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Review Article

MICROBIAL BIO-FILM AN UNPREDICTABLE TROUBLE ON MEDICAL DEVICES

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ABSTRACT
Biofilm is a gathering of the microbial cells that is irretrievably linked with a surface and typically enclosed in a polysaccharide matrix. Biofilm formation is a major virulence factor contributing for the chronic infections. Biofilms pose a serious problem for public health because of the increased resistance of biofilm-associated organisms to antimicrobial agents. Unwanted biofilms can create enormous increases in fluid frictional resistances, unacceptable reductions in heat transfer efficiency, product contamination, enhanced material deterioration, and accelerated corrosion. In this mini-review, the current knowledge on the problems related to medical biofilms; concepts of biofilm formation, importance of exopolysaccharides and emerging nanotechnology for controlling medical biofilms.

Keywords: *Biofilms, Exopolysaccharides (EPS), Medical Biofilms, Clinical, Resistance to Antimicrobial Agents, Quorum Sensing, Nanotechnology*

INTRODUCTION
In the seventeenth century, a dry-goods merchant named Antonie van Leeuwenhoek first observed "animalcules" swarming on living and dead matter (Costerton, 1999). This biofilms are not easily defined as they vary greatly in structure and composition from one environmental niche to another. Microbial biofilms are extremely complex microbial ecosystems consisting of microorganisms attached to a surface and embedded in an organic polymer matrix of microbial origin (Davey and O'toole, 2000). It is estimated that over 5 million medical devices or implants are used per annum in the U.S. alone (Bryers, 2008). Microbial infections have been observed on most, if not all, such devices, including: prosthetic heart valves, orthopedic implants, intravascular catheters, artificial hearts, left ventricular assist devices, cardiac pacemakers, vascular prostheses, cerebrospinal fluid shunts, urinary catheters, ocular prostheses and contact lenses, and intrauterine contraceptive devices (Bryers, 2008). Biofilm formation and persistence has profound implications for the patient, because microorganisms growing as biofilms are significantly less susceptible to antibiotics and host defenses than are planktonic forms of the same microorganisms (Bryers, 2008). Many biofilm infections are notoriously difficult to resolve and they commonly manifest as chronic or recurrent infections. The susceptibility of biofilms to antimicrobial agents cannot be determined by means of standard micro dilution testing, since these tests rely upon the response of planktonic (suspended) rather than biofilm (surface-associated) organisms (Donlan and Costerton, 2002). The biofilm mode of growth confers on the associated organisms a measurable decrease in antimicrobial susceptibility. For example, biofilm-associated *Escherichia coli* required 1500 times the MIC of ampicillin to provide a 3-log reduction (Donlan, 2001; Ceri *et al.*, 1999). Williams *et al.*, found that *Staphylococcus aureus* biofilms required 110 times the MBC of vancomycin to provide a 3-log reduction (Williams *et al.*, 1997). The effect on susceptibility may be intrinsic (i.e., inherent in the biofilm mode of growth) or acquired (i.e., caused by the acquisition of resistance plasmids). About 99% of the world's population of bacteria is found in the form of a biofilm at various stages of growth and the films are as diverse as the bacteria are numerous (Garrett *et al.*, 2008). Over the past few decades biofilm growth has been observed in many industrial and domestic domains. Unfortunately, in most cases the growth of biofilms has been detrimental. Many industries suffer the ill-effects of biofilm growth of one type or another, which can result in heavy costs in cleaning and maintenance. Examples of such industries include the maritime, dairy, food, water systems, oil, paper, opticians, dentistry and hospitals. Perhaps the

