

Measures to minimize cross-contamination risks in Advanced Therapy Medicinal Product manufacturing

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ABSTRACT

Current European regulations define *in vitro* expanded cells for clinical purposes as substantially manipulated and include them in the class of Advanced Therapy Medicinal Products to be manufactured in compliance with current Good Manufacturing Practice. These quality requirements are generally thought to be elaborate and costly. However, they ensure three main product characteristics: safety, consistency, and absence of cross-contamination. The term cross-contamination is used to indicate misidentification of one cell line or culture by another. The Good Manufacturing Practice Guidelines suggest some recommendations in order to prevent cross-contaminations and require a demonstration that the implemented actions are effective. Here we report some practical examples useful both to minimize cross-contamination risks in an Advanced Therapy Medicinal Products production process and to evaluate the efficacy of the adopted measures.

INTRODUCTION

Proposed employments for cell-based medicinal products are nowadays quite impressive [1]. Fields of application are musculo-skeletal tissue regeneration, autoimmune disorders, myocardial infarction, gastrointestinal diseases, urogenital system diseases, nervous system disorders, wound healing, plastic surgery, organ transplantation, graft versus host disease, and diabetes. Current European cell therapy laws have recently classified cell manipulation types, according to potentially associated risks. Induction to proliferation, non-homologous use (not intended to be utilized for the same essential functions in the recipient as in the donor), and an eventual association with scaffolds or medical devices have been termed "substantial manipulations". This has defined the class of Advanced Therapy Medicinal Products (ATMPs) [2] that have to be mandatorily produced in proper facilities

and in accordance with current Good Manufacturing Practice (GMP) Guidelines [3]. Such quality requirements are generally thought to be elaborate and costly. However, they ensure three main ATMP characteristics: safety, consistency, and absence of cross-contamination. The term cross-contamination is used to indicate misidentification of one cell line or culture by another [4]. This is a major problem to consider when producing ATMPs. For example, in autologous cell therapy, if accidental cross-contamination between two cultures from two different patients occurs during production in a GMP facility, the relative mixed-up ATMPs would carry potential implications for donor engraftment. To this end, the GMP Guidelines suggest some recommendations in order to prevent cross-contamination and require a demonstration that the implemented actions are effective.

Here we report some practical examples useful both to minimize cross-contamination risks in an ATMP production process and to evaluate the efficacy of the adopted measures.

TECHNICAL, ORGANIZATIONAL, AND BEHAVIORAL MEASURES

Actions to be implemented to minimize cross-contamination risks during ATMP production are described in general in Chapter 5 of Volume 4 – EU Guidelines for Good Manufacturing Practices for Medicinal Products for Human and Veterinary use, Current Edition. In the practical applications they may be adapted or implemented, depending on cell type, manipulation process, or therapy.

Environment

The GMP facility should be designed and subsequently built in order to reduce to minimum the recirculation or re-entry of untreated or insufficiently treated air. Pressure differential and airlocks should ensure flow reversal containment and

prevention, without creating turbulence. Appropriate air extraction should be provided in each clean room, depending on classification (A, B, C, and D areas) [5]. Pass boxes for raw materials handling should be constructed. Dedicated and self-contained zones should be available for particular products such as highly sensitizing materials or biological preparations intended for injection. GMP facility-specific parameters like air filtration and ventilation, temperature, relative humidity, differential pressure, number of air particles, and bacterial colony forming units should be standardized and continuously monitored.

Personnel

Only trained and equipped operators can enter the GMP facility. Personnel should wear disposable sterile garments that both ensure protection against hazardous dry particles, aerosols, liquids, and small size particles and represent a physical barrier for cross-contamination (Figure 1).

Cleaning procedures

Ineffective cleaning of environment and equipment is a common source of cross-contamination. Therefore, decontamination and cleaning procedures should be validated in order to verify their effectiveness (tests for product residues presence) [6]. A rigorous cleaning program should be applied for the routine activities by trained dedicated personnel. Validated extraordinary procedures should be followed after accidental cross-contamination occurs.

Production process

Different cell-based products should not be manipulated simultaneously. Production in segregated areas is recommended. When this is not possible, due to the structure small dimensions, a production by campaign (separation in time) followed by appropriate and validated cleaning procedures is suggested. Closed, automated systems of production should be preferred. When culture operations are manual more rigorous procedure should be developed and followed.

