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Uropathogenic *Escherichia coli* biofilms

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ABSTRACT

Urinary tract infection (UTI) is one of the most common infectious diseases, with a global annual incidence of ~175 million cases. Uropathogenic *Escherichia coli* (UPEC) is the major cause of UTI (>80%) and increasingly associated with rising antibiotic resistance. UPEC form biofilms during infection of the urinary tract, either on the luminal surface of the bladder, intracellularly within bladder superficial epithelial cells, or on the surface of indwelling catheters. This lifestyle of sessile growth promotes enhanced resistance, persistence and increased rates of recurrent UTI. UPEC employ a range of virulence factors to form biofilms, including fimbrial adhesins for attachment and autotransporters to promote cell-to-cell aggregation. In addition, UPEC biofilms are encased in an extracellular matrix comprised of proteins such as curli amyloid fibres and polysaccharides such as cellulose, which together form a hydrating glue that provides structural support for the biofilm and protects its component cells. Here, we describe the key features of UPEC biofilms and their importance for UPEC pathogenesis of the urinary tract.

Keywords: fimbrial adhesins, UPEC biofilms, UPEC pathogenesis, urinary tract infections, uropathogenic *Escherichia coli*.

Urinary tract infections

Urinary tract infection (UTI) involves infection of the bladder (cystitis) and kidney (pyelonephritis) and can lead to life-threatening sepsis. Approximately 25% of women who suffer UTI will experience a recurrence within 6 months of the initial infection,[1] either with the same strain or a new organism.[2] Overall, recurrent UTI is associated with increasing antibiotic resistance, treatment failure, decreased quality of life and mounting pressure on our healthcare system. Catheter-associated UTIs (CAUTIs) are also a major problem, particularly in the hospital setting. In Australia, it is estimated that the economic burden of antibiotic-resistant UTIs could mount to A$1.6 billion per annum by 2030 if nothing is done to halt increasing rates of infection.[3]

Uropathogenic *Escherichia coli*

UTI is caused by a diverse range of pathogens, the most common being uropathogenic *Escherichia coli* (UPEC), which is responsible for >80% of all infections.[4] UPEC possess multiple virulence factors that enable infection of the urinary tract and promote disease pathogenesis. These include: (i) adhesive organelles that mediate attachment to uroepithelial cells in the bladder (e.g. type 1 fimbriae) and the kidney (e.g. P fimbriae) and facilitate colonisation[5,6]; (ii) autotransporter proteins (e.g. Ag43) that mediate aggregation[7,8]; (iii) iron acquisition systems involving siderophores and heme-binding proteins that scavenge iron from the host to enable survival in the iron-poor urinary tract[9]; (iv) toxins such as cytotoxic necrotising factor-1 and hemolysin that damage host cells, thereby enabling penetration into deeper tissue layers[10]; (v) surface polysaccharides such as the capsule and O-antigen that provide protection against soluble and cellular mediators of host innate immunity[11]; and (vi) flagella that promote motility, enabling ascension from the bladder into the kidneys.[12] UPEC also exhibit high rates of antibiotic resistance, including resistance to last line carbapenems and polymyxins, and frequently carry conjugative plasmids that facilitate the rapid dissemination of antibiotic resistance genes.[13]

UPEC biofilm formation

UPEC can exist in extracellular and intracellular niches in the urinary tract, both of which are associated with the formation of biofilms. UPEC form biofilms on the luminal surface

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of the bladder and on the surface of catheters. In addition, UPEC can form biofilm-associated intracellular bacterial communities (IBCs) within superficial bladder epithelial cells, where they establish tightly packed aggregates comprising 10^4–10^5 cells enclosed in an extracellular matrix.[14] The pathway of IBC formation has been described extensively in mice,[14] and evidence for IBC formation in human patients has also been reported.[15] The formation of biofilms by UPEC during infection enables the establishment of a physical barrier that protects against host innate immune defences (e.g. neutrophils and antimicrobial peptides such as cathelicidins) and antibiotics.[16] In laboratory mice, UPEC intracellular growth within bladder epithelial cells results in the persistence of infection despite treatment with antibiotics with different target specificities and from different classes.[17] The close proximity of bacterial cells within biofilms also contributes to the exchange of DNA, including antimicrobial resistance plasmids. Overall, the impact of biofilm formation by UPEC is associated with increased resistance, persistence and recurrent infections.

