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FACILITY MANAGEMENT OF A GOOD MANUFACTURING PRACTICE LABORATORY FOR HUMAN CELL AND TISSUE THERAPY: UKM MEDICAL CENTRE EXPERIENCE

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

[1]Tissue Engineering Centre, Universiti Kebangsaan Malaysia Medical Centre (UKMMC), 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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*Corresponding Author:
Ruszymah Bt Hj Idrus
Email: ruszyidrus@gmail.com, ruszy@medic.ukm.my

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ABSTRACT

Tissue engineering is an emerging field of regenerative medicine aiming at therapeutic application to patient. Translation from research and development to clinical application required the product to be manufactured in a controlled environment of a good manufacturing practice (GMP) facility to ensure the quality, safety and efficacy of the product. We would like to share our experience in managing a GMP laboratory for human cell and tissue therapy in UKMMC. The GMP laboratory for cell and tissue therapy was completed at the end of 2012 and has since been operated with reference to PIC/S and Therapeutic Goods Administration (TGA) guidelines for cells and tissue product. Facility design and size of the cleanroom is critical as it determines the operation and maintenance efficiency. In optimizing the space area, the facility has dedicated grade A, B, C and D areas with supporting anterooms and office adjacent to the cleanroom. Material, personnel and waste flow are being strictly controlled. Critical equipments must undergo qualification procedures and periodic calibrations. Customized cleaning and environmental monitoring programme are adopted for classified and unclassified areas. Personnel microbiological surveillance is performed regularly to maintain sterility. Only trained and approved personnel are allowed in the facility. All facility, equipments, and personnel training records must be documented and audited for consistency.

1.0 Introduction

Tissue engineering is a well known cross-disciplinary field of regenerative medicine aiming at the replacement, repair or restoration of normal function to diseased organs or tissues by the delivery of safe, effective and consistent therapies composed of living cells, administered either alone or in combination with specially designed materials [1]. After its formation in 2000, the tissue engineering laboratory UKMMC has been awarded as a centre of excellence in research by the Ministry of Higher Education in 2008. The laboratory have focuses on several cell and tissue types such as skin, cartilage and bone have since proven its ability to grow into engineered tissue construct *in vitro* and *in vivo* animal model [2-5]. Our first patented product is skin tissue engineering known as MyDerm™, an autologous bilayered human skin substitute with the potential to treat non-healing skin loss such as diabetic ulcers, burns and trauma injuries [5].

Preclinical study must be done before embarking on clinical trial [6,7]. In our laboratory this has been done extensively and presented to our local ethical committee as proof of

concept. However the most challenging part is building a facility for cell and tissue therapy in accordance to national and international standard. After several design changes to accommodate manufacturing requirements and recommendation from the National Pharmaceutical Control Bureau (NPCB), Ministry of Health (MOH) Malaysia, construction were started in 2010 and completed at the end of 2012.

Following the completion of the facility, two audits have been conducted; firstly by national GMP authority, under the national pharmaceutical control bureau (NPCB), Ministry of Health Malaysia and the international certification by the Australian Red Cross Blood Service (ARCBS), an Australian Government Agency. The assessments are basically following the PIC/S Guide to Good Manufacturing Practice for Medicinal Products (PE 009-9) and Therapeutic Goods Administration (TGA) Australian Code of Good Manufacturing Practice - Human Blood and Tissues (2000). These lengthy processes and development are excruciating learning curves for the centre in aspect of designing, constructing and maintenance. We would like to share our own experience in building the facility and managing a GMP laboratory for human cell and tissue therapy.