Raw materials

Sterile and disposable plastic wares must be utilized. Cell culture media sterility should be certified to be negative for aerobic, anaerobic bacteria, fungi, and endotoxins. Animal origin reagents need to be tested also for mycoplasma and viruses presence. If fetal bovine serum is used, its origin from countries with negligible Bovine Spongiform Encephalopathy (BSE) and Transmissible Spongiform Encephalopathy (TSE) risks should be certified [7]. In general, the material's full batch documentation/certification should be carefully evaluated to attest fulfillment with specific current regulations.



Figure 1. Personnel should wear disposable sterile garments that both ensure protection against particles, aerosols, and liquids, and represent a barrier to cross-contamination.

Donor screening

Donors should be evaluated according to current legislation [8]. In particular, blood samples should be harvested to evaluate the presence of transmissible pathologies by means of HIV-1/HIV-2 Antibody, Hepatitis B Surface Antigen, Hepatitis B Core Antibody, Hepatitis C Virus Antibody, and syphilis screening. In addition, nucleic acid techniques (NAT) for HBV DNA, HCV RNA, HIV DNA detection should be performed to reveal virus presence also during the window period. Positive donors' cultures should be manipulated in segregated areas to avoid cross-contamination risks. For instance, it has been

demonstrated that Human Bone Marrow Mesenchymal Stem Cells in culture [9] are able to fully support HBV infection, replication, expression, and secretion. If the GMP facility does not display the possibility to treat infected cells in segregated areas, positive patients should be previously excluded from the treatment.

HOW TO PROVE THE ABSENCE OF CROSS-CONTAMINATION IN AN ATMP

In general, GMP compliance protects from cross-contamination risks. However, it is required to demonstrate that the adopted measures are effective. An option that has been already proved to be useful for this aim is the DNA profiling technique [10, 11]. This is a highly sensitive and specific method routinely used in forensic caseworks for human identification [12] and applied to reveal and monitor micro-chimerism after stem cell transplantation [13]. DNA profiling is the determination of unique genetic characteristics that make an individual distinguishable from all other humans, including closely related ones. In particular, this technique allows the identification of short, highly specific, tandem-repeated (hypervariable) genomic sequences (short tandem repeats, STRs), that do not contribute to gene function. Based on a specific statistical approach, the random match probability is equated with the probability that a match would occur by chance. The random match probability is the unlikely coincidence that an unrelated person would by chance have the same DNA profile and can be determined by calculating the frequency of the observed profile in a reference population database [14].

As a practical example, we report the use of DNA profiling in the context of the Autologous Chondrocyte Implantation (ACI) technique [10], a worldwide applied cell therapy. Regenerative procedures, including ACI, can be used as second-line measures to repair chondral or osteochondral defects. ACI therapeutical approach was first used to treat full-thickness chondral defects of the knee and later applied to the ankle [15]. In the original procedure, small grafts of normal cartilage removed from non-weight bearing areas of the knee were treated in a GMP facility. Chondrocytes were isolated, expanded in culture for several weeks, and then retrieved as a suspension for re-implantation. Recent experimental and clinical research has been directed toward the development of procedures using suitable scaffolds which act as carriers for the implantable cells, maintaining at the mean-time phenotype stability [16]. In this case, DNA profiling need to be performed both on blood samples of patients undergoing arthroscopy for ACI and on final ATMPs (cells harvested at the end of the culture period). Specific and validated kits analyzing 15 tetranucleotide STRs plus amelogenin locus for sex determination (internal control) can be utilized. If the results evidenciate that the unique DNA profile of each individual blood sample is maintained unaltered in its corresponding ATMP, this is an evidence of cross-contamination absence between different chondrocyte cultures. In fact, the

genetic profile of each patient detected in blood sample controls before manipulation is unaltered in the cells until the end of the production process [10].

CONCLUSION

When producing ATMPs it is mandatory to implement and give evidence of all the possible measures to apply to prevent/minimize cross-contamination risks.

If accidental cross-contamination occurs or is revealed by evidence tests (i.e. DNA profiling), extraordinary cleaning procedures should be applied and an investigation performed in order to find the causes, if any. Preventive actions and monitoring should be implemented in order to avoid or reduce this risk in the future. In general, contaminated ATMPs should be eliminated.

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COMPETING INTERESTS

The authors declare no competing interests.

PUBLISHING NOTES

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