environment where people are exposed to biofilms most frequently is the domestic environment (Garrett *et al.,* 2008). Hospital-related infection (nosocomial infection) periodically provokes sensationalist headlines, for good reason. Surgical instruments and fluid lines, e.g. scalpels, drips and catheters, are common sources of biofilm growth and subsequent infection. Biofilm forming Methicillin-resistant Staphylococcus aureus (MRSA) is particularly important due to its ubiquity in the National Health Service (NHS) and repeated resistance to all but a few antibiotic programs (Bookstaver *et al.,* 2009). In order to control biofilm formation on medical devices and all costs associated, a large number of new strategies and approaches have been developed in the last few years, including: antimicrobial locks (in the case of catheters) (Bookstaver *et al.,* 2009); surface modification of biomaterials with antimicrobial coatings (Knetsch and Koole, 2011); the use of quorum sensing inhibitors (Lönn-Stensrud *et al.,* 2009); antimicrobial peptides as a new class of antibiotics (Batoni *et al.,* 2011); enzymes that dissolve biofilms (Donelli *et al.,* 2007), nitric oxide (Regev-Shoshani *et al.,* 2010), Nevertheless, nanoscale materials have recently appeared as one of the most promising strategies to control biofilm infections related to indwelling medical devices, especially due to their high surface area to volume ratio and unique chemical and physical properties (Rai *et al.,* 2009). The use of silver nano-particles (NPs) is now considered as one of the most promising strategies to combat biofilm infections related to indwelling medical devices (Gong *et al.,* 2007). Endoscope equipment is used in specialized services with a high demand for exams. Because of their high cost, their inventory tends to be restricted (Balsamo *et al.,* 2012). Reuse of the equipment is approved, despite its complex structure, with long channels internally covered with polytetrafluorethylene and small luminal diameter, favoring the attachment of organic material and microorganisms and, consequently, the formation of biofilm (Balsamo *et al.,* 2012). Although a different specialized society have well established gastrointestinal endoscope cleaning and is infection recommendations, various studies discuss that the transmission of microorganisms or adverse effects in patients submitted to gastrointestinal endoscopes may be due to the formation and permanence of biofilms, making them responsible for cross-transmission of bacteria and viruses. As biofilm formation is unavoidable in structures like endoscope channels and a causal link exists between the current causes of exogenous infections related to flexible endoscopes and bad processing quality (Balsamo *et al.,* 2012). For bacteria, the advantages of biofilm formation are numerous. These advantages include: protection from antibiotics, disinfectants, and dynamic environments (Garrett *et al.,* 2008). Intercellular communications within a biofilm rapidly stimulate the up and down regulation of gene expression enabling temporal adaptation such as phenotypic variation and the ability to survive in nutrient deficient conditions (Garrett *et al.,* 2008). Bacterial adhesion to a material surface can be described as a two-phase process including an initial instantaneous and reversible physical phase (phase one) followed by a time-depended and irreversible molecular and cellular phase. The factors involved in both phases of bacterial adhesion as well as the techniques and theories used to study this adhesion are well reviewed (Katsikogianni and Missirlis, 2004). For these reasons and the emergence of restrictive legislation regarding the effects of cleaning agents on the environment and to user health and safety (Commission Regulation EC No. 1048/2005), there is a lot of industrial interest in developing materials and methods which can remove and actively prevent the formation of biofilms. This article represents an overview of the process of biofilm formation and factors affecting its formation.

Biofilms Configuration

Tolker-Nielsen and Molin noted that every microbial biofilm community is unique although some structural attributes can generally be considered universal (Tolker-Nielsen and Molin**,** 2000)**.** The major components are typically water and the bacterial cells, followed by the EPSs of the matrix, which provides (i) a physical barrier against the diffusion of antibiotics, defense substances, or other important compounds from the host; and (ii) protection against environmental stress factors, such as UV radiation, pH changes, osmotic stress, and desiccation (Bogino *et al.,* 2013). Remarkable discoveries have occurred in biofilm research during this past decade. The application of new microscopic and molecular technologies to biofilm investigations has opened our eyes to this underappreciated area of microbial biology. Using these technologies, researchers have shown that biofilms are not simply organism-

