Sensing the unreachable: challenges and opportunities in biofilm detection

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Bacteria can attach to essentially all materials and form multicellular biofilms with high-level tolerance to antimicrobials. Detrimental biofilms are responsible for a variety of problems ranging from food and water contamination, bio-corrosion, to drug resistant infections. Besides the challenges in control, biofilms are also difficult to detect due to the lack of biofilm-specific biomarkers and methods for non-destructive imaging. In this article, we present a concise review of recent advancements in this field, with a focus on medical device-associated infections. We also discuss the technologies that have potential for non-destructive detection of bacterial biofilms.

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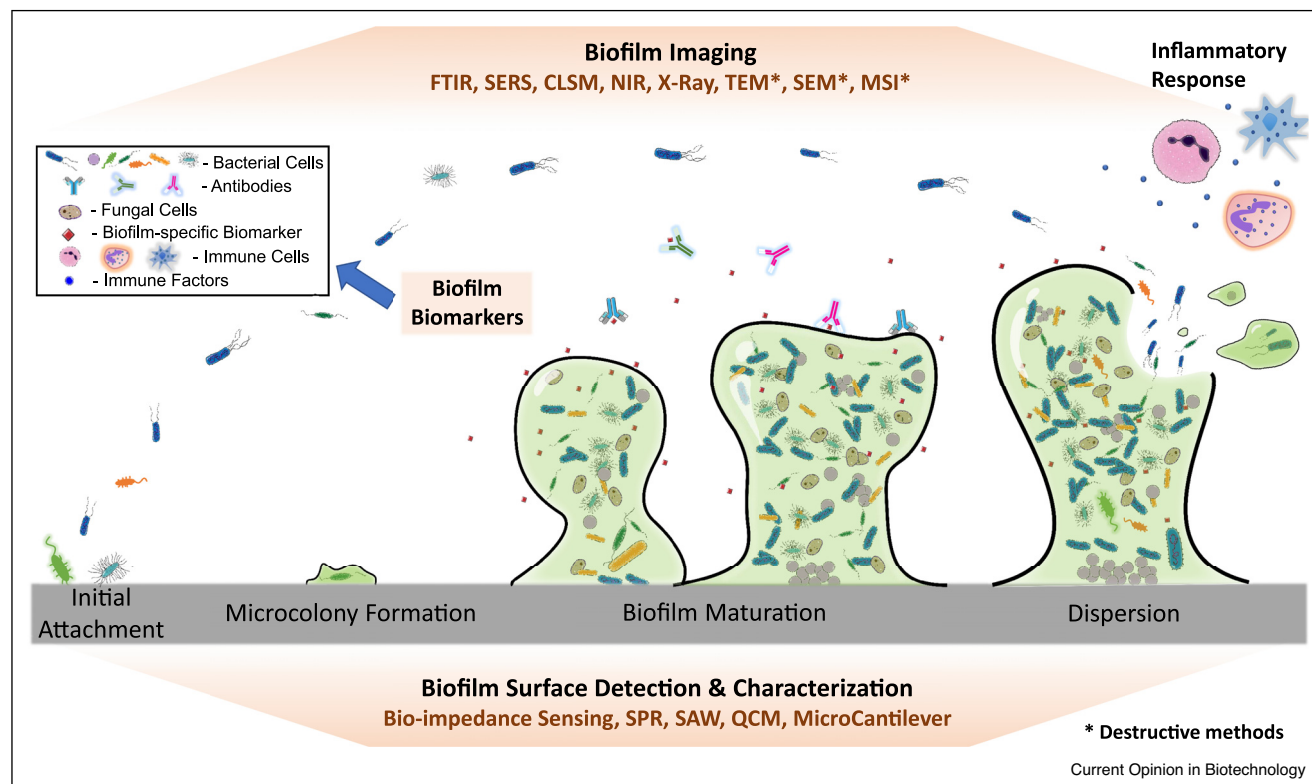
Bacterial biofilms

As the oldest life form on earth, bacteria have developed remarkable capabilities to survive in harsh environments. One such strategy is to colonize surfaces and form biofilms, which are complex structures of bacterial cells embedded in an extracellular matrix comprised of polysaccharides, proteins, DNA and lipids produced intrinsically by these bacteria [1]. Biofilm cells are up to 1000 times more tolerant to antibiotics than their planktonic counterparts, rendering biofilm-related infections largely unresponsive to antibiotic therapies [2]. Thus, detrimental biofilms are of great concern especially when formed by pathogenic species on implanted medical devices and biomaterials such as indwelling catheters, orthopedic implants, cochlear implants, among others [3–6].