GMP Guidelines

GMP is an acronym that describes a set of principles and procedures which help to ensure that the products manufactured will have the required quality. It 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 [8]. In Malaysia, GMP for pharmaceuticals, cosmetics, health supplements and veterinary products are regulated by NPCB. According to the Control of Drugs and Cosmetics Regulations 1984, compliance to GMP is a pre-requisite for the application of a manufacturing license as well as product registration [9]. Medicinal products (MP), advance therapy medicinal products (ATMP) and biologics need to comply with the highest available international standard to ensure the safety, efficacy and quality of products manufactured. In order to ensure this, activities associated with all stages of the sample collection, processing, testing, storage and release of product, and the training and development of personnel need to be controlled with particular attention given to traceability and quality control of the process and the product [10].

To date the most current GMP standards specifically defined for cell and tissue therapy products are regulated by the Food and Drug Administration (FDA) USA, European Medicines Agency (EMA) European Union (EU) Directives and Therapeutic Goods Administration (TGA) Australia [8, 11, 12]. However the GMP regulations did not state the how-to in procedural approach and in which it only prescribe the minimum specifications required [13].

Facility Design

Apart from the Standard Operating Procedures (SOPs) development and validation processes for manufacturing protocols, the need for infrastructure and facility design which facilitate GMP operations are of equal importance. The facility located at the 12th floor of UKM Medical Centre has the advantage of hospital support in terms of waste management, general security, engineering, pest control, cleaning service, sterilization service, medical gas, laboratory disposables supply and in-house patients sample management. It shares the common cold water supply, electricity, telephone and network connection, elevator service and building maintenance service. Any disruptions in these shared services will somehow affect the operation and operational cost of the GMP facility, thus maintaining close connection with hospital management is important.

The design of the facility and cleanrooms are based on several factors i.e, classification of the graded area, gowning requirement, entry and exit workflow, product, material and waste flow. The intended operation capacity for example the number of products, patients and manufacturing shift will project the equipments needed, hence the size and number of cleanrooms required.

Classification of a cleanroom is described in the US Federal Standard 209E, which is superseded with ISO 14644 (1999) cleanrooms and associated controlled environments, classification of air cleanliness [14]. EU GMP Annex 1 is better suited for daily GMP processes where the limit of the number of particles and microbiological contaminations have been clearly classified in a scale of grade A, B, C and D as tabulated in Figure 1 [12, 13]**.** CTT Laboratory is designed to have dedicated grade A, B, C and D areas as depicted in Figure 2.

Our facility has three grade B cleanrooms of 26 to 35 square meters in sizes with grade A class II biological safety cabinet (BSC) and grade B gowning room and exit areas. Open cells and tissue manipulation, processing, material storage (temporary for same day procedure) and preparation are performed in the grade A room. It is supported by grade C area which contains a storage room termed Post-Quarantine Room, a cleaner's sluice and a grade C change room. There is a grade D change room designated for changing street clothes

EU GMP, PIC/S Grade	ISO 14644-1 Classification Numbers (N)			
	At Rest		In Operation	
A	ISO 5		ISO 5	
B			ISO 7	
C	ISO 7		ISO 8	
D	ISO 8		Not defined	
EU GMP, PIC/S Grade	Maximum allowed particles 1000 litre (1 m3) of air sample volumes			
	At Rest		In Process	
	0.5µm	5.0µm	0.5µm	5.0µm
A	3,520	20	3,520	20
B		29	352,000	2,900
C	352,000	2,900	3,520,000	29,000
D	3,520,000	29,000	Not Defined	Not Defined

Fig1. Clean room classification relevant for cell therapy facility based on ISO 14644-1 and EU GMP, PIC/S.

to grade C attire upon entry to grade C area. Other supporting rooms such as quality control laboratory, autoclave room, control room and receiving room are located in the unclassified area. Cleanroom size should not be too big as maintaining it will be very costly and laborious, or too small as that would limit space for personnel and equipments, causes difficulties in maintaining the airborne particulate level required.