containing slime layers on surfaces; instead, biofilms represent biological systems with a high level of organization where bacteria form structured, coordinated, functional communities (O'toole *et al.,* 2000). Bacterial mobility is enabled by two types of protein growths on the cell surface, flagella and fimbriae. Flagella are long, spiral growths that enable bacteria to float in liquid medium, and fimbriae are short, straight growths that enable limited, twitching movements of bacteria on substrate surface. *Escherichia coli* and *Pseudomonas aeruginosa* showed that both kinds of bacterial mobility are necessary for biofilm formation (O'toole *et al.*, 2000). Bacterial mobility enabled by flagella is necessary for establishing the connection between the bacteria and the surface, while the mobility enabled by fimbriae is necessary for the formation of microcolonies. Basic structural units of a biofilm are microcolonies, separate communities of bacterial cells embedded into EPS matrix. These microcolonies are in most cases mushroom-shaped or rodlike and they can consist of one or more types of bacteria (Macleod *et al.,* 1990). Depending on bacteria type, microcolonies consist of 10–25% of cells and 79–90% of EPS matrix (O'toole *et al.,* 2000). EPS matrix protects biofilm cells from various negative environmental conditions, such as UV radiation, abrupt changes in pH values, draining. Between microcolonies, there are channels through which water flows (O'toole *et al.,* 2000). These water channels function in a biofilm as a simple circulatory system distributing nutrients to microcolonies and receiving harmful metabolites (Costerton, 1995). Biofilm is polymorphic and it can adjust its structure to changes in the amount of nutrients, which was de-monstrated by experiments with different glucose concentrations. When glucose concentration is high, microcolonies grow fast and consequently biofilm thickness increases significantly. When glucose concentration is decreased, biofilm biomass is reduced and the former structure is restored. Studies of biofilm in different hydrodynamic conditions, such as laminar and turbulent flow, have shown that biofilm structure changes depending on the flow type. In laminar flow bacterial microcolonies become round and in turbulent flow they extend in downstream direction (Stoodley *et al.,* 1998).

Role of Extracellular Polymeric Substances

The extracellular polymer matrix (EPS), or glycocalyx, is composed of polysaccharides, proteins, lipids and extracellular DNA (Neethirajan *et al.,* 2014; Abee *et al.,* 2011). If cells reside at a surface for a certain time, irreversible adhesion forms through the production of extracellular cementing substances. As mentioned earlier, this extracellular material, associated with the cell has also been referred to as glycocalyx, a slime layer, capsule or a sheath (Costerton *et al.,* 1978). If biofilms can be metaphorically called a "city of microbes", the EPS represent the "house of the biofilm cells (Flemming *et al.,* 2007). The EPS influence predator-prey interactions, as demonstrated in a system consisting of a predatory ciliate and yeast cells, in which grazing led to an increase in biofilm mass and viability, with EPS as the preferred food source (Joubert *et al.,* 2006). The extent to which polysaccharides are involved in the adhesion process remains open to question. Some evidence suggests that excess polymer production may even prevent adhesion, although trace amounts of polysaccharide might be required initially for adhesion (Brown *et al.,* 1977). EPS is highly hydrated, and can be both hydrophilic and hydrophobic with varying degrees of solubility. The polysaccharide content of EPS has a marked effect on the biofilm as the composition and the structure will determine their primary conformation (Sutherland, 2001). Bacterial EPS contains backbone structures of 1, 3- or 1, 4-b-linked hexose residues, which are rigid and generally poorly soluble or insoluble, whereas other EPS molecules are more readily soluble in water. EPS provides many benefits to a biofilm including the promotion of cohesive forces, increased absorption of nutrients and heavy metals, the sequestration of microbial products and other microbes, protection of immobilized cells from environmental changes and the provision of a medium for intercellular communication and transfer of genetic material (Characklis and Cooksey, 1983). In environmental biofilms, polysaccharides are frequently only a minor component. Unfortunately, it remains a substantial challenge to provide a complete biochemical profile of most EPS samples. It is often difficult to purify EPS matrix constituents apart from other components such as cells or other macromolecules transiently associated with the EPS (Nielsen and Jahn, 1999). EPS influences the physical properties of the biofilm, including diffusivity, thermal conductivity and rheological properties. EPS, irrespective of charge density or its ionic state, has some of the properties of diffusion barriers, molecular sieves and adsorbents, thus influencing

physiochemical processes such as diffusion and fluid frictional resistance. EPS has little effect on uncharged molecules including potential nutrients such as sugars. However, biofilm bacteria are thought to concentrate and use cationic nutrients such as amines, suggesting that EPS can act as a nutrient trap, especially under oligotrophic conditions (Costerton *et al.,* 1981). Such mechanisms are crucial for preventing the washout of enzymes, keeping them close to the cells that produced them and allowing for effective degradation of polymeric and particulate material. This leads to the concept of an "activated matrix." Activation is made even more dynamic and versatile by the release of membrane vesicles. These highly ordered nanostructures act as "parcels" containing enzymes and nucleic acids, sent into the depth of the EPS matrix (Schooling and Beveridge, 2006).

