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Clinical and laboratory evidence for *Neisseria meningitidis* biofilms

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Abstract

Neisseria meningitidis is the etiologic agent of meningococcal meningitis. Carriage of the organism is approximately 10% while active disease occurs at a rate of 1:100,000. Recent publications demonstrate that *N. meningitidis* has the ability to form biofilms on glass, plastic or cultured human bronchial epithelial cells. Microcolony-like structures are also observed in histological sections from patients with active meningococcal disease. This review investigates the possible role of meningococcal biofilms in carriage and active disease, based on the laboratory and clinical aspects of the disease.

Keywords

biofilm; capsule; HrpA; *Neisseria meningitidis*; two-partner secretion

Bacterial biofilms

Biofilm research has gained considerable attention in the last 10–15 years in both the clinical and research communities. A biofilm is a community of microorganisms attached to a surface and encased in an exopolymetric matrix. Biofilms are formed under conditions of environmental stress, and are often associated with shear forces. The members of the biofilm are afforded more protection from environmental factors than if they were in an individual planktonic state. This includes protection from antimicrobials, changes in osmolarity, predation, UV light, reactive oxygen species and dehydration [1]. This added protection allows the bacteria living in the biofilm to persist in environs such as water pipes in industrial settings, as well as in the human host in chronic infections. A model organism for biofilm development is *Pseudomonas aeruginosa*, which forms biofilms in the lungs of cystic fibrosis patients. For this organism, biofilm formation occurs in five stages:

- Loose association of the bacterium with a surface;
- Tight bacterial adherence and transcriptional changes to adapt to the new environment;

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- Bacterial aggregation and microcolony formation, as defined by a small number of bacteria encased in a matrix;
- Mature biofilm development, often seen as mats or tall structures that have channels to disperse nutrients to all the bacteria;
- Detachment of bacteria from the biofilm to colonize other locations [1].

The importance of biofilms in infectious disease was established when it was demonstrated that the biofilm phenotype endows an increased level of antibiotic resistance [2]. There are various hypotheses as to why biofilms promote antibiotic resistance. The first is that the matrix traps antibiotics or possibly other host immunodefensive substances, such as immunoglobulins, complement components and reactive oxygen or nitrogen species [2]. This would protect bacteria on the inside of the biofilm but still may sacrifice bacteria on the outer portions of the biofilm. The second way biofilms could aid in antibiotic resistance is that some members of the biofilm take on characteristics of stationary-phase bacteria where metabolism is reduced to low levels [2]. Many antibiotics act on metabolic pathways and would therefore have little affect on a quiescent bacterium within a biofilm. The third mechanism through which biofilms resist antibiotics is with persisters [2]. These are bacteria that have natural resistance to the antibiotic or other adverse environmental conditions and would therefore survive even if the other members of the biofilm perish. The final mechanism is related to the third. Biofilms of *P. aeruginosa* contain DNA in their matrix and genetic transfer between organisms in the biofilm is established [3,4]. The biofilm sets up an environment where DNA from the matrix or conjugation with adjacent bacteria could provide a source of antibiotic resistance genes and therefore increase the chances of survival to members of the biofilm population.

Biofilms are implicated in several infectious diseases and merit study to reduce morbidity. The biofilm state is often associated with chronic infections and not acute infections. In cystic fibrosis, chronic lung infections are due to *P. aeruginosa*, *Staphylococcus aureus* and *Haemophilus influenzae*, which reside in the airway in biofilms [5,6]. Otitis media is caused by nontypeable *H. influenzae* living within a biofilm [7]. Endocarditis is considered to be a biofilm-related infection caused by staphylococci or streptococci, and urinary tract infections are often caused by biofilms formed by uropathogenic *Escherichia coli*, *Proteus mirabilis* or *Klebsiella pneumoniae* [8,9]. Implant- and catheter-related infections are caused by many of the aforementioned organisms [8].

