Pharmaceutical BIOPROCESSING

The role of microbiology in the design and development of pharmaceutical manufacturing processes

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The role of microbiologists in pharmaceutical development and manufacturing has become more visible in recent years due to the QbD and risk-based approaches promoted by regulatory authorities and industry [1]. Microbiology, microbiological control and contamination control are indispensable in the manufacture of sterile and nonsterile products as well as biologic drug substances, as evidenced by multiple conference presentations and publications. Terms such as objectionable microorganisms, organisms of concern, alert and action limits, environmental monitoring and sterility are dominating our daily work. Lack of sterility assurance is the number one reason for recalls of sterile drug products, and presence of objectionable organisms is the number one reason for recalls of nonsterile products [2,3]. While no one disputes the role of microbiologists in the design and development of pharmaceutical manufacturing processes, microbiologists still appear 'confined' in the laboratory of the manufacturing facility long after a process has been established and approved. They are not visible as equal partners in the development process along with chemists and pharmacists. However, microbiology issues frequently arise and critical decisions must be made. The science of microbiology and its applications have a large impact on microbiological and contamination-control strategies for robust and consistent processes with infrequent failures and contaminations. End-product testing is no longer considered acceptable for assuring product quality.

Back to the basics

When expectations and practices seem to drift away from science and reach scientifically unjustifiable terrain, we need to go 'back to the basics' and the science of applied microbiology. This is even more necessary when regulatory expectations impose scientifically insupportable requirements and expectations. Training and mentoring microbiologists in the basic principles of microbiology is critical. Microorganisms do not visit facilities out of nowhere; there is a source associated with their origin and introduction into a facility and manufacturing process. Once introduced, favorable conditions are required for a microorganism's growth. Such conditions must be evaluated to understand whether this microorganism could represent a hazard to the product and patient. Microbiological control centers primarily on preventing the introduction of microorganisms into a manufacturing process. If introduction cannot prevented, then control is achieved by destruction, removal, inhibition of microorganisms, or a combination of these approaches. Drug product sterility is based on destruction or removal of microorganisms and is indicated for products that bypass the human body's natural defenses (e.g., parenteral administration). In the case of nonsterile products, APIs, excipients and biologic drug substances, small amounts of microorganisms are acceptable provided that they do not replicate in the final material during storage and provided that these microorganisms are not pathogenic and do not create other objectionable conditions. Nonsterile drug

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products are acceptable for routes of administration that pass through the human body's defenses (e.g., oral administration).

Product quality cannot be achieved through end-product testing as an extremely small amount of product is sampled and tested. Furthermore, the test methods are limited in their detection and quantitation capabilities. Microorganisms are living entities and are not homogeneously distributed. Their detection and measurement represents detection and measurement at a specific time point only as conditions can change following sampling and testing. Retesting of the same material may not provide the same result.

Conditions for microorganism survival, growth & persistence

Microorganisms are living entities, and therefore, highly variable. Like other living entities, they have specific requirements for survival and growth. They need water and nutrients as well as favorable pH and temperature conditions. The longer they stay under these conditions, the happier they are and will thrive. When conditions become unfavorable, some will die, some will form spores, some will shrink their cell size and some may find other ways to survive such as biofilm formation. As a general rule of thumb, most microorganisms will grow and be happy in mild temperature conditions in an aqueous environment near neutral pH. When these conditions are not met, they may die or survive (in a different form or without growth) awaiting more favorable conditions to occur.

Some microorganisms can produce toxins and other by-products when they are actively growing and reach a certain threshold or mass. These toxins can remain in the material and may require specific means to be destroyed or removed, so that the toxins are not present in the final drug product. Many microorganisms can also produce enzymes that may degrade the drug substance or drug product over time. Even if a microorganism is not present in the final material but was present at some point and was provided with the nutrients to thrive, its by-products may be present in the final material, if not destroyed or removed. One example is bacterial endotoxin, which can be produced by certain actively growing microorganisms and can be destroyed by depyrogenation.