Sensing Medical Biofilms

The "holy grail" of biofilm infections is an "early-warning" diagnostic method that would allow for non-invasive detection of the early stages of tissue or biomedical implant infection and an expedient response. Such diagnostics are only now just emerging (Bryers, 2008). Currently, only upon the onset of a cyclical fever in an implant recipient, will a patient receive a battery of blood tests meant to detect any infecting microorganisms; such as colony-forming plate count assays that typically take anywhere from 48–72 h and are only capable of detecting planktonic not sessile cells (Bryers, 2008). Emergence of PCR techniques have shorten the time period but they also sample body fluids (blood, saliva, urine), which will not provide an accurate estimate of the actual biofilm flora colonizing an implant (Bryers, 2008). Two important biofilm-forming bacterial pathogens, *S. aureus* and *P. aeruginosa*, were made bioluminescent by insertion of a complete *lux* operon (Kadurugamuwa *et al.,* 2003). These bacteria produce significant bioluminescent signals for both in vitro studies and in an in vivo model, allowing effective real-time assessment of the physiological state of the biofilms. In vitro viable counts and light output correlated well for 10 days or longer, provided that the growth medium was replenished every 12 h (Kadurugamuwa *et al.,* 2003). Recovery of the bacteria from the catheters of infected animals showed that the bioluminescent signal corresponded to the CFU and that the *lux* constructs were highly stable even after many days in vivo (Kadurugamuwa *et al.,* 2003).

Scheming Medical Biofilms

Since native immunity can be circumvented or compromised (by drugs or disease), the medical profession has been attempting to eradicate biofilm-based infections by resorting to disinfectants and antibiotics (Bryers, 2008). These are mostly synthetic compounds evaluated for the most part on their ability to inactivate or kill *suspended* bacteria but exhibit little efficacy when applied to biofilm infections (Bryers, 2008). However, biofilm bacteria are significantly less responsive to antibiotics and antimicrobial stressors than planktonic organisms of the same species (Gilbert *et al.,* 2002). Recent studies have shown that sub-lethal doses of antibiotics can actually enhance biofilm formation. The genes coding for alginate biosynthesis were induced by exposure to the β-lactam antibiotic, imipenem (Bagge *et al.,* 2004). Exposure to subinhibitory concentrations of imipenem caused structural changes in the biofilm, for example, an increased biofilm volume and alginate polymer matrix. Increased levels of alginate matrix production may be an unintended adverse consequence of imipenem treatment in cystic fibrosis patients (Bryers, 2008). Similarly, Hoffman *et al.,* (2005) report that subinhibitory concentrations of aminoglycoside antibiotics (e.g., tobramycin) induced biofilm formation in *P. aeruginosa* and *E. coli*. Enhanced biofilm formation in the presence of antibiotics may be one universal defense mechanism of bacteria in avoiding the lethal effects of antibiotics (Hoffman *et al.,* 2005).

Supervision of Biofilm Infections

Viewing bacteria from the perspective of multicellular behavior is altering our view of microbiology and of Koch's postulates (Percival *et al.,* 2010). It is evident that 99.9% of organisms prefer attachment, and that bacterial cells have the ability to aggregate into particular three-dimensional assemblages (Davey and O'toole, 2000). Biofilms have been recognized as being important in human disease and the number of biofilm-associated diseases seems to be increasing (Davies, 2003). It is important to understand the characteristics of the biofilm mode of growth and the various aspects of biofilm formation. To successfully treat biofilm infections, knowledge of the phenotype of the bacterial population is required.

This is important, as antibiotic treatment may not be totally effective if more than one phenotype exists. Some of these cells might remain intact serving to re-infect the host once the antimicrobial treatment has finished (Davies, 2003). A key factor to combating biofilm infections is to understand the physiology of biofilm development. Davies (2003) suggested that chemotherapeutic agents could be developed to promote or prevent transition from one stage of biofilm maturation to the next by targeting unique biofilm regulatory or signalling molecules. Specific agents might be discovered or developed which will interfere with the production of virulence factors, or promote or inhibit the shedding of biofilm bacteria (Davies, 2003). As mentioned before, biofilm resistance depends on aggregation of bacteria into multicellular communities. Therefore, one antimicrobial strategy might be to develop therapies to disrupt the multicellular structure of the biofilm. It could be that host defenses might be able to resolve the infection once the multicellularity of the biofilm is reduced, and then the effectiveness of antibiotics might be restored (Stewart and Costerton, 2001). For in vivo indwelling device-associated infections, effective, preventive and therapeutic strategies still need to be developed. One such therapy could be the production of materials with anti-adhesive surfaces, for example, heparin (Tenke *et al.*, 2004). On heparin-coated catheter stents, no biofilm formation was evident between 6 and 8 weeks, whereas uncoated tubes were obstructed within 2–3 weeks. Heparin coating seems one possible solution, but further development of materials resisting bacterial colonisation is needed (Tenke *et al.*, 2004).