Biofilm formation by pathogenic *Neisseria*

Neisseria spp. are obligate human pathogens/commensals. The nasopharyngeal flora members, *Neisseria meningitidis* and *Neisseria lactamica*, are able to infect the human hosts for long periods of time without obvious symptomatic disease [10,11]. Likewise, infection by *Neisseria gonorrhoeae* often results in long-term asymptomatic infections in women [12]. The ability to survive for long periods of time in the human host despite a response by both innate- and adaptive-immune defense mechanisms, indicates a specialized lifestyle that resists clearance. The authors hypothesize that *Neisseria* spp. carriage in the human host is made possible by its ability to form biofilms on human mucosa. A brief overview of the literature pertaining to what is known regarding biofilms formed by *N. gonorrhoeae* is given below. A detailed review of the literature pertaining to *N. meningitidis* biofilms and clinical data that may indicate the relevance of *N. meningitidis* biofilms in the infection and carriage process will be discussed later in the text.

N. gonorrhoeae biofilms

N. gonorrhoeae (gonococcus) is the etiologic cause of the sexually transmitted disease, gonorrhea. In men, gonococcal infection results in acute urethritis 2–5 days postinfection

[12]. In women, gonococcal infection is often asymptomatic. The undetected infection can ascend the female genital tract and cause more serious infections such as pelvic inflammatory disease, which can lead to sterility. The ascending infection can also escape the female reproductive tract to cause disseminated gonococcal infection [12].

The ability of the gonococcus to remain undetected in women led to speculation that *N. gonorrhoeae* persists as a biofilm. Gonococcal biofilms can form *in vitro* on cervical tissue and can persist up to 8 days without obvious damage to the cervical tissue [13]. These biofilms are also maintained in a flow cell on glass [13]. Gonococcal biofilms are observed *in vivo* on cervical biopsies, as well as in mixed biofilms on indwelling intrauterine devices of women with reproductive tract infections [14,15].

Electron micrographs of gonococcal or meningococcal biofilms show the presence of long membrane-like structures throughout biofilms grown *in vitro* on glass or on transformed human airway epithelia in flow cells, as well as on archival cervical biopsies of gonococcal culture-positive patients (Figure 1). These membrane-like structures on the gonococcal biofilms label with antibody 2C3, which reacts to the outer membrane protein H.8 present in both the gonococcus and meningococcus [15]. The pathogenic *Neisseria* are known to bleb the outer membrane and release it into the extracellular environment. A mutation in the *msbB* gene results in a penta-acyl lipid A and has a reduced ability to blebbing. This mutant also has a reduced ability to form biofilms in flow cells [15]. The experimental evidence obtained using the *msbB* mutant demonstrates that the blebbing process aids in matrix formation, stabilizing the gonococcal biofilm structure. Autolysis is also known to occur in *N. gonorrhoeae* [16–19]. Membrane from the lysed organisms as well as intracellular contents, such as nucleic acids, may aid in matrix formation. Nucleic acids are known to contribute to biofilm matrix in *P. aeruginosa* [4].

Research is underway to learn more about the gonococcal biofilm environment. Mutational analyses demonstrate that the manganese and zinc transport system, MntABC, is needed to protect the organism against oxidative stress in biofilms grown on glass in flow cells [20]. Further analyses found that mutants lacking *oxyR* (global regulator of redox response), *prx* (peroxiredoxin/glutaredoxin), *gor* (glutathione oxidoreductase) and *trxB* (thioredoxin reductase) are also deficient in biofilm formation [21,22]. This further emphasizes that neutralization of reactive oxygen and nitrogen species is important in gonococcal biofilm formation. It is unknown at this time if defense from oxidative stress is important in biofilm formation of *N. meningitidis*.

***N. meningitidis* biofilms**

Carriage is characterized by the presence of meningococcus in the nasopharynx, an antibody response to the carriage strain and the absence of active meningococcal disease [23,24]. In the USA, 5–10% of the civilian population are carriers [25]. In studies of the civilian population, encapsulated meningococci are isolated from carriers up to 86% of the time and average carriage length is reported to be 9.6 months [25]. The ability of a carrier to pass the carriage strain to close contacts varies from 8–50% in studies [25,26]. This wide range in transmission efficiency may be caused by differences in strain characteristics or in carrier behaviors [27]. A stable biofilm population may explain long-term carriage, the typical humoral response against the carriage strain, as well as provide a population to aid in aerosol transmission [25, 28].