Mechanisms of UPEC biofilm formation

UPEC biofilm formation involves several stages of progression: (i) adhesion, (ii) aggregation and early development of the biofilm structure, (iii) biofilm expansion and maturation, and (iv) dispersal of cells from the biofilm (Fig. 1). Initial attachment requires the expression of fimbriae such as type 1 fimbriae that mediate specific binding to mannosylated bladder superficial epithelial cells by a tip-located FimH adhesin, and subsequent invasion and the formation of IBCs.[14] UPEC also produce other fimbriae that contribute to adhesion by interaction with different receptors, including P, F1C, F9, Afa, and type 3 fimbriae.[18–20] After attachment, UPEC proliferation leads to the formation of aggregates or clusters by cell-to-cell interactions. One important UPEC surface factor that drives aggregation and biofilm development is the autotransporter protein Ag43,[8] but other autotransporters also promote similar phenotypes.[21,22] A key process that occurs during UPEC biofilm maturation is the production of an extracellular matrix, a glue composed of protein and polysaccharide that functions as an external support to maintain the structural integrity of the biofilm. The primary building blocks of the UPEC biofilm extracellular matrix are curli and cellulose. Curli are extracellular amyloid fibres that form connections between cells and facilitate interactions with UPEC-produced cellulose.[23] Other polysaccharides such as β-1,6-*N*-acetyl-D-glucosamine (PGA) and colanic acid also function as extracellular matrix components that shape the architecture of the developing biofilm. The last stage of UPEC biofilm maturation involves detachment, leading to dispersal of cells from the biofilm and the capacity to seed new biofilms at distal sites.

A molecular mechanism that triggers the transition of UPEC from a sessile (attached) state to a planktonic

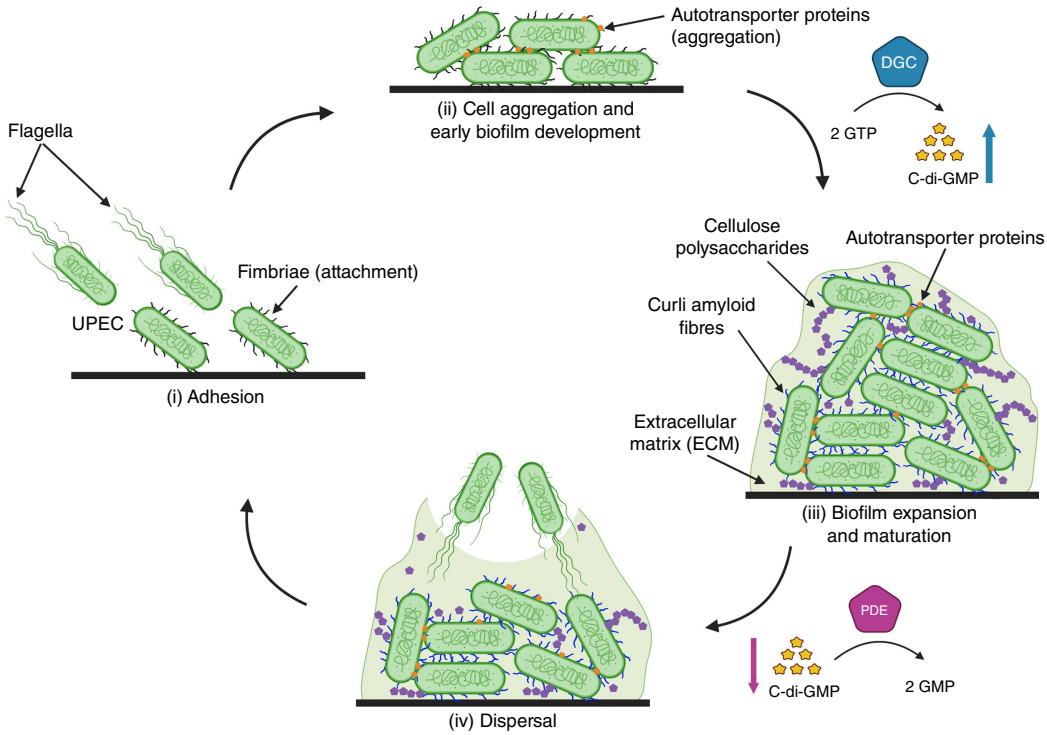

Fig. 1. Schematic diagram of biofilm formation and dispersal. Biofilm developmental process involving: (i) surface attachment of uropathogenic *Escherichia coli* (UPEC), (ii) cell aggregation and early development, (iii) expansion and maturation, and (iv) dispersal that can result in the formation of new biofilms at distal sites. Diguanylate cyclases (DGC) synthesise c-di-GMP from two GTP molecules, increasing levels of c-di-GMP that promotes biofilm formation. In the reverse step, c-di-GMP is degraded into two GMP molecules by phosphodiesterases (PDE), reducing c-di-GMP levels and promoting motility and dispersal from biofilms. Image was created with Biorender.