Biofilm Study through Microscopy

Scanning electron microscopy (SEM) allows visualization of surface structures with a three-dimensional appearance and at different resolutions. In the case of biofims, the highly hydrated glycocalyx is greatly distorted and only proteinaceous structures can be visualized. Gold-immunolabelling techniques allow quantification of certain proteins (Hannig *et al.*, 2008). Transmission electron microscopy (TEM) is the standard electron microscopic technique used for the evaluation of ultrathin sections. Cryo-electron microscopy allows the evaluation of ultrathin sections of biological samples in a hydrated state. The formation of vitreous (non-crystalline) ice preserves the appearance of the sample. However, this technique requires a complex processing of samples and specialized equipment (Bouchet-Marquis and Fakan, 2009). Confocal scanning laser microscopy (CSLM) is an epifluorescence microscope that creates a thin plane-of-focus. Laser light is scanned across the specimen to provide excitation energy for intrinsic or extrinsic fluorophores, with subsequent detection of the resulting fluorescence. CSLM images of implants can be difficult to interpret because they are often acquired at low resolution to maximize the viewable area of the implant (Gorman *et al.*, 1994). Environmental scanning electron microscopy (ESEM) is a descendant of the conventional SEM that overcomes a clear disadvantage of this technique in the biological field: the need for altering the characteristic of biological samples to visualize them in the high-vacuum chamber. Biological samples are highly hydrated and exhibit low conductivity. The ESEM incorporates two design modifications that allow visualization of poorly conductive biological samples in their natural hydrated state (Danilatos, 1993).

Clinical Significance of Biofilms

Microbial biofilms often develop on, or within indwelling, medical devices, e.g. contact lenses, central venous catheters, mechanical heart valves, pacemakers, peritoneal dialysis catheters, prosthetic joints, urinary catheters and voice prostheses (Percival and Kite, 2007) and a number of microorganisms can produce biofilms on these surfaces. Implantation of mechanical heart valves causes tissue damage, and circulating platelets and fibrin tend to accumulate where the valve has been attached. The resulting biofilms develop on the heart tissue surrounding the prosthesis or the sewing cuff fabric used to attach the device to the tissue (Donlan, 2001). Serious and lethal processes such as endocarditis, infections in cystic fibrosis and infections of permanent indwelling devices such as joint prostheses and heart valves may also be associated with biofilms (Lewis, 2001). Biofilms associated with urinary catheters are particularly important because they cause infections in 10–50% of patients who undergo catheterisation (Stickler, 2002). *Proteus mirabilis, Morganella morganii, P. aeruginosa, K. pneumoniae* and *Proteus vulgaris* are commonly found in urinary catheter biofilms. A number of these bacteria (e.g. *P. mirabilis*) express urease, an enzyme which hydrolyses urea found in the urine, resulting in the production of ammonia.

Ammonia causes an increase in the pH of the urine, which in turn allows mineral precipitation including that of calcium and magnesium phosphates, leading to blockage of the catheter and infection (Tunney *et al.,* 1999). Unfortunately, urinary catheters also provide a passageway for bacteria from a heavily contaminated external skin site to a vulnerable body cavity. Verifying catheter biofilm as a primary source of urinary tract infection relies on clinical signs of infection, direct visual observations of catheter biofilm, and urine analysis. Similarly, by better understanding the clinical signs of wound biofilm and utilizing future point-of-care tools to confirm wound biofilm, management practices and patient care can be optimized (Hurlow *et al.,* 2015). Polymicrobial communities will eventually develop, but initial infections are usually by single bacterial species (Lewis, 2001).