A multitude of factors are likely to trigger the transition to meningococcal disease from the normal carriage state. Carriage of *N. meningitidis* and the development of a protective antibody response decreases the likelihood of later disease onset by a different strain [29]. In a survey of three companies of military recruits, five out of 13 soldiers who had no bactericidal

antibodies to serogroup C meningococci at the beginning of basic training developed invasive disease from serogroup C *N. meningitidis* during training, while no subject with high bactericidal antibody titers prior to training developed disease [28]. Development of meningococcal disease occurs within 1–14 days of acquisition of a new strain while carriers of meningococci develop a protective antibody response to the organism 9–10 days following infection [23,30]. Bactericidal antibodies developed to a particular strain also have bactericidal properties to some strains of other serogroups [29]. There is evidence that a respiratory tract infection 7–10 days prior to acquisition of a new *N. meningitidis* strain in the nasopharynx increases the chances of developing meningococcal disease [23,31]. However, the enhanced possibility of disease with coinfection is more pronounced in adults than in children [31]. These seminal studies in military recruits and civilian populations demonstrate the importance of carriage to prevent active meningococcal disease through the development of a protective antibody response. Chronic infection or carriage is commonly associated with a biofilm mode of growth. Studies that suggest *N. meningitidis* has the ability to form biofilms *in vivo* and *in vitro* are described below.

Clinical evidence of *N. meningitidis* biofilms in the carrier state

Clinical evidence of meningococcal biofilms during carriage is limited. A comprehensive study of tissue excised during tonsilectomy, adenoidectomy or in surgery to repair sinus passages would be beneficial. A small-scale study was performed that used nasopharyngeal swabs to identify carriers of *N. meningitidis* in 32 patients who were to have their tonsils removed. This method revealed that 10% of patients were carriers. However, immunolabeling for the PorA protein of the meningococcus on 5 µm frozen sections of the tonsils revealed that 45% of the patients had the meningococcus in their tonsillar tissue [32]. Interestingly, the labeled bacteria appear to be in microcolonies just below the epithelial surface, which could explain why as few as 10% were identified by nasopharyngeal swab. The finding of microcolonies just under the epithelial surface is interesting considering that streptococci can attach to damaged heart valves and form biofilms below the fibrin matrix [33]. It is possible that *N. meningitidis* can form biofilms in deeper tissues in a similar manner as a result of damage to respiratory tissue from inflammation caused by coincidental bacterial or viral infection, or environmental stresses, such as smoking. The prevalence of meningococcal carriage is known to be higher in individuals exposed to tobacco smoke or recent respiratory tract infection [23,31,34]. The finding of microcolonies within the tonsillar tissue instead of on the surface also raises the possibility of intracellular bacterial communities or intracellular biofilms, as recently demonstrated for uropathogenic *E. coli* and *K. pneumoniae* [35]. Uropathogenic *E. coli* subverts host defenses by living in these intracellular communities. A recent study of meningococcal biofilms on human bronchial epithelial (HBE) cells demonstrates the possibility of meningococci living within the HBE cells at 48 h postinfection [36]. It is possible that *N. meningitidis* could live in intracellular communities as well, which would allow for persistence and immune evasion [32]. Sim *et al.* noted that *N. meningitidis* has homologs to genes of *Legionella pneumophila* that are important to the intracellular survival of *Legionella* [32].

Device-related *Neisseria* biofilms

Bacteria commonly colonize prosthetics and implant-related devices. The bacteria live on these implants in biofilms and the infection can jeopardize the prognosis of the patient. *N. gonorrhoeae* can colonize intrauterine devices in a mixed biofilm with other bacteria as described previously [14]. Laryngotracheal stents are often colonized with bacteria after implantation and the bacteria grow in biofilms on the surface of the stent. A clinical study examined 21 patients who had an average age of 37 months for biofilm formation on laryngotracheal stents. All stents were positive for 2–5 different bacterial organisms and 52%

were *Neisseria*. No effort was made to establish if these bacteria were *N. meningitidis*, *N. lactamica* or one of the oral *Neisseria* [37].

