Endotoxin Testing as a Detection Method for Bacterial Biofilms

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***Dr. Tim Sandle** has over twenty-five years experience of microbiological research and biopharmaceutical processing. This includes experience of designing, validating and operating a range of microbiological tests including sterility testing, bacterial endotoxin testing, bioburden and microbial enumeration, environmental monitoring, particle counting and water testing. In addition, Dr. Sandle is experienced in pharmaceutical microbiological risk assessment and investigation. Dr. Sandle is a tutor with the School of Pharmacy and Pharmaceutical Sciences, University of Manchester for the university's pharmaceutical microbiology MSc course. In addition, Dr. Sandle serves on several national and international committees relating to pharmaceutical microbiology and cleanroom contamination control (including the ISO cleanroom standards). He is a committee member of the Pharmaceutical Microbiology Interest Group (Pharmig); serves on the National Blood Service advisory cleaning and disinfection committee; and is a member of several editorials boards for scientific journals.Dr. Sandle has acted as a consultant, expert witness and technical advisor to sterile and non-sterile manufacturing facilities, microbiology laboratories, the medical device industry and hospitals. Dr. Sandle has also undertaken several technical writing and review projects. Dr. Sandle has written over four hundred book chapters, peer reviewed papers and technical articles relating to microbiology. In addition, Dr. Sandle has written several books. Dr. Sandle has also delivered papers to over fifty international conferences and he is an active journalist.*

Introduction

Microbial biofilms - structured consortium of bacteria that are embedded in layers of self-produced polymer matrices, largely composed of polysaccharide, protein and DNA – are well described and known problems for pharmaceutical water systems and medical devices. What is less well-researched is the association of biofilms with endotoxin, especially within the pharmaceutical and medical device context. Here the association of biofilms and endotoxin is of significance to the risks presented by biofilms to water systems and for patient risks in relation to medical devices. With water systems the detection of endotoxin may provide an early warning of a biofilm problem. While the screening of Water-for-Injection systems for endotoxin is a GMP requirement, other types of pharmaceutical grade water are not commonly sampled for endotoxin testing. The introduction of this type of testing may prove useful where there is a concern about biofilm formation. The same may also apply to medical devices, especially given the risk posed from endotoxin. Detachment of cells or cell aggregates, production of endotoxin, increased resistance to the host immune system, and provision of a niche for the generation of resistant organisms are all biofilm processes which could lead to infection.[1]

In both cases the impact of endotoxin will be variable given that lipopolysaccharide size and composition are highly dynamic and vary according to the strain and growth conditions which contribute to the way by which bacteria adapt to changing environments.[2] Nevertheless, endotoxin can potentially provide earlier warnings about biofilm developments than are possible with techniques like bioburden testing and assessments can also assist with the design of materials, especially those that do not readily bind endotoxin, and with in-use assessments.

This article discusses the association of biofilms and endotoxin; looks at the challenges this association poses for water systems and medical devices; and considers whether tests for endotoxin can function as part of a detection method to support an endotoxin control strategy.

Biofilms

Microorganisms are often found in dense communities called biofilms, and the biofilm is recognized as the most common state of bacteria given it is an adaptive mechanism against environmental stresses. Protection is provided to the microbial community through an array of secreted molecules termed extracellular polymeric substances that lead to a three-dimensional architecture, made up of polysaccharides, proteins, lipids, and extracellular DNA.[3]

Within a biofilm the behaviors of organisms are often different compared with the non-biofilm state (particularly planktonic cells) as a result of different genes being turned on or turned off. Differences with this stable microenvironment include the mechanisms of communication (like quorum-sensing-regulated mechanisms); development of mutations; and with the competition and co-operation between strains and species, both of which impact upon community function.[4] Another change that can occur with biofilms is with modifications occurring in lipopolysaccharide, the major component of all Gram-negative bacterial outer membranes and the release of which is commonly referred to as 'endotoxin'. Modification to the molecule can occur through the incorporation of a palmitate acyl chain into the lipid A part of lipopolysaccharide, as shown with Pseudomonas aeruginosa strains.[5] The significance of this to reduces host inflammatory response (of importance to the discussion about medical devices below) and to enhance the survival of biofilm communities when subjected to treatments.

Biofilms are not easy to detect, which is a factor of the slow release of cells and a reflection of conventional cultivation methods only being capable of measuring the number of living cells capable of growing on the chosen agar under the selection incubation conditions. Conventional bioburden