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PROCESS DEVELOPMENT FOR THE PRODUCTION OF HUMAN CELL AND TISSUE IN ACCORDANCE TO GOOD MANUFACTURING PRACTICE: OUR EXPERIENCE IN UKM MEDICAL CENTRE

Khairul AK[*1], Manira M[*1], Seet WT[1], Ahmad Irfan AW[1], Ruszymah BHI[1,2]**

[1]Tissue Engineering Centre, Universiti Kebangsaan Malaysia Medical Centre, Jalan Yaacob Latif, Bandar Tun Razak, 56000 Cheras Kuala Lumpur. Malaysia.
[2]Department of Physiology, Faculty of Medicine, Universiti Kebangsaan Malaysia, Jalan Raja Muda Abdul Aziz, 50300 Kuala Lumpur. Malaysia

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*Both authors contributed equally to this paper
**Corresponding Author: Ruszymah Bt. Hj. Idrus

Email:
ruszy@medic.ukm.my
ruszyidrus@gmail.com

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ABSTRACT

Public awareness on cell and tissue therapy treatment has inspired many research laboratories to produce cell and tissue based technology in treating patients. Translation from research base products to clinical application requires a set of internationally recognized guideline to be followed. Good Manufacturing Practise (GMP) is a guideline to ensure the quality and safety of products offered to the consumer meet the standards. Production aspects in a GMP compliant laboratory built for production of human cell and tissue will be discussed briefly in reference to the Pharmaceutical Inspection Convention/ Scheme (PIC/S) Guide and Good Distribution Practice (GDP) Guide. Critical materials that will be used in the production must be clearly defined. Production personnel dedicated to perform the manipulation must be trained to achieve the competency level required. Final product must be handled in a proper manner to maintain the quality and safety of the product. GMP is an internationally recognized guideline and has been followed by manufacturers worldwide. Therefore it is essential to set the production standard in accordance to this guideline so that the product can be distributed in the international market. In this article, we will describe our experience for the establishment and development of the processes involving cell culture and tissue reconstruction in accordance to PIC/S Guide to GMP for Medicinal Products; PE 009-9; 1September 2009 and GDP; Malaysia; First Edition January 2011.

Introduction

Tissue engineering is a relatively new field that uses living cells, biocompatible materials, and suitable biochemical (e.g., growth factors) and physical factors (e.g., cyclic mechanical loading), as well as combinations thereof, to create tissue-like structures. Most frequently, the ultimate goal is implantation of this tissue constructs into the body to repair an injury or replace the function of a failing organ. The critical functions may be structural (e.g., bone, cartilage), barrier and transport-related (e.g., skin, blood vessels), or biochemical and secretory (e.g., liver and pancreas) [1]. In order to produce a high quality and safe product, the translation of cell manipulation from research laboratory to Good Manufacturing Practice (GMP) setting is required. Thereafter, standard operating procedures (SOP) are drawn up for each step of the production process (patient's selection, cell collection, processing, characterization, evaluation and release of the final product), with particular attention given to traceability and quality control of the process and the product

[2]. Each step written in the SOP has to be validated to prove the effectiveness of the production steps and it can also be used for scientific evaluations. Therefore, the establishment of culture condition for cell and tissue intended for therapeutic or clinical application should follow the GMP guidelines.

Good Manufacturing Practice can be defined as the quality assurance which ensured products such as foods, drugs, medical devices, cells and tissues are consistently produced and controlled in such a way to meet the quality and safety standards appropriate to their intended use as required by the regulatory authority. For clinical grade cells, European Medicines Agency and, the Food and Drug Administration required that the GMP guideline to be followed as it offers optimal defined quality and safety in cell transplantation. This guideline will apply on the development of the building or premises, hiring and training of personnel or staff, standard operating procedures, line of production and quality control, validation of equipments and materials, and packaging and releasing of the final products. It requires that all raw materials are traceable and that production follows validated SOP [3].

In Tissue Engineering Centre, Universiti Kebangsaan Malaysia Medical Centre, Cell Tissue Technology Laboratory, a GMP compliant laboratory with three clean rooms is set up to ensure quality and safety of the final products. The environmental conditions and equipment in this laboratory have been validated and have met specific standards. All critical materials selected for production were suitable for clinical use. The production process will need to be assessed with media fill validation before the actual process can be carried out on patient samples.