The classified area is supplied by cooled and clean air from 3 dedicated Air Handling Units (AHU) located at the service floor, above the facility. The heating, ventilation and air condition (HVAC) system temperature is set at 20 ± 4 ˚C for grade B, 22 ± 4 ˚C for grade C area, relative humidity (RH) of 50 ± 10% for all classified area and differential pressure of 15 Pa for room across different grades. Rate of air change per hour is set at 80 for grade B, 40 for grade C and 20 for grade D with 85:15 ratio of re-circulated and fresh air.

The pre-quarantine room, served as the receiving counter for critical material and tissue sample, is specially supplied with cooled air from grade C AHU. This is to ensure room temperature and humidity are within the acceptable limit of the critical materials stored temporarily in the room. Unclassified area which includes quality control room, autoclave room, control room and support corridor is supported with normal ceiling cassette air conditioning operating at ambient room parameters. The essential power supply is provided via a generator set located in utility room and also an uninterruptible power supply (UPS) unit in the control room. The generator set supplies backup power for critical systems of the facility such as HVAC and lighting in the event of main supply failure whereas UPS ensures uninterrupted power supply. There is also a cryopreservation room equipped with liquid nitrogen freezer tanks for storage of cells and a storage room for non temperature controlled materials.

Material, Personnel and Waste Flow

Material, personnel and waste follows must have a specific arrangement of flow in and out of the facility. Material must be passed through checks via pre quarantine room before being passed through a series of dynamic pass boxes across different grades into the cleanroom. The pass boxes use High-Efficiency Particulate Air (HEPA) filter and is certified for corresponding cleanliness grade. Personnel enter and exit the classified area of the facility in bidirectional or two-way flow via grade C and D change rooms suite which has interlocking function. From grade C area, personnel enters the grade B cleanroom in a unidirectional or one-way flow by using grade B gowning room and exits via grade B airlock and degown room. Waste is brought out from the facility via a dynamic pass box labeled as waste hatch in cleaners sluice located in grade C area.

Facility Management

A GMP facility for cell and tissue production should be designed and built to ensure product safety, to minimize risk of injuries, contamination and manufacturing errors, and to permit efficient cleaning and maintenance. The entry access must be controlled by trained and authorized personnel. To ensure maximum security in our facility, the access entry and

Cleanliness Grade	Nominated areas
A	Class II Biosafety Cabinet
B	Immediate environment surrounding a grade A area e.g. Cleanroom.
B	Gowning room, degowning room and grade B exit corridors
C	Transfer corridor, classified storage room, change room and cleaners sluice
D	Change room

Fig2. Cleanliness grade of the nominated area for the facility.

exit is being controlled by digital key lock. Different access levels either for full, partial and visitor, are programmed via the door access system with different personnel in charge based on their role, training and competency. General facility areas are kept clean with scheduled pest control, domestic waste, biohazard waste disposal and regular cleaning. AHU maintenance works are performed periodically followed by a third party cleanroom certification service. The certification services are performed 6 monthly for grade B area and annually for grade C and D area.

Environmental Control and Monitoring

Critical rooms and equipment parameters are monitored by the Building Monitoring System (BMS) Topkapi, Europe and Equipment Monitoring System (EMS), GE Kaye ® Labwatch ® Monitoring System, USA which will record all reading for audit trail and traceability purposes. Alert will be sent out to our Facility Manager if one of the parameters goes out of range. In a cleanroom setting, the manufacturing area should have a relatively higher pressure compared to their adjoining areas. This is to ensure that clean air always flow outwards to minimize the risk of contamination. Clean air circulation is maintained in the Grade D, C and B areas by means of a HEPA filter at particle size of 0.3 µm in order to maintain the viable and non-viable airborne particles in accordance to the specification of PIC/S Guide to Good Manufacturing Practice (PE009-9 Annex 1) and ISO 14644-1:1999 [8, 10].

In EU GMP guidelines it was stated that the air quality standard during the processing of tissue and cell is a key factor that may influence the risk of tissue or cell contamination. It is recommended that an air quality with particle counts and microbial colony counts equivalent to those of grade A with the background environment at least equivalent to GMP grade D is generally required for the processing of the cell or tissue [12]. Our facility is designed to this recommendation, in which the processing area in grade A environment with the grade B background environment.