Clinical evidence of *N. meningitidis* biofilms in overt disease

Biofilms are commonly observed in chronic infections. However, recent clinical evidence of meningococcal biofilms in overt disease has been described. Purpura fulminans is a disease state associated with meningococcal sepsis, in which cutaneous, hemorrhagic lesions occur. Immunolabeled skin biopsies have been taken from these lesions to determine if capsule, pili and PorA are expressed during septic infection. All three virulence factors are expressed and interestingly the bacteria are mostly grouped in microcolonies in these lesions [38]. It is unclear if capsule, pili and PorA are important in the formation of the microcolony structure *in vivo*, or if they are important for other aspects of the disease state. This study indicates the possibility of biofilm formation during symptomatic disease and not just chronic nasopharyngeal infection, however, more work needs to be performed to establish if the presence of microcolonies allows for the progression to mature biofilms *in vivo*.

In another study of symptomatic meningococcal sepsis, histological sections demonstrate microcolonies of meningococci in brain capillaries, which are likely to have a low blood flow [39]. Further *in vitro* studies show that meningococci cannot attach to surfaces at high flow rates, however, attachment is achieved at low flow rates. Once attached the bacteria can withstand higher shear forces. *In situ* imaging of rat brain capillaries demonstrates that meningococci can attach when blood flow temporarily decreases and can then multiply into microcolony-like structures in these capillaries [39]. The studies of brain tissue and purpura fulminans demonstrate the ability of meningococci to form small biofilms *in vivo* during septic infection [38,39]. However, none of the above studies provide high resolution images that would allow us to determine if a matrix is present in these microcolonies and if the matrix is composed of membranes as established for gonococcal biofilms *in vivo* [15].

In vitro study of *N. meningitidis* biofilms Pilus

The role of pilus in meningococcal biofilms has been established on glass. Two studies demonstrate that pilus is important for twitching motility, resulting in the aggregation of meningococci into microcolonies and aiding in 3D architecture of the biofilm [40,41]. A pilus-associated protein (PilX) is responsible for autoaggregation and plays a role in twitching of *Neisseria* [42]. Meningococcal mutants of *pilE*, (which encodes the main pilus subunit), or *pilX*, still form biofilms on glass but have a flat architecture [40]. This indicates that the pilus is not necessary for biofilm formation on glass. Currently, it is not known if the pilus has a role in biofilm formation on human tissue other than its established role as the initial adhesin for pathogenic *Neisseria* [43].

Capsule—Static biofilm assays in a 96-well polystyrene plate demonstrates that encapsulated meningococci are deficient in biofilm formation, however, a mutant deficient in capsule or a lipooligosaccharide-truncation mutant has an increased biofilm mass and height compared with the wild-type strain [41]. A study that utilized flow cells also demonstrates that encapsulated meningococci are impaired for biofilm formation [40]. These studies suggest that there is a need for hydrophobic interactions to allow for biofilm formation and that the hydrophilic capsule and possibly lipooligosaccharide, block this interaction. Greiner *et al.* postulate that hydrophobic interactions may be important to *Neisseria* biofilm formation in a study of *N. gonorrhoeae* biofilms after demonstration of extensive membranous material in the biofilm matrix [13].

The finding that encapsulated meningococci do not form biofilms is paradoxical. If biofilms are present *in vivo* and the organisms do not express capsule, then why would meningococcal

