells or those expressing adaptive stress responses (such as the stringent response in *P. aeruginosa* and *Escherichia coli*) are less susceptible to antimicrobial killing (30, 33, 34). Finally, gram-negative bacteria and fungi have been reported to overexpress antimicrobial efflux pumps. The relative contribution of these mechanisms to biofilm antimicrobial tolerance varies for individual organisms and antimicrobials.

Microbes growing in biofilms readily evade the host immune system through multiple different mechanisms. *In vitro* studies suggest that biofilm microorganisms are less readily recognized by the immune system (35–37), and are resistant to neutrophil phagocytosis and killing (38–40). For example, the *P. aeruginosa*

exopolysaccharide Psl reduces opsonophagocytosis by inhibiting surface complement deposition, and promotes intracellular bacterial survival, while alginate protects against phagocyte activation, uptake, and killing (41, 42). *Staphylococcus epidermidis* PNAG also hinders antibody binding and opsonic killing (43). Similarly, β-glucans in *C. albicans* biofilm matrix act as decoy molecules that prevent efficient microbial recognition by neutrophils, leading to impaired oxidative burst and neutrophil killing (37, 38). GAG in *A. fumigatus* biofilms conceals β-glucans from recognition by Dectin-1, an innate immune pattern recognition receptor, and protects hyphae from neutrophil extracellular traps (19, 20). Finally, biofilm growth is also associated with down-regulation of flagellin expression and motility in bacteria such as *P. aeruginosa*. Because flagellin is a ligand for Toll-like receptor 5, a major innate immune pattern recognition receptor, and flagellar motility facilitates host cell invasion and phagocytosis, loss of flagellar motility in biofilm cells promotes immune evasion (44).

The biofilm environment facilitates polymicrobial interactions (45), cooperative metabolic functions (46), and cell–cell communication (47). Because many biofilms are polymicrobial, microbial interactions that are cooperative or antagonistic may significantly affect microbial virulence, host interactions, and antimicrobial resistance (48).

Diagnosis of Biofilm Infections

Because biofilm infections have no definitive diagnostic marker, their recognition can be challenging. They are typically defined on the basis of a combination of microbiological, clinical, and microscopic features: localized and difficult-to-eradicate infections, and/or detection of microbial biofilms on direct examination of infected tissues (49). Biofilm growth can also impede microbiological diagnosis as microorganisms are not readily recovered for culture without physical disruption of surface-attached biofilms. Sampling of *in vivo* biofilms is thus difficult, and traditional diagnostic methods often lack sensitivity and specificity (50).

Furthermore, *in vitro* antimicrobial susceptibility tests are of limited value in predicting activity against biofilms. Conventional antimicrobial susceptibility assays test the activity of antimicrobials against microorganisms in the planktonic, not biofilm, state. Although antimicrobial susceptibility assays for biofilm-grown bacteria have been developed (51, 52), they have not been found to predict microbiological or clinical outcomes when evaluated in patients with cystic fibrosis infected with *P. aeruginosa* (53, 54). Standardized antimicrobial susceptibility assays for fungal biofilms have not yet been developed.

Biofilm Formation in Experimental Model Systems

Given the dearth of *in vivo* biofilm models and our limited ability to probe a microbial growth phenotype that is lost on *ex vivo* culturing, our understanding of how biofilms form and behave in the human body remains limited (55). Our current knowledge is largely extrapolated from *in vitro* systems (e.g., microtiter plates, continuous flow chambers), which best model the formation of surface-attached biofilms (56). Although biofilms take on distinct characteristics in different growth environments and experimental systems, they typically share the following features: attachment to surrounding surfaces (biotic or abiotic), or self-aggregation; production of an extracellular matrix; and reversion to a planktonic phenotypic state on dispersion or release from biofilms.

Surface attachment is the first and necessary step for the formation of surface-associated biofilms, and a critical determinant of abiotic surface colonization. The initial attachment, a reversible nonspecific adhesion, is largely determined by interactions between microbial adhesion factors or appendages, and the abiotic surface atomic structure and chemical composition. Irreversible attachment follows and is usually mediated by specific protein adhesin–receptor interactions, as well as the synthesis of EPS components (57). Surface proteins, such as LecA and LecB lectins in *P. aeruginosa* (58, 59), fibronectin-binding proteins (60), protein A (61) or Bap (62) in *Staphylococcus aureus*, or the glycosylphosphatidylinositol-linked cell wall proteins (e.g., Hwp1) and agglutinin-like sequence proteins Als1 and Als3 in *C. albicans* (63), promote cell surface contact during biofilm formation. In gram-negative bacteria, cell appendages such as flagella, pili, or fimbriae mediate adherence to biotic and abiotic surfaces (64, 65). After these initial adhesion events, the elaboration of biofilm matrix by microorganisms serves to increase surface adherence and stability.