The guideline in EU GMP does not specify the methods for monitoring the microbiological and particulate cleanliness of air and surfaces. Therefore, we have developed the procedures in accordance to ISO standard i.e. ISO 14698:2003 for cleanroom, associated controlled environment and biocontamination control [15, 16]. We have established the in-house environment monitoring (EM) plans which include non-viable airborne particle monitoring and viable airborne particle monitoring via analysis of microbial content in the air and surfaces by using air sampling and TSA (Tryptone Soya Agar) contact plate method. In order to rationalize the EM procedure, the samplings are done at critical areas which are at higher risk of contamination. These include the surface of BSC, where open manipulations take place and the interior surface of pass boxes where all critical materials are transferred into the cleanroom. The sampling of other non critical area is performed quarterly.

Cleaning

Routine cleaning of the working areas should be performed at the end of the working shift. Weekly, fortnightly and quarterly clean is performed according to a pre-determined schedule. Cleaning should include equipments, work surfaces, floor, windows and ceilings. Appropriate cleaning and disinfection reagents suitable for the facility are used and regular rotation of disinfectant is practiced in order to achieve the needed full sterility. In ensuring that the cleaning is effective, non-viable airborne particle counting, microbial air sampling and surface sampling with TSA contact plates are performed after each quarterly clean.

Equipment Management

Every laboratory should have adequate equipment to perform the processes stated in the laboratory mission, or in our case,

the quality objectives [17]. The basic critical equipments available in our cleanroom are the class II BSC (BioAir Safemate, Italy), CO_2 Incubator (New Brunswick Galaxy, USA), centrifuge and micropipettes (Eppendorf, Germany), pharmaceutical refrigerator with freezer and -20 ˚C freezer (Panasonic Sanyo, Japan), shaker incubator and heating block (Stuart, UK), water bath (Benchmark Scientific, USA), inverted and light microscope (Olympus, Japan), air sampler (SAS Super IAQ PBI International, Italy) and particle counter (HandiLaz Mini Particle Measuring System ,USA).

The equipments are calibrated and qualified (Installation Qualification - IQ/Operational Qualification - OQ/Performance Qualification - PQ) accordingly before they are put into service. A routine maintenance, preventive maintenance, calibration, performance checks and cleaning procedure are set up to ensure that they are kept in good working conditions [6]. All equipment in our facility has a corresponding SOP detailing the basic function and operating instructions for easy reference.

Personnel and Training

Personnel are important resources in a laboratory. In order to establish and maintain a satisfactory quality management system, all personnel must be competent to carry out the tasks in accordance with documented procedures. Staff must understand the importance of their tasks and should be able to perform their duties with integrity [11, 18]. Our facility provides learning and development programs which comprise of training plan, training session and competency assessment. All our staff members are trained and their competency assessed accordingly before they are allowed to perform the associated tasks in the classified area. They have to undergo proper aseptic technique training such as hand washing, open donning and gowning before they can enter the clean room facility to prevent contamination and ensure the safety of the product [19]. Staffs are also made aware of the hygiene requirement of a GMP facility such as no make-up, perfume, nail polish, jewellery and deodorant inside the manufacturing areas. Regular meeting and up to date training is mandatory to create continuous awareness and most importantly elevating the morale of the staff.

5.0 Conclusion

The design, consultation, development and establishment phase of the GMP laboratory for cell and tissue therapy has taken us more than 5 years before our first clinical trial could be initiated. Being one of the pioneers in developing such facility and no definitive national guideline is available, more time was required to finalize the design for such facility. Once accredited achieved the task of operating and maintaining such facility is an uphill battle. We felt that it is important in developing a GMP facility with a product in mind which will then operate in a cost effective manner.

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