Challenges in biofilm detection

Although a number of methods have become quite routine for biofilm characterization in controlled laboratory settings [7•], biofilm detection in clinical settings has multiple challenges [8–10]. In general, biofilm associated infections (BAI) are chronic and remain local to the infection sites such as an implant. Additionally, clinical evidence suggests that for long periods of time biofilms can produce occult, or subclinical, infections, in which the inflammatory symptoms are less pronounced than acute infections, only revealing themselves when biofilm cells shed off resulting in bacteremia [11]. Usually BAIs involving foreign bodies are not confirmed until the device is explanted and even then often a diagnosis of biofilm is based on anecdotal evidence [9]. When a biofilm is formed on an implanted medical device, it cannot be directly sampled without a surgical procedure. This essentially precludes the detection using standard microbiological methods such as bacterial culturing on agar plates until intra-operative access. Even if the cells can be sampled, slow-growing variants of bacteria and dormant persister cells [12] may not form colonies under routine culturing conditions and thus cause a false negative result [13]. Culturing methods also face challenges with the highly heterogeneous distribution of biofilms and the involvement of fastidious strains and/or mixed-species biofilms that require specific growth factors [9]. A standardized, reliable method for the detection of biofilms in clinical settings is still missing. Besides BAI diagnosis *in vivo*, microbial detection on explanted medical devices is also challenging if the cells are difficult to sample (if trapped in crevices such as those in endoscopes) or culture (if dormant or missing growth factors). Sampling may not be effective without removing bacteria using a stronger force such as sonication [14]. In this article, we provide a concise review of recent advancements (focusing on the past three years) in biofilm detection in medical settings and possible future directions (Figure 1).

Figure 1

Schematic of biofilm formation and potential targets for detection. Biofilm formation causes significant changes in bacterial gene expression and metabolism, triggers host immune response, and alters the chemical/physical properties of the substrate material. These changes are potential targets for biofilm detection.

Molecular methods

Compared to culturing methods, DNA-based analyses are more sensitive in detecting microbes including unculturable cells and samples with mixed species. Conventional polymerase chain reaction (PCR)-based approaches target ribosomal RNA for detection [15]. Inclusion of specific gene targets can reveal additional information such as antibiotic resistance [16••]. Compared to PCR tests that target individual genes, meta-genomic sequencing can reveal more information regarding antibiotic resistance, virulence factors and other important physiological traits. Recent development of whole-genome sequencing (WGS) has drastically improved the throughput and accuracy, and lowered the cost [17•]. DNA-based technologies do have limitations, one being the lack of information about the viability of cells and not being able to distinguish whether the organisms are in a biofilm or planktonic phenotype (if the cells cannot be separated during sampling). Combining DNA detection with messenger RNA analysis for biofilm-specific gene expression may be able to distinguish the phenotype of the organisms. However, contaminating DNA from the clinical environment (including the patient's skin, surgical instruments, gloves and irritants) are also a concern.

Biofilm associated biomarkers

Although biofilms formed on implanted devices cannot be directly sampled, biofilm growth may produce unique molecules, or stimulate biofilm-specific host responses, that can be detected using standard methods. Antibodies may not be detectable during acute infections, but can be used to help with chronic BAI diagnosis. For example, alpha defensin, an antimicrobial peptide produced by the body to fight infection, has been found in the synovial fluid of infected hip and knee joints and shows good sensitivity and specificity for periprosthetic joint infection (PJI) [18•]. However, like antibody tests, alpha defensin is not biofilm specific.

Besides host factors, identifying biofilm-specific markers on bacterial cells will enable effective detection of biofilms. Since the discovery of biofilm-associated protein (Bap) in *Staphylococcus aureus*, Bap homologs have been found in many bacterial species. These proteins are present on bacterial surfaces and many are involved in biofilm formation [19] and chronic infection in mammary glands [20]. Recombinant subunits of *Acinetobacter baumannii* Bap has been shown to stimulate an immune response in mice [21]. However, *bap* was not detected in

S. aureus isolates from patients with urinary tract infections (UTI) [22]. Further studies are needed to identify markers that are unique to bacterial biofilms, especially those present in multiple bacterial species.

