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Review

In vitro and *in vivo* model systems to study microbial biofilm formation

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ABSTRACT

Biofilm formation is often considered the underlying reason why treatment with an antimicrobial agent fails and as an estimated 65–80% of all human infections is thought to be biofilm-related, this presents a serious challenge. Biofilm model systems are essential to gain a better understanding of the mechanisms involved in biofilm formation and resistance. In this review a comprehensive overview of various *in vitro* and *in vivo* systems is presented, and their advantages and disadvantages are discussed.

Contents

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1. Introduction

Since 1943, when marine microbiologist Claude ZoBell described the so-called "bottle effect" (referring to the phenomenon that the number of free-living microorganisms in fresh sea water gradually declines when the water is kept in a glass bottle, while the number of attached microorganisms increases) (ZoBell, 1943) we have been aware of the fact that microorganisms are capable of living their life attached to a surface. However, it then took more than 30 years (and the paradigm-changing work of Bill Costerton and colleagues) to accept that for microorganisms (both bacteria and fungi) the biofilm mode of life is the rule rather than the exception (Costerton et al., 1978, 1999). Biofilms are defined as consortia of microorganisms that are attached to a biotic or abiotic surface. Biofilm formation is a multi-stage process in which microbial cells adhere to the surface (initial reversible attachment), while the subsequent production of an extracellular matrix (containing polysaccharides, proteins and DNA) results in a firmer attachment (Sauer, 2003; Stoodley et al., 2002). Cells embedded in this matrix communicate with each other and show a coordinated group behaviour mediated by a process called quorum sensing (QS) (Zhang and Dong, 2004). Sessile (biofilm-associated) cells are phenotypically and physiologically different from non-adhered (planktonic) cells and one of the typical properties of sessile cells is their increased resistance to antimicrobial agents (Donlan and Costerton, 2002; Mah and O'Toole, 2001; Stewart and Costerton, 2001). Biofilm formation is often considered the underlying reason why treatment with an antimicrobial agent fails and as an estimated 65–80% of all infections is thought to be biofilm-related, this presents a serious challenge (Costerton et al., 1999; Hall-Stoodley et al., 2004; Parsek and Singh, 2003). Biofilm formation can also have detrimental effects in industrial systems. Biofouling is especially problematic in systems in which materials come into contact with water, including heat exchangers, ship hulls and (marine) fish cages (Braithwaite and McEvoy, 2005; Coetser and Cloete, 2005; Flemming, 2002). Of particular relevance to human health is biofilm formation in drinking water reservoirs and distribution systems as these biofilms hinder the efficient operation of these systems. In addition, they may also pose a health risk to the users, providing a habitat for pathogenic miroorganisms like *Legionella pneumophila* and *Escherichia coli* (Flemming, 2002; Juhna et al., 2007). On the other hand, there are many (potential) applications of microbial biofilms, in processes as diverse as bioremediation (Singh et al., 2006), production of fine chemicals (Li et al., 2006), fermentation (Kunduru and Pometto, 1996), biofiltration (Cohen, 2001), wastewater treatment (Nicolella et al., 2000), biofuel production (Wang and Chen, 2009) and generation of electricity in microbial fuel cells (Rabaey et al., 2007).

In order to increase our knowledge concerning biofilm biology, biofilm model systems to be used for the study of the often complex communities under controlled conditions are indispensable (Doyle, 1999; Hamilton et al., 2003; Wolfaardt et al., 2007). In this review we present an overview of *in vitro* and *in vivo* model systems and discuss their advantages and disadvantages. The focus of this review is on tools to study medically-relevant biofilms, but many of the models can of course also be used to mimick biofilm formation in other settings.