Risk assessment for microbial hazards

Quality risk management in the pharmaceutical industry is still rather new. A guidance was introduced in 2005 [4] and the pharmaceutical industry is under the implementation phase. Therefore, the level of sophistication, systematic use and tangibility of results seen in other industries have not yet materialized. Risk assessment, however, is indispensable for microbiological control as it provides a system for identifying and assessing microbial hazards to prevent them from occurring in the process and affecting product quality. Hazard Analysis Critical Control Point (HACCP) has been used as the primary risk assessment tool in other industries to predict introduction and persistence of microorganisms in the manufacturing process. This is an area where microbiologists must receive good training to be able to serve as experts in the conduct of risk assessments to design processes that are sufficiently controlled to achieve microbiological product quality.

Microbial growth potential

All materials and components should be evaluated for their microbial growth potential – their ability to promote and sustain microbial growth. This is integral for process design and validation.

The microbiological quality of raw materials can vary depending on their origin, starting materials and manufacturing process. In addition, shipping and distribution conditions can have an adverse effect on quality, if not appropriately managed. Frequent questions that arise pertain to microbial and endotoxin limits and specifications for APIs and excipients. A microbiologist should be engaged early on during development to conduct a risk assessment along with members from other disciplines to determine the appropriate microbiological quality of these raw materials and the risk for microbial growth. Special attention should be provided to the origin of materials, particularly animal-derived materials that can harbor viruses. Drying, heating and solvent steps in the manufacture of the raw material can reduce microbial load. Certain materials are naturally inhibitive to microbial growth and this should be considered in the acceptability of the material for the process and for developing a monitoring/testing program with scientifically justifiable acceptance criteria.

The drug product formulation itself should be evaluated for its potential to support microbial growth depending on its composition and route of administration. The risk increases for aqueous formulations. In general, tablets and capsules are not conducive to microbial growth due to their low water activity – there is not enough unbound water to be used for microorganism growth. A microbiologist should be enlisted early on to conduct studies to determine how different organisms behave in the formulations under development. These studies can be a USP <51> test or modified versions of this test using lower inocula and different intervals for enumeration of microorganisms [5–7]. ATCC strains but also typical environmental isolates should be evaluated, if possible. Growth or survival of each microorganism should be determined. Based on these studies, aqueous

multidose drug product formulations may not require a preservative, or low preservative amounts may be sufficient, if there are bactericidal or bacteriostatic properties. A bioburden-based approach may be employed for moist heat sterilization instead of an overkill approach that may not be suitable for product stability leading to a terminal sterilization process versus an aseptic fill manufacturing process. A terminal sterilization process is less variable and can be more effectively controlled because it is less open to the environment and there are fewer personnel interventions compared with a typical and manual aseptic process. Worst case and bracketing approaches can be followed for process validation by selecting the most growth-promoting materials.

The composition of other materials and/or solutions used in a manufacturing process should also be considered for their potential to support microbial growth. For example, coating solutions used in tablet manufacturing processes can be conducive to microbial growth depending on the storage conditions. Solutions used in drug product manufacturing processes before providing the final formulation may also promote microbial growth depending on the storage conditions. In biologic drug substance operations, there are multiple solutions that are typically growth promoting such as in-process intermediates following harvest of the bioreactor, bioreactor media and buffer solutions used in purification steps. Knowing the most growth-promoting solution assists in designing the process appropriately and validating hold times using worst case and bracketing approaches. Furthermore, bactericidal or bacteriostatic solutions may not require frequent in-process monitoring or may not need to be monitored at all. More filtration steps may be necessary to control bioburden in a biologic drug substance process or an aseptic process (prior to sterile filtration), if solutions tend to promote growth and there are open operations or connections where microorganisms can be introduced into the process stream.