Numerous studies have described *in vitro* biofilm formation to be a tightly regulated and stepwise process governed by cell–cell signaling (e.g., quorum sensing in *P. aeruginosa* and *S. aureus*), intracellular signaling [e.g., cyclic-di-GMP and (p)ppGpp], and biofilm-specific transcriptional factors (57, 66, 67). Cyclic-di-GMP has emerged as an intracellular secondary messenger critical to the transition from motile planktonic to sessile adherent biofilm lifestyles in a wide range of bacteria (68). In particular, cyclic-di-GMP down-regulates bacterial motility while up-regulating EPS production to promote bacterial surface adherence and biofilm formation. *In vitro* biofilms such as those observed in *P. aeruginosa* can also take on complex architecture, with a "mushroom-like" structure when biofilms are cultured under continuous flow conditions (66). Whether this ordered process occurs during *in vivo* biofilm formation remains to be determined, as such ordered structures are not observed in human samples (55).

Biofilms: A Microbial Reservoir for Nosocomial Infections

Microbial biofilms are ubiquitous in natural and human-made environments, and likely encompass the majority of bacteria in their natural habitats. Most water distribution systems are colonized with biofilms (69) and this may contribute to the nosocomial transmission of *P. aeruginosa*, for example (70). Contaminated surfaces in health-care settings are also increasingly recognized as a reservoir for transmission for pathogens such as *P. aeruginosa*, *Acinetobacter baumannii*, and *S. aureus*, particularly within the intensive care unit (ICU) (71, 72). Common biocides such as chlorhexidine and triclosan are ineffective at killing biofilm bacteria, including *P. aeruginosa* and *S. aureus* (73), and biofilms have been recovered from sanitized hospital surfaces (72). The ability of biofilm-living organisms such as *Acinetobacter* to

survive desiccation for weeks likely further contributes to their propensity to transmit within health-care settings (74).

Concepts in Antibiofilm Strategies

Conventional antimicrobial drugs have limited activity against biofilms, and great effort has been invested in developing treatments that specifically target biofilms by inhibiting biofilm formation, disrupting established biofilms, or enhancing the activity of conventional antimicrobials (25). To counter surface-attached biofilm formation, many studies have focused on the development of novel biomaterials with surface modifications that alter biophysical cell–surface interactions or prevent biofilm growth. This avenue of research has led to a wide range of novel surfaces coatings with antimicrobials, cationic antimicrobial peptides, or metal nanoparticles being applied to medical devices such as indwelling catheters and endotracheal tubes (75). For example, bismuth thiols or silver-containing molecules have potent antibiofilm activity and have been extensively studied for their potential clinical use.

Interrupting bacterial signaling pathways that coordinate gene functions implicated in biofilm formation is also a compelling approach. For example, inhibitors of (p)ppGpp (76, 77), cyclic-di-GMP (78), and quorum sensing (79) signaling show promising antibiofilm activity *in vitro* in multiple bacterial species and could lead to novel therapeutic strategies to prevent biofilm formation.

Various structural components of the biofilm matrix are also potential targets for enzyme-based therapies. For example, PelA and PslG, glycosyl hydrolases from *P. aeruginosa*, disrupt established *P. aeruginosa* biofilms and enhance biofilm susceptibility to antibiotics and neutrophils (80). β-1,3-Glucanase targets *C. albicans* β-1,3-glucan–rich EPS and increases fluconazole activity *in vivo* (28). Finally, Sph3, a glycoside hydrolase that degrades GAG, inhibits the formation of adherent *A. fumigatus* biofilms and disrupts preformed fungal biofilms *in vitro* (18). DNA can be an important matrix component of biofilms formed by many microbial species including *P. aeruginosa* (81), *S. aureus* (82), *C. albicans*, and *A. fumigatus* (83). In both fungal species, degradation of extracellular DNA with recombinant DNase destabilizes biofilm structural integrity and increases antifungal activity against biofilms *in vitro* (84, 85).

Although similar observations have also been made in bacterial biofilms *in vitro* (81, 86), these effects vary by experimental conditions, and the role of extracellular DNA and DNase therapy *in vivo* remains equivocal. Inhaled recombinant DNase I (dornase alfa) is routinely used in the management of cystic fibrosis lung disease, but primarily for its mucolytic properties. Although disruption of biofilms *in vivo* carries the potential risk of causing disseminated disease, this approach may be considered in combination with conventional antimicrobial therapy that will effectively target microorganisms released from biofilms.

