STERILITY AND CROSS CONTAMINATION CHALLENGES IN TISSUE ENGINEERING AND REGENERATIVE MEDICINE

Massimiliano Cesarini

Team Leader for Regenerative Medicine Solutions

Comecer Group

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Abstract

Regenerative Medicine today is in wide expansion. With increasing research efforts in this field, commercial manufacturing aspects for bio-product are getting more attention. From a GMP perspective, the most critical challenge due to specific factors characterizing cell manipulation is the asepsis of the process. This paper also highlights other critical factors in the process to present a complete view of the risks connected with this activity. A possible solution to these challenges is Isolation Technology, commonly used in the pharmaceutical industry and suggested by the regulatory guidelines in the field.

Introduction

Processes involving cell manipulation have a wide variety of application, from human tissue engineering to stem cells research and adoption. The scientific community acknowledges the enormous potential of this technology within Regenerative Medicine and Gene Therapy. On the other hand, today's reality is more than just research and involves manufacturing capabilities subject to regulatory assessment.

European GMP (Good Manufacturing Practice) concerning bio-materials are described in Annex 2 (Manufacture of Biological active substances and Medicinal Products for Human Use) where the requirements on the asepsis of the process are set forth and strictly relate to what is written in Annex 1, the fundamental regulatory basis for sterile manufacturing.

So the important question is: are sterility and cross contamination important challenges in cell manipulation?

Problem Definition

The sterility or asepsis of the cell manipulation process can be assessed by examining a series of important factors in the adopted procedures.

1 - Cell manipulation is substantially a manual process composed of different complex steps during which cells are often directly exposed to the external environment. In classified environments, the first source of contamination comes from the human body; that is why strict procedures on operators gowning need to be implemented and frequent training sessions are required for all those people who need to work in a clean room.

2 – Extensive use of media is necessary for the proliferation of cells. Antibiotics are sometimes added to the media in order to reduce the risk of microbial contamination but this is not always possible and also does eliminate the risk. Paradoxically the conditions of incubation are exactly the most appropriate for bacteria proliferation as stated in the Annex 2 of the GMP:

> *"Since materials and processing conditions used in cultivation processes are designed to provide conditions for the growth of specific cells and microorganisms, this provides extraneous microbial contaminants the opportunity to grow."*

3 – Laboratory equipment is not usually designed to undergo an automated decontamination cycle. Most of the time the lab equipment used, such as incubators, centrifuges, refrigerators and microscopes, is decontaminated manually by wiping with sanitizing agents. This is because the adoption of oxidizing agents like Vaporized Hydrogen Peroxide capable of usage in automated decontamination processes, nowadays very common in the pharmaceutical industry, is not so popular within laboratory equipment manufacturers.

4 – Starting material (cells) comes from hospital operating rooms where aseptic procedures are not under strict control due to technical limits and lack of training. This means the contamination can also be brought into the process from the original cells.

Given these four factors, **sterility in cell manipulation represents the most important challenge from a GMP manufacturing perspective** and definitely the risk of contamination is higher than in common aseptic processing, used for example in the preparation of injectables.

The second part of the question concerns **cross contamination**. In normal pharmaceutical practices, this issue relates to contamination between different batches. In regenerative medicine, the batch can be a single product dedicated to a single patient, so this kind of risk is extremely high. Most of the time the environment in which live cells are exposed is shared with the origin cells of others without proper cleaning between them. Regarding this issue, ***Annex 2*** points the attention on the air-handling units:

> *"11. Air handling unit should be designed, constructed and maintained to minimize the risk of cross-contamination between different manufacturing areas and may need to be specific for an area.[...]"*

The case of viral vector preparation for gene therapy is the typical worst-case scenario for the risk of cross contamination, while one major challenge is the manufacturing of pathogenic organisms. The potential danger of exposure to these organisms from an HSE standpoint requires them to be treated according to the Biosafety Level associated with them and that is why Annex 2 highlights:

> *"7. [...] Dedicated production area should be used for the manufacture of pathogenic organisms (i.e. Biosafety level 3 or 4)."*

This means that a **containment strategy** (i.e. protection of the operator from the product) is also needed for these particular operations.

From these observations we can conclude that we do have important sterility challenges, in terms of both aseptic processing compliance and cross contamination.

So, what can be done? Is it possible to improve the asepsis in cell manipulation? If so, how?

Solution Proposal

The first thing we did was to look at what we are commonly doing in the pharmaceutical industry for aseptic processing applications, sometimes involving also hazardous substances: **integration of all the critical areas under isolation technology**.

Isolators are intended as sealed enclosures that provide a physical and aerodynamic barrier from the external environment for the specific application of aseptic processing.

The primary characteristics of Isolators designed to reach a Class A environment (EU GMP Classification, Class 100 FDA Classification) are:

- complete assembly in stainless steel 316L
- operation in positive pressure related to the environment
- possibility of laminar airflow
- equipped with an automated decontamination system using Hydrogen Peroxide or Peracetic Acid.

Given their characteristics, Isolators can be installed in a class D environment, while being validated internally as Class A. This fundamental ability is what primarily constitutes the revolution of this technology compared to the clean room.

Conclusion

In the pharmaceutical industry, the adoption of isolators for aseptic processing is already a well-established technology with clear advantages in terms of asepsis of the process, energy efficiency, and better working conditions for operators. All these benefits are bringing reduced operational costs and a higher quality for the finished product. This is why from a regulatory perspective the Annex 1 of the GMP is also suggesting the use of this technology when applicable:

> *"21. The utilization of isolator technology to minimize human interventions in processing areas may result in a significant decrease in the risk of microbiological contamination of aseptically manufactured products from the environment."*

If this sounds like an effective solution to the risk of contamination in cell processing, we would just need to verify if the specific involved processes could be integrated within isolation technology.

Fortunately, the answer is yes. It is possible. Results are impressive.

If you wish to find out more about our proposed solution and the difference between clean room and isolators, fill out the form at www.comecer.com/cell-processing-sterility

Comecer is a confirmed leader in isolation technology for pharmaceutical, chemical, and food industry applications.

Its product portfolio covers various standard applications, but also high-quality customised solutions thanks to specific expertise acquired over the years.

Continuous technological updating and the specific client requirements contribute to ensure operator safety and product sterility.

For containment: isolators to handle highly active principles or excipients (ATEX, where required), isolators to load reactors, multi-stage isolators for chemical synthesis or laboratory operations.

For asepsis: isolators for sterility tests and formulation of sterile drugs, isolators or RABs integrated into the liquid or powder filling lines.

Solutions aimed at Grade A pharmaceutical continuity is then added:

RABs, laminar flow trolleys, transfer hatch (with peroxide sterilisation, where required).

Comecer's objective is to respect the fundamental safety and quality requirements, not only internally but especially towards the end client.

COMECER S.p.A.
Via Maestri del Lavoro, 90
48014 Castel Bolognese (RA) - Italy

Phone +39 0546656375
Fax +39 0546656353
E-mail: comecer@comecer.com
www.comecer.com

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