App Note: F400-01-01

Evaluation of the anti-biofilm properties of ciprofloxacin

Deborah Diehl, and Nina Bionda, PhD., iFyber LLC, Ithaca, NY

INTRODUCTION

Persistent and chronic bacterial infections have been directly linked to the presence of microbial biofilms [1, 2]. Microbial biofilms are sessile communities of one or more microorganism that reside within a self-produced extracellular matrix (Figure 1). These cellular aggregates can form on living and non-living surfaces, soluble and in-soluble materials, are fairly ubiquitous in natural ecosystems, and have serious implications to human health. The inherent characteristic of microbial biofilms is a remarkable tolerance to treatment with antibiotics traditionally effective against planktonic (free floating) bacteria [3]. Although the existence of biofilms has long been recognized, the realization that these microbial communities are the root of many health care problems occurred only a few decades ago. Nowadays, microbial biofilms are considered to be main contributors to over 80% of all clinical infections, with estimated 1.7 million people yearly falling victim to a biofilm-related infection in the U.S. alone. Of this number, around 100,000 patients die annually [4, 5]. The recognition of the importance and the implications of microbial biofilms has brought about the need for novel antimicrobial technologies and hence development and implementation of methods for anti-biofilm efficacy screening.

MBEC ASSAY FOR ANTI-BIOFILM ACTIVITY

A Minimum Biofilm Eradication Concentration (MBEC) assay is a widely used method of choice for evaluating antimicrobial efficiency against microbial biofilms formed on a solid substrate [6]. This method utilizes a 96-well microtiter plate with a lid containing 96 pegs which support formation of the bacterial biofilm (Figure 1). The assay allows for the simultaneous screening of multiple antimicrobial compounds and concentrations against a chosen bacterial species. The high-throughput format of the MBEC assay also allows for evaluation of test compounds with respect to differential bacterial load, early vs. mature biofilm, and assessment of synergistic effects, among other properties.

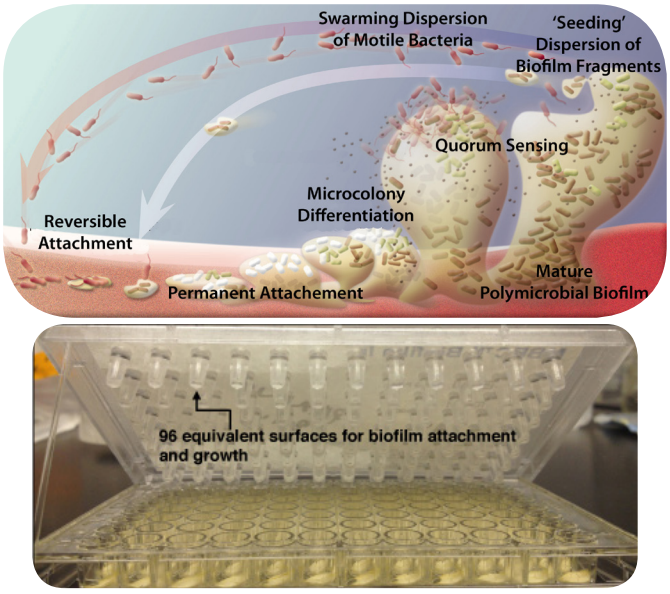

Figure 1. (Top) Schematic representation of the life cycle of a bacterial biofilm, and (Bottom) an MBEC assay device used for the *in vitro* assessment of biofilm formation.

iFyber has implemented the MBEC assay to evaluate biofilm formation of several prominent pathogenic strains implicated in persistent chronic infections, and has successfully used this in vitro assay to study the anti-biofilm properties of both currently marketed products and experimental technologies.

APPLICATION NOTE: ANTI-BIOFILM ACTIVITY OF CIPROFLOXACIN

Effects on established *E. coli* biofilms: Ciprofloxacin is a broad spectrum fluoroquinolone antibiotic used for the treatment of a wide variety of infections caused by Gram-positive and Gram-negative bacteria. *E. coli* is one of the main pathogens present in biofilms, which are often found on urinary catheters and considered to be the main cause of persistence of urinary tract infections [7]. iFyber has utilized the MBEC device to assess the activity of ciprofloxacin with respect to both prevention and eradication of *E. coli* biofilm. Using iFyber

standard operating procedures, *E. coli* biofilm was established by 24 h incubation of the bacterial inoculum in the 96-well MBEC plate. The resulting biofilm was subsequently treated with a serial dilution of antibiotic followed by enumeration of both planktonic and biofilm-associated bacteria. This assay revealed that at the minimum inhibitory concentration (MIC, 4 ng/mL) ciprofloxacin does not affect the E. coli biofilm. However, at concentrations above the MIC, ciprofloxacin is able to significantly reduce biofilm-related bacterial load, i.e. reduction of 4 log-units at concentrations 5xMIC (0.04 μg/mL), Figure 2.