Biofilm-Associated Infections

Indwelling Catheter–Associated Infections

Catheter-associated infections are frequent complications of central venous catheter use in the ICU and are associated with increased cost, length of ICU stay, and mortality. *In vitro* experimental systems provide a good model of catheter-associated biofilms, showing that microbial cells adhere to abiotic surfaces under static or continuous flow, and form surface-attached biofilms that resist host immunity and antimicrobials. Once dispersed from biofilms, organisms may disseminate to cause bloodstream infections and further colonization (87, 88).

Catheter-associated infections are most commonly caused by skin commensal bacterial organisms (e.g., coagulase-negative *Staphylococcus* species and *S. aureus*), although enteric gram-negative bacilli and opportunistic fungi (predominantly *Candida* species) are encountered in immune-compromised and ICU patients (89). The ability to form biofilms on abiotic surfaces is likely a major mechanism of virulence for commensal organisms such as *S. epidermidis* and *Candida* species in catheter-associated infections (90, 91).

Strategies to prevent catheter-associated infections have focused on surfaces coated with antimicrobials and antiseptics to inhibit microbial adhesion and biofilm formation. For example, catheters coated with chlorhexidine–silver–sulfadiazine or minocycline–rifampin have reduced bacterial colonization and bloodstream infections (92). Catheters impregnated with amphotericin B are also effective against *C. albicans* biofilm infections in animal models and avoid the systemic toxicity of this compound (93).

The treatment of catheter-associated infections relies on two principles: disruption of biofilms, and antimicrobial treatment to eliminate viable organisms. At present, disruption of biofilms is largely limited to the removal of colonized catheters, and this is recommended whenever feasible because of the limited activity of antimicrobials in eradicating established biofilms. Although clinical and *in vivo* efficacy data are limited, antibiotic lock therapy, which consists of instilling high concentrations of antibiotic within the catheter to eradicate the adherent biofilm, may be used as adjuvant therapy to salvage permanent indwelling catheters in some situations (94). Although *C. albicans* biofilms show *in vitro* resistance to echinocandins, these drugs display excellent activity *in vivo*, where they can disperse preformed catheter-associated biofilms (95), enhance immune recognition and fungal killing (27, 96–98), and completely eradicate catheter-associated infections (99). This antibiofilm effect is likely mediated through depletion of β-1,3-glucan from the biofilm matrix by these competitive inhibitors of β-1,3-glucan synthase. Although the clinical efficacy of echinocandins supports their use as the treatment of choice for *C. albicans* biofilm infections, current guidelines nonetheless recommend removing the infected catheter whenever possible (100).

Endotracheal Tube Colonization and Ventilator-Associated Pneumonia

Ventilator-associated pneumonia is a major nosocomial infection associated with significant morbidity and mortality. Biofilms readily grow on the surface of endotracheal tubes, and bacterial colonization occurs within hours of endotracheal intubation (101). On aerosolization of biofilms during mechanical ventilation or disruption during tracheal suctioning, bacteria are released and can cause pneumonia (102, 103). Biofilms formed on endotracheal tubes, although not sufficient to cause ventilator-associated pneumonia, are likely the major

microbial reservoir (104–106). In a study of patients undergoing mechanical ventilation, biofilms were detected by scanning electron microscopy in 72 of 75 endotracheal tubes. In 50% of ventilator-associated pneumonia cases, the same pathogens were identified in the bronchoalveolar lavage fluid and endotracheal tube biofilms, and this occurrence was associated with treatment failure (107).

Studies using both culture-based and culture independent methods show that endotracheal tube biofilms are polymicrobial and are composed of many of the organisms found within the oropharyngeal and enteric flora (101, 107–109). This observation suggests that retrograde colonization or aspiration of secretions into the subglottic area is a significant route for bacterial colonization of the distal endotracheal tube (102, 103, 110). Members of the oral flora (e.g., *Streptococcus* and *Prevotella* species) are most common, but ESKAPE organisms (*Enterococcus faecium*, *S. aureus*, *Klebsiella pneumoniae*, *A. baumannii*, *P. aeruginosa*, *Enterobacter* spp.) are also frequently recovered from endotracheal tube biofilms (101, 107, 110).

Coaggregation and other cooperative interactions between various microbes may promote biofilm formation and enhance antimicrobial resistance, as observed with dental biofilms (111). Although oral commensal organisms are traditionally considered nonpathogenic, experimental studies of polymicrobial infections challenge this idea. For example, interactions between oral commensal organisms and *P. aeruginosa* may cause increased virulence in lung infections (112). Moreover, ventilator-associated pneumonia caused by oral commensal organisms in the context of polymicrobial infections are likely underestimated by current standard microbiological approaches.