Selection of Critical Material

The substitution of research grade reagents with the appropriate clinical or "for further manufacturing" grade reagents were recommended in order to produce a final product which is suitable for clinical use. According to Pharmaceutical Inspection Convention/ Scheme (PIC/S) and World Health Organization (WHO), the source, origin and suitability of the critical material used in the GMP laboratory should be clearly defined [4]. All critical material must have their product certification such as certificate of analysis, certificate of quality or certificate of sterility. This certificate is important to show that the materials had undergone and passed certain level of quality testing to meet the purposes of the usage. The certificate should include, but not limited to, the product name, product code, batch/lot number, manufacturing date, expiry date and test results if applicable. In our production process, we prefer to use chemically-defined reagents and human/microbial recombinant alternatives that are produced in a controlled production environment rather than animal-derived products. This is because the current good manufacturing practice (cGMP) applies strict qualification process for animal-derived products e.g. herd qualification, livestock processing establishment qualification, assessment of viral safety data or Transmissible Spongiform Encephalopathy (TSE) risk. GMP products guarantee an acceptable level of consistency, potency and purity for these key components of our processes [2]. For safety reasons, autologous human serum will be used in place of fetal bovine serum (FBS) as supplements to our culture medium since we have proven in earlier studies that human serum is equal if not superior to FBS in terms of maintaining cell proliferation and specific genes expression [5]. Validations of critical materials such as reagents and mediums may be performed to ensure that the quality and functionality is maintained after delivery. Representative samples from the delivery are taken and put through a "use test" to ensure that their specific function is maintained. The release criteria of the test must be defined and only materials that have met the release criteria will be use in the production process.

Operator Competency

It is mandatory for any manufacturer to provide adequate and competent personnel [6] for production. Personnel competency levels are evaluated by the trainings and assessments that they have attended and passed. All personnel must pass the operator related competency assessments before they can perform work inside a clean room facility. Frequency of the assessment must be defined clearly and followed strictly by the personnel. The aim of the assessment is to ensure that the operators are familiar with the appropriate aseptic techniques in order to prevent contamination being introduced into the facility and to ensure that the sterility and safety of the product is controlled. All personnel have to undergo proper aseptic technique training such as hand washing, open donning and gowning before they can enter the clean room facility. All the training must be observe by trainers who have adequate knowledge of the processes. James L. Howard and Arlen D. Hanssen, 2007 stated in their study that major contributor of microbial contamination in a clean facility is from the production personnel [7]. Personnel that are involved in the production of medicinal products should be appropriately trained in the requirements specific to the type of the product. They should pass the operator aseptic validation before they are allowed to perform any open cell manipulation steps. Furthermore, the implementation of a detailed hygiene plan for each area within the establishment is compulsory. GMP regulations also defined that within the clean room production unit; only one production process is allowed at a time to avoid cross-contamination among different samples. This would present a major obstacle in a common research lab with limited space and with many people working on different projects simultaneously [3].

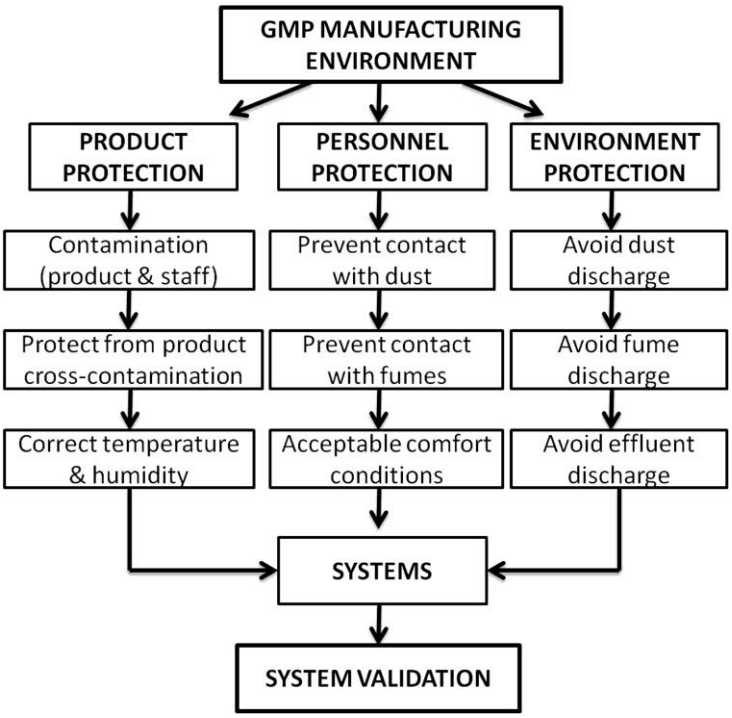

Fig. 1 Primary aspects in GMP. World Health Organization Technical Report Series 937, Geneva 2006.