Biofilm prevention/induction potential:
More recent literature reports [8] show that different antibiotic classes can elicit both biofilm-preventive and biofilm-inducing activity, depending on the antibiotic concentration. Using a range of concentrations, both above and below the MIC, iFyber has evaluated the activity of ciprofloxacin on formation of *E. coli* biofilm over 24 h and found that at concentrations above and close to the MIC (5 ng/mL), ciprofloxacin shows prevention of biofilm formation. At sub-MIC doses, ciprofloxacin did not induce formation of biofilm beyond that obtained by the control (no antibiotic added), Figure 3.

CONCLUSION

iFyber has demonstrated successful implementation of the MBEC assay in determining the anti-biofilm potential of the commercial wide-spectrum antibiotic, ciprofloxacin. The data reported here show highly consistent levels of biofilm production across the MBEC device, as well as anti-biofilm activity of ciprofloxacin in agreement with literature reports. Moreover, these studies highlight that this 96-well plate model is highly amenable to the assessment of multiple treatments in a single plate, as well as evaluation of different aspects of anti-biofilm activity, including inhibition of biofilm formation, biofilm eradication, and the effect on planktonic bacteria formed upon biofilm dissemination.

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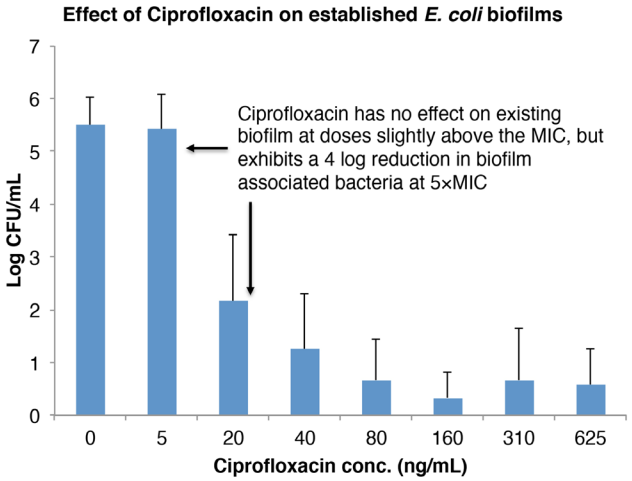

Figure 2. MBEC assay results for eradication of an existing biofilm. Biofilm associated *E. coli* that remain on the peg lids of the MBEC assay device are determined after 24 h incubation in ciprofloxacin. Bacteria are enumerated through standard plate counts of colony forming units (CFUs), and reported as CFU/mL as a function of ciprofloxacin concentration.

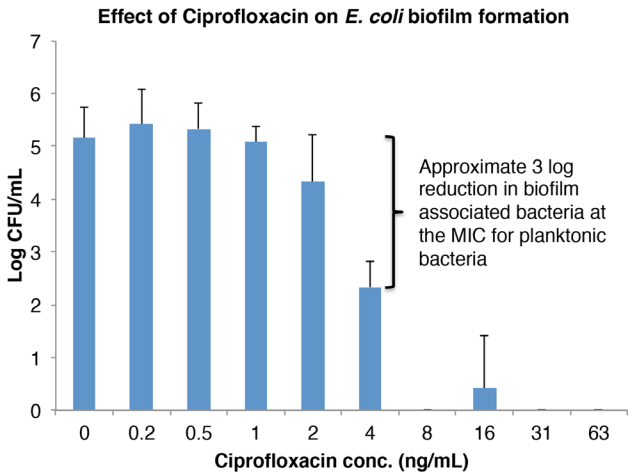

Figure 2. MBEC assay results for biofilm prevention. Pegs of the MBEC assay device were incubated for 24 h in the presence of bacteria and varying concentrations of ciprofloxacin.

Contact Us
Nina Bionda
nbionda@ifyber.com
607.330.2307