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Clinical Microbiology 2015: Chips for antimicrobial drug discovery and diagnosis- Anand Ramasubramanian- University of Texas at San Antonio

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Abstract
We have an interest within the development of microscale technologies for applications in drug development and diagnostics for infectious diseases. We've developed a high-density microarray platform ('chips') consisting of nano-liter volumes of microbial pathogens on chemically modified glass slides employing a robotic microarray. We've successfully grown 1200 individual cultures of 30 n-L volume on a typical glass slide consisting of either single or polymicrobial cultures of Candida albicans, Pseudomonas aeruginosa or Staphylococcus aureus as biofilms. These nano bio-films display morphological complexity, three dimensional architecture and drug resistance almost like conventional cultures in well-plates or flasks. i will be able to demonstrate the suitability of the chip for single and combinatorial screening of small molecule libraries. i will be able to also demonstrate an adaptation of the chip as a diagnostic tool for pathogen identification and antimicrobial susceptibility testing in clinical samples of MRSA. In summary, our chip platform cuts reagent use and analysis times, minimizes or eliminates labor intensive steps and dramatically reduces assay costs and thus opens a replacement chapter in microbial culture.

IMPORTANCE

With an estimated 80% of infections being related to a biofilm mode of growth and therefore the ensuing recalcitrance of those biofilms with reference to conventional antibiotic treatment resulting in high mortality rates, there's a dire and unmet need for the event of novel approaches to stop, treat, and control these infections. Both bacteria and fungi are capable of forming biofilms that are inherently fragile and sometimes polymicrobial in nature, which further complicates treatment. During this work, we showcase a nanobiofilm chip as a convenient platform for culturing several many mono- or polymicrobial biofilms and for susceptibility testing. This platform enables true ultra-high-throughput screening for antimicrobial drug discovery or diagnostics or for addressing fundamental issues in microbiology.

KEYWORDS: high-throughput screening, antimicrobial agents, biofilms

INTRODUCTION

Biofilm-associated infections (BIs) are notoriously difficult to treat as they demonstrate 100-fold to 1,000-fold increases in antimicrobial resistance compared to their planktonic counterparts. BIs are the most explanation for morbidity and mortality related to biomedical-device-related infections, adding over 1 billion dollars to hospitalization costs annually within the us alone. Biofilms are three-dimensional (3D), dynamic microbial communities consisting of attached cells encased during a self-produced exopolymeric matrix. The cells within the biofilms show increased antibiotic resistance through multiple mechanisms and are shielded from the host defenses due partially to the presence of extracellular matrices, thus making biofilm infections most difficult to treat. Experimental models of biofilms that mimic the natural environment provide convenient thanks to understand biofilm biology. During a natural disease setting, biofilms are formed when cells adsorb to surfaces (such as implantable catheters) that are coated with host serum proteins, replicate, and release an exopolymeric matrix that encases the cells. Several in vitro experimental models simulate this in vivo process where the cells are attached to a two-dimensional surface precoated with plasma or a protein of interest. Abiotic surfaces like flasks, well plates, or filters are coated with serum proteins to initiate cell adhesion, and therefore the growth conditions are optimized to facilitate the method of biofilm formation . Although these models are useful in expanding our knowledge of biofilms, a number of the main disadvantages are that these models incorporate low-throughput processes which the inherently fragile biofilms require delicate handling during the washing and analysis steps, thus challenging the high-throughput automation, reproducibility, and reliability of the biofilm assays. To deal with these issues, we recently developed a completely unique platform for fungal biofilm culture consisting of Candida albicans cells encapsulated in nanoliter volumes of hydrogel matrices on glass slides during a microarray format. We demonstrated that the benefits of this high-throughput fully automated platform include (i) production of many spatially distinct but identical "nanobiofilms" on one glass slide; (ii) formation of biofilms displaying phenotypic properties like those of macroscopic biofilms; (iii) the likelihood of culturing of cells for prolonged periods of your time without additional media; (iv) firm attachment of biofilms to the substrate without detachment against multiple washings; and (v) rapid and sensitive fluorimetric analyses.

In this work, we expanded the utilization of our platform to the culture of mono- and dual-species bacterial biofilms at the

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nanoscale level and also of mixed bacterium-fungus biofilms. To demonstrate the flexibility of our platform, we cultured both Gram-positive (Staphylococcus aureus) and Gram-negative (Pseudomonas aeruginosa) bacteria. S. aureus is that the leading explanation for nosocomial infections, since, as a commensal, S. aureus can easily colonize indwelling catheters and biomedical devices and may have quick access to circulation and therefore the vitals. P. aeruginosa biofilms cause pulmonary infections in CF patients. Infections thanks to polymicrobial biofilms have also been found to correspond to significantly higher mortality rates (70%) than are seen with infections caused by one species of microorganism (23%). Among the nosocomial infections that are polymicrobial in nature, S. aureus, C. albicans, and P. aeruginosa were identified because the most ordinarily occurring microorganisms contributing to the high morbidity and mortality rates related to such infections. Hence, this nanobiofilm platform provides versatility and adaptability suitable for the formation of bacterial and fungal also as polymicrobial biofilms and allows the implementation of ultra-high-throughput applications, including susceptibility testing and screening for novel antibiotics, which could rather be impossible to realize using traditional culture systems.

RESULTS

For any given microorganism, the successful fabrication of a nanobiofilm microarray requires a transparent definition of the specifications of the requirements of the platform and therefore the proper design to satisfy those specifications. Briefly, the key specifications are that the chip should hold firmly several many spatially distinct and robust biofilms resembling conventional macro scale biofilm cultures and will enable rapid, reliable, and reproducible analyses of those biofilms with a typical microarray scanner. These specifications were achieved employing a factorial design of experiments wherein the acceptable combinations of abiotic and biotic variables were determined for optimal biofilm culture and analysis, as described before by our group. These principles guided the development of the bacterial biofilm chips described below.

Biofilm formation depends on several factors like the composition, pH, ionic strength, and temperature of media and therefore the physicochemical properties of the substrate. In case of biofilm microarrays, the 2D substrate is replaced by the 3D encapsulating hydrogel. To obtain fully formed biofilms within self-supporting hemispherical hydrogel spots, we optimized the culture conditions by employing a two-level factorial design method described intimately elsewhere.

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