capsular conjugate vaccines affect carriage as demonstrated in the UK [44]? It is important to demonstrate the ability of encapsulated *N. meningitidis* to form biofilms on tissues in order to explain what is known about long-term meningococcal carriage, evasion of innate immune defenses and active disease [38,45,46]. To address this question, a modified flow cell that accommodates a coverslip with a monolayer of transformed airway epithelial cells was used to establish if encapsulated meningococci can form biofilms and to identify bacterial factors involved in biofilm formation. These studies found that encapsulated meningococci can form mature biofilms on SV-40 transformed HBE cells in the flow cell by 48 h. These biofilms had significantly more biomass and a taller average height than biofilms grown on collagen-coated coverslips or on glass alone in the same chambers. *In situ* immunolabeling of the biofilms as well as ELISA of biofilm material against the meningococcal capsule demonstrate that organisms within the biofilm are encapsulated [36]. This is important because capsule production is phase variable, as well as being regulated by CrgA [47,48]. Unencapsulated *N. meningitidis* bind to tissue better than encapsulated meningococci [45]. If the only organisms that were phase-off for capsule production bound the HBE cells, then it would be possible that the majority of the organisms that produced the biofilm would also be phase-off. Since capsule production is regulated, it is also possible that organisms in a biofilm environment have capsule production downregulated. The studies on transformed respiratory epithelia demonstrate that the majority of meningococci in the biofilms are encapsulated, showing that phase variation and regulation do not have a major role in capsule production of organisms at the 48 h biofilm time point. Furthermore the average biofilm height of wild-type and isogenic unencapsulated mutants were statistically indistinguishable at 48 h on HBE cells. These studies demonstrate the need for a proper substrate for the meningococci to adhere to and form biofilms. This finding is significant as meningococcal infection, as a biofilm of encapsulated organisms, would make a population of bacteria fitter than unencapsulated organisms as they would be more able to evade host-immune clearance as well as cross the epithelial or endothelial cell layer [45,49].

Two-partner secretion—Two-partner secretion (TPS) is important for biofilm formation in *Bordetella bronchiseptica* [50]. The secreted member of the TPS system of *B. bronchiseptica*, filamentous hemagglutinin (FHA), enhances static biofilm formation on 96-well microtiter plates by enhancing attachment to the plate surface [50]. The two-component regulatory system (BvgAS), which regulates FHA production in *Bordetella pertussis* and *B. bronchiseptica*, also enhances biofilm formation on glass when the BvgAS system is turned on and inhibits biofilm formation when turned off [51]. This BvgAS-enhanced biofilm formation corresponds to the conditions in which BvgAS induces FHA production. Studies of the *N. meningitidis* TPS system demonstrate that the secreted TPS member, HrpA, enhances biofilm formation in flow cells on transformed airway epithelial cells [52]. This was the first study of *N. meningitidis* biofilms to demonstrate a factor involved in the evolution of the biofilm structure on a relevant substrate. HrpA is implicated in different studies in cell association at 5 h following infection, as well as intracellular escape from vacuoles at 7 h following infection [53,54]. Biofilms at the 6 h time point on transformed airway epithelial cells show no difference in biomass between wild-type, mutant or complemented strains. This indicates that differences in initial cell association were not a factor at the 6 h time point, further implicating HrpA as an adhesin. Biofilms grown on collagen-coated coverslips for 48 h also demonstrate reduced biofilm biomass and average height in the *hrpA* mutant, which shows that egress from the intracellular environment is not a major contributor to the biofilm and not the cause for the *hrpA* biofilm-associated phenotype. These findings also demonstrate that HrpA has a different role in biofilm formation for *N. meningitidis* than FHA has for *B. bronchiseptica*.

The exact function of HrpA within the biofilm is not yet understood, but HrpA processing from a 220 kDa form to a 180 kDa form is enhanced in anaerobic conditions [52]. The oxygen gradient that occurs in large biofilms would produce an environment in which the processed HrpA would be predominant in the deeper recesses of the biofilm. This processed HrpA may

have a role in holding the structure of the biofilm together, presumably through bacteria–bacteria interactions. The oxygen gradient may be key to the production of active HrpA and formation of large mature biofilms, as observed in smaller biofilms in the *hrpA* mutant strains [52]. It is interesting to note that *N. gonorrhoeae* lacks a functional HrpA and yet, is shown to grow biofilms *in vivo*, further demonstrating that biofilm formation by the pathogenic *Neisseria* is multifaceted and not dependent on a single factor.