2. *In vitro* biofilm model systems

2.1. Microtiter plate-based model systems

Microtiter plate (MTP)-based systems are among the most-frequently-used biofilm model systems (see for example Cerca et al., 2005; Christensen et al., 1985; Coenye et al., 2007; De Prijck et al., 2007; Gabrielson et al., 2002; Krom et al., 2007; Miyake et al. 1992; Peeters et al., 2008a,b,c; Pettit et al., 2005; Pitts et al., 2003; Ramage et al., 2001; Shakeri et al., 2007; Stepanovic et al., 2000; Toté et al., 2008; Silva et al., 2010; Uppuluri et al., 2009b; Walker and Sedlacek, 2007). In these systems, biofilms are either grown on the bottom and the walls of the microtiter plate (most commonly a 96-well plate) or they are grown on the surface of a coupon placed in the wells of the microtiter plate (most commonly a 6, 12 or 24-well plate). MTP-based systems are closed (batch reactor-like) systems (Fig. 1), in which there is no flow into or out of the reactor during the experiment (Heersink and Goeres, 2003). As a consequence, the environment in the well of a MTP will change during the experiment (e.g. nutrients become depleted, signalling molecules accumulate, etc), unless the fluid is regularly replaced.

The multitude of advantages offered by these straightforward and (generally) user-friendly systems explains their widespread use. Firstly, MTP-based assays are fairly cheap as only small volumes of reagents are required, they provide the opportunity to perform a large number of tests simultaneously and this system is ideal for screening purposes (Niu and Gilbert, 2004). MTP-based model systems have been used to distinguish biofilm-deficient mutants from biofilm-forming wild type strains (Heilmann et al, 1996; O'Toole and Kolter, 1998) and to screen for the antimicrobial and anti-biofilm effects of various antibiotics, disinfectants, chemicals (including quorum sensing inhibitors) and plant extracts (Ali et al., 2006; Amorena et al., 1999; Pitts et al., 2003; Quave et al., 2008; Ramage et al, 2001; Shakeri et al., 2007; Peeters et al., 2008b, 2008c; Brackman et al., 2009; Vandenbosch et al., 2010). Secondly, a profound examination of the effects of modification, coating or impregnation of materials on various stages of biofilm development can easily be performed in microtiter plate model systems (Chandra et al., 2001; De Prijck et al., 2007, 2010b; Imamura et al. 2008; Mowat et al., 2007). Thirdly, this system also allows researchers to easily vary multiple parameters including the composition of growth media, incubation temperatures, humidity, presence or absence of shear stress and O_2 and CO_2 concentrations (Krom et al., 2007; Stepanovic et al., 2003).

Ceri et al. (1999) developed a variation of the traditional MTP model system. The "Calgary Biofilm Device" was introduced as a rapid technology to determine the antibiotic susceptibility of biofilms and it has been commercialized as the MBEC Assay ("Minimal biofilm eradication concentration" assay) by Innovotech. In this system, pegs are attached to the top lid of a microtiter plate and by closing the microtiter plate, these pegs will be immersed in the media present in the wells of the 96-well MTP. Following biofilm growth, the lid can be transferred to a second plate, which contains various (antibiotic) solutions. After the treatment, the top lid can either be transferred to a new microtiter plate containing media to allow regrowth, or the pegs can be clipped from the top lid and the biofilm biomass or the number of sessile cells present in the biofilm can be quantified using traditional viable plate counting or microscopic techniques. This rapid and miniaturized biofilm assay is mostly applied to evaluate the effects of various antimicrobial agents on biofilm eradication (see for example Aaron et al., 2002; Bardouniotis et al., 2001; Ceri et al., 1999; De Kievit et al., 2001; Finelli et al., 2003; Hill et al., 2005; Arias-Moliz et al., 2010; Melchior et al., 2007; Harrison et al., 2005), but it has also been used to assess the influence of quorum sensing on biofilm formation (Tomlin et al., 2005).

Another MTP-based commercially available method is the Biofilm Ring Test (BioFilm Control SAS) (Chavant et al., 2007). With this technology, the immobilisation of inert paramagnetic beads included in the culture medium during the formation of the biofilm is measured. A magnet is used to collect the non-immobilised beads into a single spot which is then quantified through specialised image algorithms. This technology has been used to study the kinetics of biofilm formation of *Listeria monocytogenes*, *E. coli*, *Staphylococcus carnosus* and *Staphylococcus xylosus* (Chavant et al., 2007), to determine the influence of matrix components on *Leuconostoc mesenteroides* biofilm formation (Badel et al., 2008), to confirm that AI-2 based quorum sensing affects biofilm formation in *Streptococcus mutans* (Huang et al., 2009), to evaluate the effect of co-administration of antibiotics on *Pseudomonas aeruginosa* biofilms (Tré-Hardy et al., 2009), to compare biofilm formation between