As with vascular catheters, strategies that incorporate materials into endotracheal tube biomaterials to inhibit bacterial adhesion and/or biofilm formation have been tested clinically (113). Most promising and extensively studied, silver-coated endotracheal tubes are associated with significantly reduced biofilm formation, bacterial lung colonization, and risk of ventilator-associated pneumonia (114, 115). Other approaches include endotracheal tube surfaces coated with antiseptics (116) or metal nanoparticles (117). Although potentially promising, the lack of cost-effectiveness and safety data on some of these devices still precludes their routine use.

Chronic Bacterial Lung Infections in Cystic Fibrosis

Patients with cystic fibrosis (CF) suffer from abnormal mucociliary clearance and other impaired host defenses caused by mutations in the cystic fibrosis transmembrane conductance regulator gene. This leads to chronic lung disease characterized by persistent bacterial infections of the airways and destructive lung inflammation (118). *P. aeruginosa* is the major pathogen in adult patients with CF and causes lifelong chronic airway infections that resist eradication by the host immune system and antibiotic therapy. The chronic, noninvasive, and drug-recalcitrant nature of chronic infections with *P. aeruginosa* is attributable primarily to its growth as biofilms (1, 2, 119).

P. aeruginosa biofilm growth is associated with widespread changes in gene expression and with up-regulation of exopolysaccharide production, whereas acute virulence genes (e.g., type III secretion) and motility are down-regulated (4, 120, 121), leading to bacteria that cause less cytotoxicity and invasion of host cells (41). These experimental results are consistent with the clinical observations that patients with CF harbor chronic pulmonary infections with *P. aeruginosa* for decades without developing invasive disease (119), in contrast to patients with *P. aeruginosa* acute pneumonia, who may succumb within days.

Unlike catheter-associated biofilms or experimental models of surface-attached biofilms, *P. aeruginosa* forms untethered biofilm aggregates within the sputum in CF airways. Bacteria form similar biofilm aggregates within high-density gels when bacterial motility is restricted, and conditions associated with CF sputum and chronic inflammation, including the presence of neutrophil elastase, DNA, and amino acids, are sufficient to promote biofilm aggregate formation (41, 122, 123). Importantly, this nonattached biofilm growth also confers resistance to antibiotics and neutrophil killing *in vitro* (41). Unfortunately, the lack of an *in vivo* lung infection model still limits our understanding of *P. aeruginosa* biofilms in CF.

The treatment of *P. aeruginosa* chronic lung infections is significantly hampered by biofilm-mediated multidrug tolerance. Inhaled antibiotics (e.g., tobramycin) are routinely used to treat CF lung disease and are associated with improved pulmonary outcomes (124). Yet, despite achieving high pulmonary concentrations, they do not eradicate chronic *P. aeruginosa* infections. Novel compounds, such as antimicrobial peptides (125) or metal nanoparticles (126), show promising *in vitro* activity against *P. aeruginosa* biofilms but still remain far from clinical use.

Aspergillus fumigatus Pulmonary Infections

The importance of biofilm formation in the pathogenesis of *A. fumigatus*, a ubiquitous filamentous fungus, has only begun to emerge. *A. fumigatus* causes invasive respiratory infections in immunocompromised patients but also colonize the airways of patients with chronic pulmonary diseases such as CF. Histopathologic studies of human tissues and of animal models of invasive and chronic pulmonary infections have demonstrated that *A. fumigatus* grows as biofilms composed of a multicellular aggregation of hyphae embedded within an extracellular matrix (16, 127). Experimental studies have demonstrated that biofilm growth contributes to fungal virulence by promoting adherence of hyphae to host cells (19, 128) and enhancing resistance to killing by antifungals (129) and the host immune system (20). The formation of pulmonary biofilms by *A. fumigatus* may thus contribute to the high failure rate of antifungal therapy in the treatment of invasive aspergillosis. Further preclinical evaluation of antibiofilm strategies will be required to understand their full potential to improve outcomes in invasive and chronic *A. fumigatus* infections.

Table 3. Implications for clinical care

- Biofilm infections are difficult to diagnose by conventional sampling and microbiology methods
- Biofilm infections are likely to relapse or fail to respond to antimicrobial therapy
- Conventional antimicrobial susceptibility testing does not predict clinical and microbiological responses to treatment

Conclusions

Although biofilm infections are common and cause clinically significant and potentially fatal infections, our understanding of their role in pulmonary infections is still evolving (Table 3). Emerging evidence suggests that biofilms may also be implicated in other persistent infections such as those caused by mycobacteria and nontypeable *Haemophilus influenzae*. Treatment of biofilm infections is hampered by the limited antimicrobial activity of current antibacterial and antifungal drugs. The quest for effective antibiofilm therapies has already led to the discovery of novel materials and drugs with promising *in vitro* and *in vivo* activity, but considerable work remains until these discoveries enter the clinic. ■

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