Tissue destruction in meningococcal biofilms

In the flow-cell system that accommodates airway epithelia, 20% of the cells had perished by 48 h in uninfected flow cells, while 30–40% of the cells were dead in infected flow cells [36]. This may be due to suboptimal media composition or other environmental factors. There is little evidence for tissue destruction *in vivo* on nasopharyngeal tissue during asymptomatic meningococcal carriage. It is possible that the sums of the innate and adaptive immune defenses force the meningococci into a quiescent biofilm phenotype *in vivo* and this results in carriage. The flow-cell system lacks components of the innate immune system including complement and professional phagocytes, and lacks all components of the adaptive immune system. This may allow for some of the meningococci to persist with a virulent phenotype in the flow-cell biofilm system. As stated previously, 20% of uninfected airway epithelial cells died in uninfected flow cells within 48 h [36]. This raises the possibility that the airway epithelial cells were in poor health due to flow-cell conditions and the infection enhanced the cell death process that had already begun. Another possibility explaining the enhanced tissue destruction in the biofilm studies is that the meningococcal isolates used in this study were from symptomatic infections and may be more cytotoxic than carrier strains.

While tissue destruction is not known to occur in the carrier state, which we believe to be a biofilm, tissue damage does occur in overt disease. One possible factor that could alter the course of meningococcal infection from a carrier state to an infectious state could be coinfection with another respiratory pathogen. Previous studies link acute respiratory disease from adenovirus, parainfluenza, respiratory syncytial virus, rhinovirus, *Mycoplasma* species and influenza virus to meningococcal disease [23,31,55,56]. It is unclear if the concurrent infection aids *N. meningitidis* infection through tissue damage or through suppression of the immune system. As discussed earlier, meningococcal infection is more common in individuals exposed to cigarette smoke [34]. This is a factor that would also damage respiratory epithelia and possibly allow meningococci to take advantage of a weakened mucosal barrier to allow for colonization or disease.

Conclusion

Biofilm communities composed of encapsulated meningococci offer protection from innate host defenses, such as cationic antimicrobial peptides, as well as complement in the nasopharynx, and could provide a reservoir for perpetuating the infection process through aerosolization [45,57]. Histological studies, demonstrating the presence of meningococci in microcolonies on tonsillar tissue, indicate that a biofilm phenotype may be important to carriage. *In vitro* studies demonstrate that encapsulated and unencapsulated organisms can form biofilms on transformed airway epithelial cells and that capsule and HrpA are important to the composition of the biofilm community. The majority of carrier strains are encapsulated, however, some unencapsulated carrier strains do exist [58]. These are important advances in understanding how meningococci may behave *in vivo* in a carrier state. The histological study of purpura fulminans biopsies and brain vasculature indicate that microcolonies develop in these tissues during overt disease and that capsule, pilus and PorA are expressed during this process [38,39]. This knowledge of biofilm formation by *N. meningitidis* is critical since researchers are targeting meningococcal antigens for a *N. meningitidis* vaccine that is effective

against all serogroups of meningococci. It is important that vaccine researchers mimic the natural environment of the host in order to determine which antigens are produced during different phases of infection. Currently, meningococcal biofilm research demonstrates the possibility that one phase of the infection cycle of *N. meningitidis* is persistence within a biofilm and therefore, has relevance to vaccine research.

The human host is the sole reservoir for *N. meningitidis*. As such, the bacterium has developed a lifestyle that makes it capable of persisting within the human host and gives it the ability to easily transmit between human carriers. The biofilm mode of growth offers a lifestyle congruent with the notion of long-term carriage and evasion of host immune clearance. The biofilm structure may also be relatively resistant to the innate immune response as characterized by asymptomatic carriage in the nasopharynx of the host for *N. meningitidis* and in the female for *N. gonorrhoeae*. The biofilm mode of growth for resistance to oxidative stress is already established for *N. gonorrhoeae* and could also have a role in meningococcal biofilms [20–22]. The presence of encapsulated organisms in the biofilm of a carrier would provide supportive, although not conclusive, evidence of why meningococcal capsular conjugate vaccines are efficacious [44]. Further research as to how the biofilm affects antibiotic treatment options and which antigenic epitopes are present on biofilms is warranted to enhance future treatment of meningococcal disease and the development of vaccines.