Biofilm Resistance to Antimicrobial Agents

It is difficult to eradicate bacterial biofilm which is therefore the cause of numerous chronic infections. Within the biofilm bacteria are 10-1000 times more resistant to antibiotics than planktonic cells (Mah and O'toole, 2001). In some extreme cases, the concentrations of antibiotics required to achieve bactericidal activity against adherent organisms can be three to four orders of magnitude higher than for planktonic bacteria, depending on the species-drug combination (Schierholz *et al.,* 1999). Application of various molecular-biological and microscopic techniques proved that bacteria within a biofilm are physiologically heterogenous, which is highly significant for resistance to antibiotics (Huang *et al.,* 1998). Biofilm‑growing bacteria exhibit increased resistance to antibiotics and disinfectants. The effective therapeutic concentration of some antibiotics to bacteria in biofilm may be even 100-to 1000‑fold higher than that to planktonic bacteria (Marcinkiewicz *et al.,* 2013). Estimate of 1000-1500 times greater resistance for biofilm-grown cells than the planktonic cells have been suggested, although these estimates have been considered too high by some investigators. It has been shown in many studies that resistance of bacteria to antibiotics, biocides or preservatives are affected by their nutritional status, growth rate, temperature, pH and prior exposure to sub effective concentrations of antimicrobials (Socransky and Haffajee, 2002). The exopolymer matrix of biofilm, although not a significant barrier in itself to the diffusion of antibiotics, does have certain properties that can retard diffusion. In terms of microenvironment, it is likely that the same factors that adversely influence antimicrobial activity in vitro, including pH, pCO_2, pO_2, divalent cation concentration, hydration level, and pyrimidine concentration, will also produce undesirable effects at the deepest layers of a bacterial biofilm (Jorgensen *et al.,* 1999), where acidic and anaerobic conditions persist. While detailed studies of these factors vis-à-vis antibiotic activity in biofilm environs are lacking, one could predict, based on disk diffusion and broth micro dilution susceptibility testing, that the activity of aminoglycosides, macrolides, and tetracyclines would likely be compromised in an acidic milieu with increased pCO_2. Also, the polyanionic nature of the alginic acid exopolysaccharide of *P. aeruginosa* (Linker and Jones, 1966) would certainly tend to concentrate divalent cations. This, in turn, would also affect the activity of aminoglycosides and tetracyclines (Jorgensen *et al.,* 1999).

Quorum Sensing and Biofilm Correlation

Quorum sensing can be divided into at least 4 steps: (1) production of small biochemical signal molecules by the bacterial cell; (2) release of the signal molecules, either actively or passively, into the surrounding environment; and (3) recognition of the signal molecules by specific receptors once they exceed a threshold concentration, leading to (4) changes in gene regulation. One common consequence of quorum sensing induction of gene expression is increased synthesis of the proteins involved in signal molecule production. Increased synthesis of the signal molecule creates a positive feedback loop, which is why quorum signals are commonly called auto inducers (Sifri, 2008). Bacteria in a community may convey their presence to one another by producing, detecting, and responding to small diffusible signal molecules called auto inducers. This process of intercellular communication, called quorum sensing, was first described in the marine bioluminescent bacterium *Vibrio fischeri* (Federle and Bassler, 2003). Cell-to-cell signaling has recently been demonstrated to play a role in cell attachment and detachment from biofilms. Xie *et al.,* showed that certain dental plaque bacteria can modulate expression of the genes encoding fimbrial expression (*fimA*) in *Porphyromonas gingivalis* (Xie *et al.,* 2000). *P. gingivalis* would not attach

to *Streptococcus cristatis* biofilms grown on glass slides. *P. gingivalis,* on the other hand, readily attached to *S. gordonii*. *S. cristatus* cell-free extract substantially affected expression of *fimA* in *P. gingivalis*, as determined by using a reporter system. *S. cristatus* is able to modulate *P. gingivalis fimA* expression and prevent its attachment to the biofilm (Donlan, 2002). It is now known that many bacteria regulate their social activities and physiological processes through a quorum sensing mechanism, including symbiosis, formation of spore or fruiting bodies, bacteriocin production, genetic competence, programmed cell death, virulence and biofilm formation. The processes controlled by quorum sensing are diverse and reflect the specific needs of particular communities. In many bacteria, quorum sensing represents a central mechanism to regulate social activities, allowing bacteria to reap benefits that would be unattainable to them as individual cells (Schauder and Bassler, 2001). Increasing evidence shows that quorum sensing-mediated social activities favor microbial interactions and are believed as major mechanisms to regulate population-level virulence of bacteria (Antunes *et al.,* 2010). Coincident with the elucidation of cell communication systems in bacteria has been the growing appreciation of the importance of biofilms in bacterial physiology and virulence. Most bacteria in the environment reside in biofilms, as do many of those involved in human infection (Costerton *et al.,* 1999). Most research supports the role of quorum sensing in biofilm formation and in the resulting characteristics of the biofilm community (Anous *et al.,* 2009).

