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Short Communication

Scaling of massively parallel patient-specific cell cultures with a transportable conditioned cell culture chamber

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ABSTRACT

Barrier Isolators, which separate the cell culture processing atmosphere from the bioburden introduced by personnel, can reduce contamination risks to a cell or tissue product. Compared to open clean room processing, isolator modules can be more readily replicated to scale-up operations. However, for processing of massively parallel patient-specific cell cultures, incubation capacity in a barrier isolator can be a bottleneck. Flexible incubation capacity can be provided by external incubators if cultures can be safely transported to and from the isolator for processing. We tested a transportable conditioned cell culture chamber (TC4) designed for this purpose. We previously published on good cell growth using this processing system to expand K562. In this study, we performed mock production runs with a highly permissive color-changing bacterial broth. We took samples of the final mock cell product and incubated them for bacterial growth. We also performed environmental monitoring of the cell processing chamber with an air sampler and contact plates. Positive control samples were all yellow and turbid. Negative samples and all test materials were negative for microbial growth. We concluded that this transport chamber could safely alleviate the bottleneck in cell production presented by the needs of massively-parallel patient specific cell incubation.

1. Introduction

The scale-up of any cell therapeutic production process does not occur evenly across each step of the entire process. The changes required to expand a rate-limiting step affect other parts of the process, touching off cascades of expensive and time-consuming adjustments and re-testing for the cell manufacturer. Barrier isolators are increasing in popularity for cGMP-compliant cell production for clinical trials [1,2]. Aseptic and modular by nature, barrier isolators provide a far more flexible facility than a stick-built clean room [3].

However, for the scale-up or scale-out of massively-parallel patient-specific cell processing in an isolator, *in vitro* incubation capacity can present a spatial challenge. More flexible incubation capacity can be provided by external incubators. The problem with using external incubators comes with maintaining the aseptic environment in the incubator and during transfers of patient cultures to and from the isolator.

The Xvivo System® has five basic types of chambers for surrounding an entire process with an aseptic and hospitable environment for cells and tissues. These chambers can be combined to fit a specific process (Fig. 1A–B). A HEPA-filtered laminar flow hood (LFH) protects the entry and exit points from unfiltered room air. Buffer chambers (BC) displace entering air with filtered, medical-grade, tanked gases (O_2, CO_2, and N_2). Incubators open only into closed processing chambers (PC) equipped with soft plastic glove fronts. Instrument chambers are customized to enclose and protect the atmosphere around specific instruments such as microscopes, cell sorters, centrifuges, and fill machines.

Personnel are the largest contributors to bioburden in a cleanroom [4]. Cleanrooms provide step-downs in particle control as personnel and materials move from uncontrolled spaces to more heavily filtered room air environments. The Xvivo System installed in an ISO-8 or Class D "clean zone" provides step-downs but with a lower risk profile (Fig. 1C). In a cleanroom, people and items move from unclassified space (UC or ISO-9) through spaces with room air filtered to EU classes D to A specifications (ISO 8–5). Even in a HEPA-filtered biological safety cabinet (BSC), the air-curtain barrier between the room and the open cell processing space can be compromised by the motions of the arms of operators [5], exposing cells to a higher risk of bioburden from people.

Abbreviations: TC4, transportable conditioned cell culture chamber.