In addition to cellular targets, biofilm matrix components provide potential markers of biofilms and may offer species identification. One of such possible biomarkers is cellulose, a major component of the biofilm matrix of uropathogenic *Escherichia coli* (UPEC). Antypas *et al.* [23•] developed an assay based on optotracing that can detect cellulose in urine in less than 45 min, which can help determine if biofilm is involved in UTI. Similarly, exopolysaccharide (EPS) is a possible marker for detecting biofilm as has been demonstrated for *Histophilus somni* biofilms [24]. Chronic lung infection by mucoid *Pseudomonas aeruginosa* biofilms leads to an IgG antibody response against *P. aeruginosa* components including alginate, lipopolysaccharides and proteins, which is currently used for diagnosis [25•]. Interestingly, many species of bacteria incorporate their own extracellular DNA (eDNA) into the matrix; and thus, cell-free DNA analysis [26] in combination with polysaccharide analysis [27] might provide evidence of biofilm and identify the species involved. Another possibility is to quantify biomass by measuring proteins in biofilms. For example, Guan *et al.* [28••] modified the o-phthalaldehyde (OPA) protein assay to achieve extraction-free detection of biofilms.

Quorum sensing (QS) is a well-known system found in numerous microbial species, which enables the cells to regulate cell density-dependent activities by sensing and responding to signaling molecules named autoinducers [29]. For lung infection by *P. aeruginosa*, QS signal profiling has been shown to be a potential biomarker for biofilm detection [30]. QS signals are also known to alter the level of immune factors and immune cell proliferation [31,32], which may help identify new biomarkers for biofilm detection. The chronic nature of BAIs indicates that host immunity is unable to eradicate biofilm cells. Thus, it is believed that the innate and acquired immune responses coexist [33•]. Deciphering the types and dynamics of immune factors involved in such infections may provide new biomarkers for better diagnosis.

Biofilm imaging

Laboratory methods for biofilm culturing, imaging and analysis have been well summarized in recent reviews [7•]. Confocal laser scanning microscopy (CLSM), fluorescence *in situ* hybridization (FISH), and later improved peptide nucleic acid (PNA)-FISH [34] and locked nucleic acids (LNA)-FISH [35], can provide spatial information about biofilms and the location of different species. But these advanced imaging techniques require a clear line of sight with the specimen, which are destructive and not applicable for *in situ* detection/diagnosis. Similar issues exist for scanning electron microscopy (SEM), time-of-flight

secondary ion mass spectrometry (TOF-SIMS), transmission electron microscopy (TEM) and Mass Spectrometry Imaging (MSI).

In comparison, Raman spectroscopy and surface enhanced Raman spectroscopy (SERS) offer non-destructive molecular detection with high sensitivity [36•]. By detecting the photons that change energy when scattering off a material (Raman scattering), Raman spectroscopy is highly sensitive in detecting low-abundant biomolecules, e.g. 5 ppb of *P. aeruginosa* pyocyanin in sputa [37] and quorum sensing signals in *P. aeruginosa* biofilms in mice [38].

For preoperative and intraoperative guidance, imaging techniques that can 'see' through tissues into the body will arguably have the greatest medical utility. A useful tool for non-invasive biofilm detection is Near Infra-Red (NIR) imaging. Systems with such capabilities have been commercialized. For example, the Spectrum *In Vivo* Imaging System (IVIS) from PerkinElmer combines 2D optical imaging and 3D NIR tomography, achieving 3D tracking of bioluminescence and fluorescence signals. Combining IVIS with biofilm-specific markers may improve biofilm diagnoses.

Hyperspectral imaging is a label-free method that has been used in wound monitoring and to detect biofilms in the natural environment. This technology provides visible and near-IR spectra in a 2D image and may have future potential for detecting color changes associated with biofilms themselves or reaction of the host tissues to biofilms.

While signs of infection can be diagnosed from observing host tissues in X-rays, direct detection of biofilm will likely require a contrast agent. For example, with appropriate contrast agents such as iron sulfate, X-ray tomography can differentiate biofilm from surrounding water and allow 3D quantification of biofilm structures [39••]. Further improvement in biofilm characterization can be achieved using X-ray micro-force computed tomography (μCT), which has been used for non-destructive analysis of biofilm grown in central venous catheters (CVC) [40]. Sellmyer *et al.* [41] synthesized [18F] fluoropropyl-trimethoprim and showed it can label bacteria but not inflammatory or cancer cells in rodents, allowing direct visualization of infection using positron emission tomography imaging. Future work to improve sensitivity and reduce background signals from bowel and bone uptake may bring exciting opportunities for non-invasive imaging of biofilm infections.

Currently medical imaging modalities do not have the spatial resolution to detect biofilms which can exist as aggregates with a diameter of 10–100 μm diameter. If biofilm specific labels can be found, radiolabeling has the potential to increase sensitivity in signal detection and would also offer theranostic potential, in which localized imaging is combined with a therapeutic benefit [42].