Process Simulation Test

Validation of aseptic processing should include a process simulation test (media fill validation) using a nutrient medium that can supports the growth of a wide variety of microorganisms. The main objective is to ensure that all steps in the sample manipulation are clean and no possible contamination factors are introduced in all the steps involved. It should imitate as closely as possible the routine aseptic manufacturing process and include all critical subsequent manufacturing steps, the equipments used and also the environments of the facility. PIC/S guideline demand that all part of the quality and facility system are running in a valid manner before the test is performed. It should also take into account various interventions known to occur during normal production as well as worst case scenario. For media fill validation, we used the ready-to-use solution of Tryptone Soya Broth (TSB) whereby all the reagents and medium used in the production process were substituted with the broth. Process simulation test should be performed as initial validation with three consecutive satisfactory passes. We performed one session of process simulation test per shift and it should be repeated at defined intervals and also after any significant modification to the HVAC-system, equipment, process and number of shifts. Normally process simulation tests should be repeated twice a year per shift and process [6].

Process Validation

According to PIC/S guideline, prospective validation that involves a series of process validation should be conducted before the actual clinical trial is started. It is a validation to ensure that the process protocols are accurate, specific and reproducible over the defined range of period. This process validation or dry run is the actual steps for manipulation of cells and tissue inside the clean room including all testing required until the final product can be released. Process validation was performed to optimize the functionality of the equipments and critical materials used during processing and also to identify any problems, analyse potential sources of errors and to evaluate the feasibility of the release tests [2]. Tissue samples must be processed within 48 hours upon delivery and must be expanded following defined cell manipulation protocols. Any open steps during cell manipulation must be done inside a bio-safety cabinet which is classified as a Grade A area. However, the final product for this dry run would not be transferred or implanted back to the patients and will only be used for validation purposes. Process validation should be performed with three consecutive satisfactory passes and needs to be repeated if there is a major change in the processing step, equipments or if the facility has under gone a major renovation.

Testing

There are a series of tests that is required to ensure the quality and safety of the final product released from this laboratory. The patients will undergo a routine blood screening for HIV, Hepatitis B, Hepatitis C, syphilis and also microbiology test for blood infection. The screening tests for infectious agents ensure that the risk of disease transmission is minimised and the manufactured products are suitable for their intended purpose [8]. The serum and plasma extract from patients' blood will be sterile filtered and sent to the hospital's diagnostic laboratory for detection of microbial growth. All reagents and medium that are assigned to each patient will be aliquoted and a sample of each aliquot will be send to the hospital's diagnostic laboratory for sterility testing. Manual cell count and cell viability test will be performed for each cell passages and the purity of the cell culture will be routinely assessed in accordance to specific SOP. The spent mediums used during cell culture will be tested for mycoplasma and endotoxin contaminations in the middle of the culturing process, while a rapid test of gram stain will be performed immediately before the product is released. Each product released from the laboratory must fulfil all the release criteria before it can be applied to patients.

Product Packaging & transportation

Released final product should be secured in a proper manner to maintain the stability, sterility and functionality of the product. Guideline in GDP released by National Pharmaceutical Control Bureau (NPCB) strictly mentioned that materials or products should be stored and transported in a control condition to avoid any incident that can affect the product **[9]**. Product packaging must be able to prevent contamination, spillage or breakage. To maintain the sterility of the final product, packaging process is perform in the bio-safety cabinet under controlled environments. The product will be sealed in a sterile plastic bag. In order to control the temperature, ice packs are put together in the product transport container before transportation. A thermometer is used to record the temperature during transportation. The details of the product and the recipient must be attached together with the package in a registered label or form **[10]**. Traceability of the product must be taken into considerations for any future purposes as suggested in PIC/S and WHO **[11]**. Product transportation should be performed by dedicated personnel with adequate training and knowledge to deliver the product in a timely manner. All possible routes for delivery must be mapped and a transport validation for temperature must be performed to take into consideration of the worst case scenario. Guidelines in Good Distribution Practice do not encourage subcontracting transportation service unless the courier service is able to provide adequate transport validation data. For our clinical trial, the final product is transported to the operating theatre which is located in the same building. The validation for transportation method assumed that the product will be transported by hand and by using the lift. Only in worst case scenario that the transporter will be required to use the emergency staircase, validation was also done to ensure that the product must still be transported within the expected temperature range and still be protected from damage and spillage.

Conclusions

The processing of human tissues and culturing of the cells are part of the processes where the final products such as tissue engineered constructs are important for clinical and therapeutic applications. Therefore, strict regulations have to be followed in order to ensure the quality and safety of the products. The GMP guidelines regulate all aspects including processes as well as documentation, training, quality control and facility. The relevant GMP guideline is in accordance to PIC/S Guide to GMP for Medicinal Products; PE 009-9; 1September 2009 and GDP; Malaysia; First Edition January 2011.

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