Nanotechnology Solutions

Nanotechnology will provide some of the most important advancements in medical devices and biomaterials in the coming years. Reduction of device related adverse events will depend on enhancing antimicrobial activity and improving biocompatibility through nanoscale modifications (Taylor and Webster, 2011). Nanomaterials are defined as having at least one dimension less than 100 nm. They provide an advantage over traditional materials because their scale is more similar to that of biological reactions occurring on the cellular level. Increased surface area to volume ratio enhances the efficacy of chemical reactions by providing a greater reaction surface. Nanoparticles are also capable of puncturing micrometer sized bacterial cell membranes without doing harm to larger host cells (Tran and Tran, 2012). Biocompatibility plays an integral role in biofilm resistance. Although several surfaces have antimicrobial activity, they may also be damaging to human cells. A summary of strategies for biocompatibility and infection resistance is provided in Table II. For instance, cationic bactericidal polymers are believed to exert their effect via membrane lysis. Unfortunately however, cationic materials are also harmful to human cells (Vasilev *et al.,* 2009). Additional modifications are necessary to make the material safe for human cell interaction, such as embedding the cationic compound into a 20 peptide MAXI hydrogel. This material was antibacterial against gram negative (*E. coli, K. pneumonia*) and gram positive bacteria (*S. aureus, S. epidermidis, S. pyogenes*) without causing harm to NIH 3T3 fibroblasts or red blood cells. The fibroblasts were able to adhere and proliferate on the hydrogel surface, and the red blood cells did not demonstrate hemolysis (Salick *et al.,* 2007). Designing materials that do not harm the host tissue is crucial to the design of antibiofilm coatings (Williams, 2008). Several metals have been recognized for intrinsic antibacterial properties, including silver, zinc oxide, titanium oxide, iron, iron oxide, copper, and aluminum oxide.

The antimicrobial properties of metals provide an alternative to antibiotics, without significant risk of resistance mutations. This is important considering that the development of new antimicrobials has been relatively unsuccessful (Pompilio *et al.,* 2012). Zinc nanoparticles have the advantage of maintaining antimicrobial activity while exhibiting a low toxicity for mammalian cells. Silver is a classic antimicrobial metal and is routinely incorporated into burn treatments and wound dressings. Silver nanoparticles have a broad range of applications, resisting biofilm formation by *E. coli*, *Enterococcus* sp., *S. aureus*, coagulase-negative *staphylococci*, and *Candida albicans* (Roe *et al.,* 2008). Titanium oxide is the most common material for orthopedic and dental implants due to its mechanical strength, chemical stability, and excellent biocompatibility. Iron oxide nanoparticles are routinely used as MRI contrast agents. Polymer coatings can be applied by dip coating, spin coating, layer-by-layer plasma polymerization or Langmuir–Blodgett extrusion (Neethirajan *et al.,* 2014).

CONCLUSION

Increasing scientific research over the past 10 years in biofilm formation has provided a wealth of possible targets with which to prevent or eradicate biofilm infections. Advances in the understanding of biofilm formation, coupled with emerging engineered biomaterials, provide many potential platforms and strategies to prevent or significantly reduce biofilm infections in susceptible populations. To date we can appreciate that biofilms are important in infectious disease processes.

The principles by which this is evident is when we consider detachment of cells or biofilm aggregates resulting in the production of emboli, bacteria may exchange resistance plasmids within biofilms, cells in biofilms have dramatically reduced susceptibility to antimicrobial agents, biofilm-associated Gram-negative bacteria may produce endotoxins and biofilms are resistant to host immune system clearance. Medical biofilms still pose as a critical issue for the clinical community, as most of the traditional therapies are not effective, due to the recalcitrant cells within these communities and the emergence of new highly resistant strains. New nano-technological strategies are being developed in order to overcome the problems associated with bacterial or /and fungi biofilm formation. It is becoming increasingly evident that quorum sensing enhances the ability of bacteria to access nutrients or more favorable environmental niches and to increase bacterial defenses against eukaryotic hosts, competing bacteria, and environmental stresses.

The physiological and clinical aspects of quorum sensing have received considerable attention and have begun to be studied at the molecular level. However, little is known about quorum sensing plays an important role in biofilm formation.

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