Unconventional methods
Bio-impedance based sensing
Biofilm formation on an implant and associated inflammation can significantly alter the environment near the device surface and device-host tissue interface, which provides an opportunity for biofilm-specific sensing. One approach to characterize such changes is to use electric impedance, which represents the retardation of a circuit under alternating current/voltage applied at specific frequencies [43]. Previous improvements in instrumentation and data analysis [44,45] have made EIS a promising technology for biofilm detection.

Recently, a CMOS on-chip impedance analysis system was engineered using lithography. These devices are highly sensitive, compact, and can be reproduced at a large scale by modern fabrication processes [46]. Single-frequency impedance spectroscopy has been developed as a label-free system for monitoring biofilm growth and treatment [43,47]. EIS could become a promising monitoring method in the future since it does not require sample preparation and can be done continuously without physically sampling the biofilm, a major advantage over many other methods.

Recent advances have also made it possible to integrate impedimetric sensors with existing electronic medical devices. The most important addition in such an integration is the electrode/probe, which has been demonstrated in the form of a microfluidic chip [48••]. This device combines real-time monitoring and a threshold activated treatment. It was later adapted by the same group into a wireless monitoring enabled urinary catheter [49]. It is worth noting that synergy between antibiotics and electrochemical treatment has been reported to have significant effect on killing biofilms including persister cells [50–52]. With the capability to combine treatment and monitoring, it is possible to engineer smart medical devices that can deliver on-demand control of biofilms.

Surface acoustic waves
Surface Acoustic Waves (SAW) are referred to a form of vibrational wave propagating through a solid material affected by its elasticity. Combing with Love Waves (LW), which travel across a surface, LW-SAW devices can provide label-free real-time monitoring of high-molecular weight molecules with high sensitivity [53].

LW-SAW sensors have been developed for early-detection of biofilm formation on surfaces with pg level sensitivity and real-time biofilm monitoring [54]. Using gold nanoparticles as a signal enhancer, LW-SAW can also be used to detect antigen at pg/mL level [55]. Such high sensitivity might be useful for detecting biofilm markers in the future.

A new device based on SAW was engineered recently, which can be clamped onto a liquid filled tube and act as an actuator to monitor soft layer deposition on the tube surface. This device is capable of distinguishing between a soft layer such as biofilm or a hard layer such as limescale in a metal tube [56]. This adaptable device has possible applications in piping inspection and medical catheter check-ups, demonstrating an alternative to integrating into devices. Instead of putting SAW sensor onto the surface of interest, it rather turns the said surface into a substrate for analysis. Besides sensing, SAW has also been used in biofilm treatment [57], presenting a future possibility to engineer smart devices.

Other approaches
With advancements in analytic biotechnology and device fabrication, researchers continuously push the boundary towards more sensitive and portable detection systems. For example, a disposable sensor consisting of a small segment of optical fiber and antibody surface coating developed for C-reactive protein sensing has a consistent linear response to the target protein between 0.01–20 µg/mL [58]. Meanwhile, Zhang et al. [59] reported the capability to monitor biofilm formation and treatment in real time using surface plasmon resonance waveguide mode. Cantilever technology can be used to form an array sensor, and achieve simultaneous detection of multiple targets of interest [60]. Although some of these are not directly applicable to biofilm monitoring in vivo, they have potential for biomarker detection and analysis of explanted medical devices. Future development in this field will also benefit from new technologies that combine molecular markers with advanced imaging [61•], and more in-depth understanding of biofilm physiology [62].

Conclusions
With the increasing challenges associated with microbial biofilms, there is an urgent need to develop the capability for non-destructive real-time detection of microbial biofilms. However, the patchy nature, size scale and lack of biofilm specific targets provide obstacles in meeting this goal. Conventional methods for microbial detection are largely based on culturing methods and the detection of immunoglobulin antibodies in the blood or other bodily fluids. Although these methods are effective in diagnosing acute infections, they commonly fail in diagnosing BAIs. Similar challenges exist in detecting microbes from explanted medical devices. Many of the future development will rely on the discovery of new biomarkers and engineering more sensitive detection systems. Most BAIs are culturing negative and involve multiple species, which require universal biomarkers rather than species-specific molecules for detection. It is also important to improve the knowledge of in vivo biofilm formation, which has significant differences than that in in vitro pure-culture systems. Understanding the dynamics and inter-species interactions will be essential for identifying the right biomarkers. Achieving effective biofilm detection also requires low-cost, easy-to-manufacture, portable/wearable/implantable devices that have a long-life span and require minimal maintenance. Addressing these challenges will bring

exciting new technologies for safer medical devices and better healthcare.

Conflict of interest statement
Nothing declared.

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