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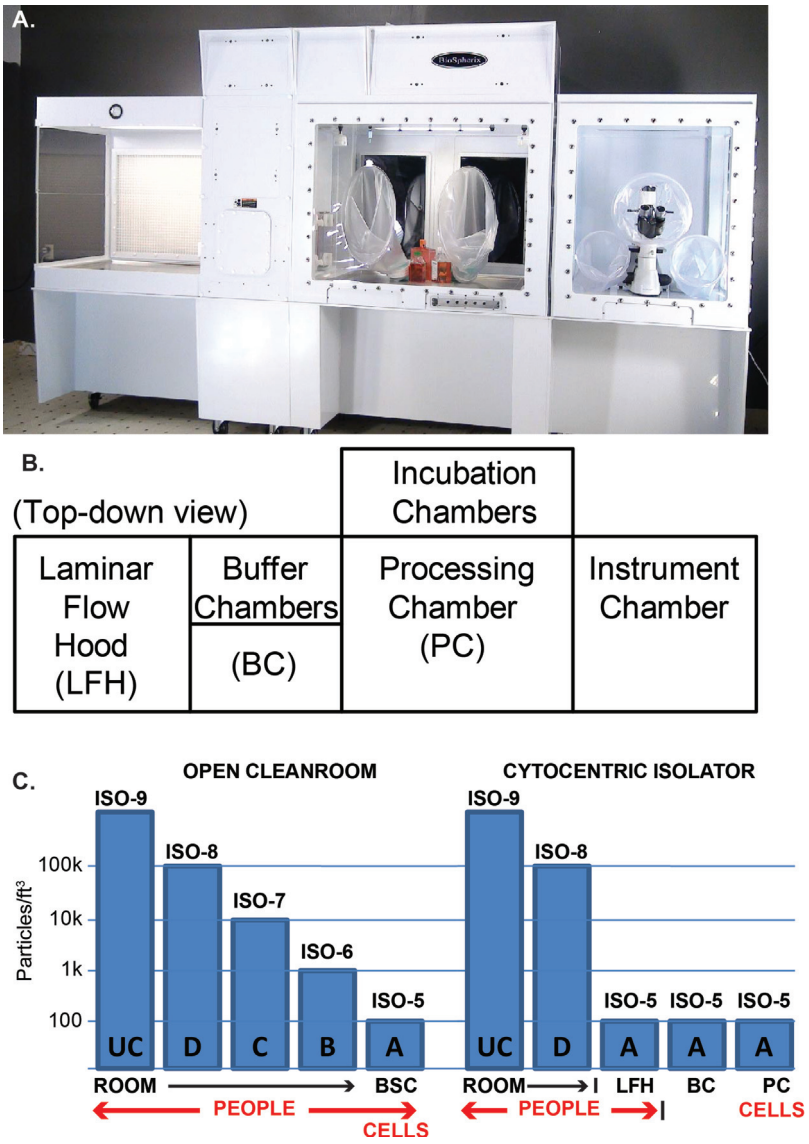

Fig. 1. Barrier isolators are a clean room alternative for full-time protection of the process.
The Xvivo System barrier isolator (A-B) is composed of five basic module types. Incubator chambers (black doors) open only into the closed aseptic cell processing chamber. Instrument chambers are designed to enclose specific pieces of equipment needed for cell processing such as microscopes, cell sorters, and centrifuges. A laminar flow hood protects the entry and exit points with HEPA filtered air flow. The buffer chamber is an airlock that allows materials to enter, but replaces any entering air with triple-filtered medical-grade tanked gases. These modules replace the biological safety cabinets, incubators, and benches in a cleanroom. They can be assembled in a custom way for the most efficient workflow for any particular process.

With the Xvivo System, HEPA-filtered air sweeps from back to front of the entrance laminar flow hood, where all entering materials are manually surface disinfected before transfer to the interior. However, unlike the cleanroom BSC, there is no open cell processing in the LFH. From the buffer chambers on, people and room air are physically excluded, and tanked gases fill the cell incubation and handling spaces. This helps provide better separation of personnel-borne bioburden and open cell processing spaces, reducing the risk of contamination to the cells.

Process changes encountered in scale-out, as a product moves through pre-clinical and early clinical stages, can be easily accommodated in the modular Xvivo System (Fig. 2A–C). New modules containing automation or additional processing space can be inserted into an existing system. However, for scale-out of massively parallel patient-specific therapeutic operations, there are limitations to how many incubators can be open into one processing chamber. This means that incubation space can be a process expansion-limiting factor. For unlimited incubation capacity, safe transport to and from external incubators can provide a flexible solution.

The ideal operation would combine the aseptic nature of the barrier isolator with the flexibility in capacity of external incubators. We performed this study to evaluate the ability of Transportable Conditioned Cell Culture Chambers (TC4) to transport cell cultures between the barrier isolator and the external incubator without compromising culture sterility. Inside the incubator, the TC4 can provide an additional impediment to microbes by keeping the cell culture flasks from contacting incubator shelves and other cultures.

2. Materials and methods

2.1. Media fill testing of the TC4/Xvivo system

Mock cell production runs were conducted using highly permissive color-changing TSB bacterial broth (bioMerieux, Marcy-l'Étoile, France). Mock non-adherent patient cultures were