(free-swimming) state is the production of the second messenger molecule bis-(3′-5′)-cyclic dimeric guanosine monophosphate (c-di-GMP).[24] High UPEC intracellular concentrations of c-di-GMP, generated by diguanylate cyclase enzymes that synthesise c-di-GMP, promote the production of adherence, aggregation and extracellular matrix components that drive biofilm formation. By contrast, low intracellular concentrations of c-di-GMP, achieved by the activation of phosphodiesterase enzymes that degrade c-di-GMP, promote the production of flagella and UPEC dispersal from the biofilm.

UPEC biofilm extracellular matrix components – curli

Curli are extracellular amyloid fibres that contribute to UPEC biofilm formation by interacting with host cell matrix proteins and enhancing adherence, aggregation and colonisation of the urinary tract.[25] The genes involved in curli production (referred to as curli-specific genes, *csg*) are found at the same location on the chromosome and arranged as two divergent operons; *csgBAC* and *csgDEFG*. The *csgBAC* operon encodes the major subunit protein CsgA, the minor subunit and nucleator protein CsgB, and the CsgC periplasmic chaperone. The *csgDEFG* operon encodes the positive regulator CsgD (which regulates both operons), the auxiliary secretion factors CsgE and CsgF, and the CsgG pore that facilitates secretion of curli subunit proteins across the outer membrane.[25]

Curli biosynthesis is regulated by a complex network involving environmental and stress-sensing mechanisms that are relayed through the curli regulator CsgD. These involve the stationary phase sigma factor RpoS, the two-component regulatory systems OmpR-EnvZ and CpxA-R, the Rcs signal transduction system, integration host factor (IHF), the histone-like nucleoid-associated protein H-NS, small non-coding RNAs and c-di-GMP. Most of our knowledge on curli regulation and biosynthesis comes from studies on commensal *E. coli* K-12 strains. We recently devised a high-resolution genetic screen employing saturated transposon mutagenesis coupled with phenotypic detection using the curli-specific dye Congo Red to define the genes involved in curli biogenesis.[23] Transposon mutants with impaired curli production, identified by their inability to bind Congo Red, were characterised *en masse* using transposon-directed insertion site sequencing (TraDIS). In addition to the known genes mentioned above, our method identified novel genes and pathways involved in curli production, including purine biosynthesis, lipopolysaccharide (LPS) biogenesis, stress and stationary phase regulation, metabolism, sodium transport and septum formation. Excitingly, we also discovered a new curli repressor that we named *rcpA* (i.e. repressor of curli production A). Overexpression of *rcpA* reduced the transcription of genes encoding the curli regulator CsgD and the curli major subunit CsgA. The *rcpA* gene encodes a protein of 93 amino acids with two predicted transmembrane helices, a cytoplasmic C-terminal domain, and a high hydrophobic amino acid ratio, all of which suggest that RcpA may localise to the inner membrane and function by sensing and responding to environmental signals. Ongoing work in our lab is aimed at understanding the function of RcpA and elucidating the molecular mechanisms by which other factors identified in our screen affect curli production.

UPEC biofilm extracellular matrix components – cellulose

Cellulose is a linear exopolysaccharide polymer comprising β-1,4-linked glucosyl residues. The genes responsible for UPEC cellulose synthesis are encoded on two divergent operons; *bcsRQABZC* encoding proteins involved in synthesis and secretion and *bcsEFG* encoding the machinery required for cellulose modification. Similar to curli, cellulose biosynthesis is controlled by a complex regulatory network, mostly through CsgD. Therefore, cellulose is often co-produced with curli, explaining why both of these molecules make up the primary components of the biofilm extracellular matrix. The second messenger molecule c-di-GMP is also a critical checkpoint for cellulose production, as it functions as a direct activator of the BcsA synthase that transfers glucosyl residues from UDP-glucose onto the growing β-D-1,4-glucan chain in cellulose biogenesis. In UPEC, cellulose is modified by the addition of phosphoethanolamine, mediated by the BcsG transferase enzyme,[26] although the functional effect of this modification remains to be properly understood. Current work in our lab is directed at defining the complete set of genes involved in UPEC cellulose production, and understanding the intersection of pathways involved in cellulose and curli synthesis and degradation.

Conclusion

The capacity to form biofilms plays an integral part in UPEC disease pathogenesis. We now have a good understanding of the virulence components that promote UPEC biofilm formation, including mechanisms of attachment, cell aggregation and extracellular matrix production. Future work will involve investigating the complex genetic networks that control these processes, with the goal to develop new therapeutics to inhibit and disrupt UPEC biofilms.

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Data availability. Data sharing is not applicable as no new data were generated or analysed during this study.

Conflicts of interest. The authors declare that they have no conflicts of interest.

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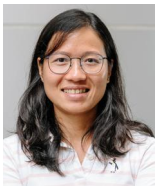
Biographies



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