Future perspective

Biofilm research with encapsulated *N. meningitidis* has demonstrated its ability to form biofilms on transformed human respiratory epithelia. This fact should lead to two sets of investigations. The first is to establish whether or not meningococcal biofilms occur *in vivo*. We believe the biofilm lifestyle is most likely the carrier state of the organism and further work is required to determine the nature of the biofilm in this environment, as well as which antigens are expressed on the surface of the biofilm in order to direct vaccine research. The second set of investigations should be on the immune response to these biofilms. The presence of meningococcal biofilms in the carrier state, composed of capsule-expressing organisms, may explain the efficacy of capsular conjugate vaccines. It is important to understand the interactions of complement, immunoglobulins and sentinel immune cells to developing, as well as established meningococcal biofilms. A minimal or inefficient response by these immune systems could explain the average carriage state of 9.6 months for this organism. However, if one type of immune response is more efficient than another, those findings could help direct future therapy against this organism. It would also be important to determine if all capsular serotypes have the ability to form biofilms and if strains with low invasive potential have increased or decreased ability to form biofilms compared with epidemic strains.

Executive summary

Bacterial biofilms

- Biofilms are formed under conditions of environmental stress, which affords microbes more protection from environmental factors than if they were in an individual planktonic state.

Biofilm formation by pathogenic *Neisseria*

- *Neisseria gonorrhoeae* forms biofilms *in vivo* and *in vitro*. The oxidative stress-response pathways are essential to this process. The oral *Neisseria* also form biofilms on the dental surface.

Clinical evidence of *Neisseria meningitidis* biofilms in the carrier state

- Histological sections of tonsillar tissue demonstrate microcolony-like structures of meningococci within the tissues. Further research is needed to determine if these structures are enveloped within a matrix, which would define them as a biofilm.

Device-related *Neisseria* biofilms

- Laryngotracheal stents are often colonized with multispecies biofilms that include the *Neisseria* genera. It is unclear at this time which of the oral or nasopharyngeal *Neisseria* is responsible for this colonization.

Clinical evidence for *Neisseria meningitidis* biofilms in overt disease

- Microcolony-like structures of *Neisseria meningitidis* are observed in sections of brain tissue and in biopsies from purpura fulminans. It is unclear at this time if these structures matured to the state of developed biofilms by becoming enveloped within a matrix.

***In vitro* study of *Neisseria meningitidis* biofilms**

- Pilus is essential for the 3D architecture of the meningococcal biofilm on glass in flow cells. Mutations in *pilE* or *pilX* result in flat biofilms.
- Capsule inhibits biofilm formation by *N. meningitidis* on plastic and glass. However, capsule does not inhibit biofilm formation on transformed human respiratory epithelia. This may have implications on the efficacy of capsular conjugate vaccines if encapsulated organisms form biofilms in the carrier state.
- Two-partner secretion contributes to biofilm formation on transformed human respiratory tissue through the secretion and deposition of HrpA on the surface of the bacterium. HrpA is thought to stabilize the architecture of the biofilm with the processed form of the HrpA protein. This processed form is most prevalent in environments of low oxygen, as may be the case within the middle of the biofilms.

Tissue destruction in meningococcal biofilms

- *N. meningitidis* biofilms on transformed human respiratory tissue in flow cells resulted in increased tissue destruction when compared with tissue in uninfected flow cells. Tissue destruction by *N. meningitidis* has also been seen in infection of nasopharyngeal organ culture. Further investigation would help determine if suboptimal media composition contributed to this effect or if this is a mechanism that contributes to invasive disease.

Conclusion

- Meningococci have the ability to form mature biofilms *in vitro*.
- More work is needed to determine if mature meningococcal biofilms form *in vivo*.
- Future research may result in better vaccine candidates and a better understanding of the immune response to meningococcal biofilms.

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Figure 1. Biofilm images of *Neisseria gonorrhoeae* and *Neisseria meningitidis* depicting a membraneous biofilm matrix
(A) *Neisseria gonorrhoeae* biofilm from an archival cervical biopsy with membraneous material as part of the biofilm. **(B)** Cryoscanning electron microscopy image of an *Neisseria meningitidis* biofilm with extracellular membraneous material forming part of the matrix. Arrows point to membraneous matrix material.