Hold times

Hold times are critical for any pharmaceutical manufacturing process as they provide an opportunity for microorganisms to grow. The microbial growth potential of the solution should be known. The temperature conditions and length of hold time will affect microbial growth. Small-scale studies can be conducted to determine optimal conditions for growth. If growth is supported, cold storage may be required and length of hold time may be short. If growth is not supported, the solution may be held at room temperature or for longer periods. Maximum hold times will need to be verified at scale during manufacturing.

Dirty hold times of equipment are especially critical for all manufacturing processes and are expected to be validated. A vessel that stays dirty for too long may not be appropriately cleaned using the routine cleaning cycle. Modified cleaning may be required to remove all residue depending on the type of residue. Biofilm can be developed that is very difficult to remove. If the material in the vessel is growth-promoting, it may limit the maximum dirty hold time. Equipment may need to be cleaned immediately.

In-process testing

A microbiologist should be consulted early in pharmaceutical development to discuss the points where a process should be monitored. While for drug product manufacture these points are standardized (e.g., prefiltration bioburden for aseptic process, bioburden prior to sterilization for terminal moist heat), they are not for other processes, particularly for biologic drug substance. In addition, the bioburden and endotoxin limits may not be clearly defined. What is an appropriate limit? What happens if an alert limit is exceeded? What if an action limit is exceeded? There are frequent questions and discussions regarding these topics.

Another topic of great interest is that of acceptable types of microorganisms in a process. What types of organisms are acceptable prior to sterilization or prior to filtration? What types of organisms are acceptable for nonsterile drug product or biologic drug substance? This is where the term 'objectionable organisms' is employed for nonsterile drug product or the term 'organisms of concern' used by some biotechnology companies for drug substance manufacture. Numerous articles have been published on this topic [8,9]. Having a microbiologist participating in the development team can help address these questions and define the criteria for the specific process and product. Objectionable organisms and organisms of concern may vary from product to product and process to process depending on the growth requirements and process conditions.

Personnel & facility/process environment

A risk assessment conducted with a microbiologist and other disciplines should assess each manufacturing process for potential entry of microorganisms and determine environmental monitoring locations. Environmental monitoring should be connected to open operations and interventions that can introduce microbial hazards into the process stream and equipment. Should such introduction occur, the environmental monitoring results can help identify the root cause.

Microbiological stability

Microbiological stability and integrity of the drug substance and drug product should be determined over its shelf life. Container-closure integrity is critical for

preventing microorganisms from entering the drug substance or product and maintaining microbiological quality. For nonsterile materials, quality should be verified during stability to assure that microorganisms present at the beginning of storage did not have an adverse effect on microbiological quality throughout the shelf life.

For sterile products that are reconstituted with diluent or further diluted and then held prior to administration, in-use studies should be conducted to determine whether potential low-level contamination during reconstitution or dilution followed by the hold period and hold temperature would result in microorganism growth. Such studies should be conducted early in development as sometimes they can affect the type of diluent to be used. The goal is to design a product that even if a patient or practitioner accidentally contaminates prior to administration and then holds for the maximum time period, will not cause harm to the patient because the diluent and product supported growth and the organisms grew to high levels during the hold period.

Summary

Having a microbiologist participate early in the drug development team can result in product knowledge regarding microbiological quality and can stir in the right direction for many important formulation design and manufacturing process design decisions. Limits and acceptance criteria for microbiological quality can be determined for drug substance, excipients and drug product based on risk. Process validation can be designed to include the most growth-promoting solutions and conditions that promote microbial growth. Furthermore, microbial risks to the process can be assessed in a preventive and not reactive mode. Microbiological control is primarily based on prevention. Remediation is time consuming, costly and may not be successful at first. Pharmaceutical development and process design require the contribution of multiple disciplines to assure product quality. Microbiological product quality cannot be achieved without the participation of a microbiologist trained in basic and applied microbiology.

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