A new automated bioreactor system for mass production of cells for cell therapy application

This white paper presents a newly patented bioreactor system for mass production of Epithelial cells for regenerative medicine applications without regular trypsin. The system can be used for mass production of cells using traditional culture method automatically by one click. It can also be used for continuous production of biologics automatically.

Continuous Mass Production of Epithelial Cells Using a Bioreactor System for Regenerative Medicine

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Keratinocytes derived from epidermis, oral mucosa and urothelium are used in the construction of cell based tissue engineering and regenerative medicine applications. Several methods (Oliveira and Hodges 2005; Bavister et al. 2005; Mignone et al. 2010; Lei and Andreadis 2008; Hodgkinson et al. 2010) are being developed to obtain cells with functional plasticity to construct artificial tissue for transplantation, to correct specific systemic diseases and as a source for cell-mediated wound healing therapies. But a method to grow adult somatic cells with maximum plasticity, from human tissue, that circumvents many of the well-known and currently debated ethical and scientific problems associated with use of embryonic derived stem cells or induced pluripotent stem cells, has not yet been developed. Traditional monolayer culture techniques utilizing trypsin for harvesting the cells results in small quantities of cells and as the cells from each monolayer are expanded by passage the ability of the daughter cells to divide is diminished (Hayflick phenomenon). Also, traditional monolayer culture techniques have several risks such as low efficiency of operations since the process is highly dependent upon manual labor; contamination of the culture and deleterious drift (genotypic or phenotypic) possibly due to the changing environmental conditions resulting from traditional manual culture techniques.

Marcelo et al 2012 has shown that human epithelial keratinocytes in primary culture can be induced by tissue culture manipulation to produce, without the use of enzymes for passaging, large numbers of small cells in a combined suspension/monolayer culture using traditional culture technique with regular T-

flasks as shown in Fig.1. They refer to the small cells as e-PUK (epithelial Pop-Up Keratinocyte) cells. They (Peramo et al 2013) also found that many other strains of cells including neonatal cells, breast cells and abdominal cells have the same pattern of producing the e-PUK cells. This method would significantly improve the production of keratinocyte cells without damage of enzymatic treatment and also enhance production efficiency. The traditional culture technique however only allows the production of e-PUKs from the first passage of keratinocyte monolayer for 7 or less days and requires generating another monolayer from the e-PUK generated from the first passage of cells to continue the subsequent production of e-PUK. The life of the subsequent monolayers and number of passages get shorter and burns out within few passages of monolayer due to the lack of ability to properly optimize the cell growing condition using these traditional techniques. Additionally these traditional techniques require substantial labor and manual operation in an open system which is subject to greater risk of contamination.

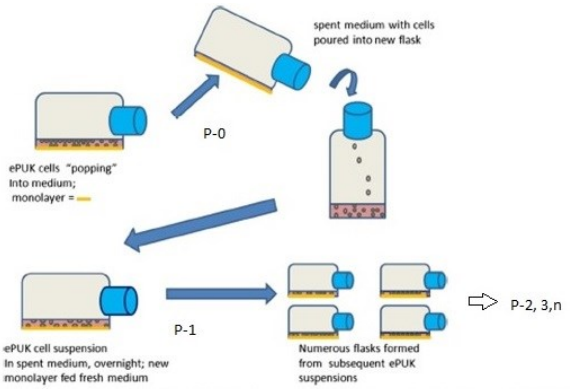

Figure 1 ePUK protocol. In the early passages there were 86,000 ±1,275 (SEM) cells/ml, with a medium number of 78,000 cells/ml, 79% viability, n=10, which is a cell production of 2.3 X10^6 cell/T-75 flask, per day.

A new culture technique utilizing a flask bioreactor was thus reported by A. Miyazawa et al (2018) using a modified T flasks with a novel programmable rocking device. The result showed that this automated bioreactor system has extended culture longevity and proliferative capacity in normal primary human keratinocytes for over 29 days with only one parent monolayer cells. As the floating ePUK cells attached the surface, the cells returned and retained the original characteristics of the keratinocytes. The schematic diagrams of this process are shown in the following Fig 2 and 3,

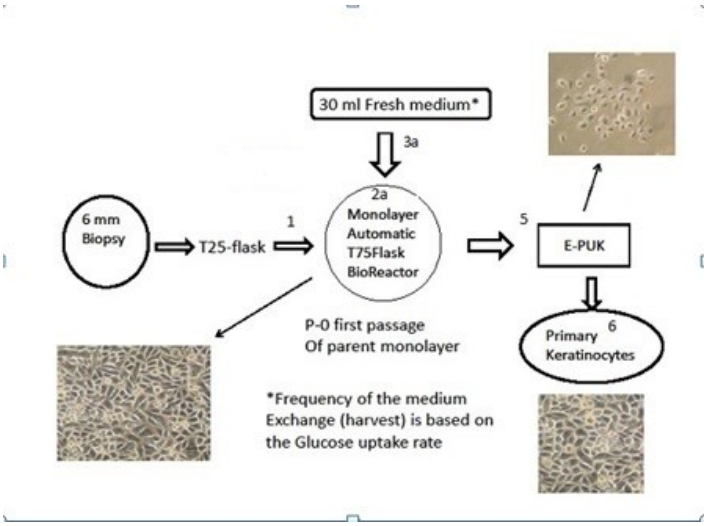

Fig 2 ePUK production diagram using T-flask bioreactor system

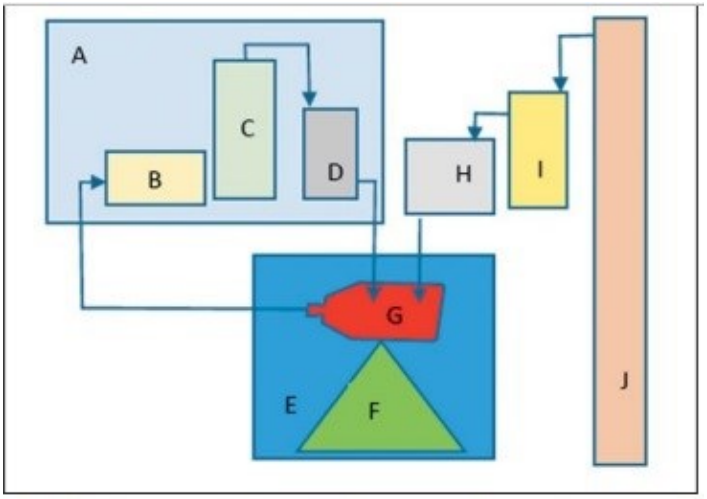

Fig 3 Bioreactor System Schematic Representation: **(A)** Laminar flow hood BSL2 houses the ePUK harvest flask **(B)**, and the fresh media reservoir **(C)**. The peristaltic pump **(D)** can be housed inside or outside of the laminar flow hood, but is depicted within the flow hood. The peristaltic pump is used to pump fresh media from the fresh media reservoir and into the bioreactor flask at each feeding interval. The incubator **(E)** maintains a temperature of 37°C and houses the rocker **(F)**, and the bioreactor flask **(G)**. **(J)** a pressurised gas cylinder with a pressure regulator that provides a constant flow of premixed gas of 5% CO_2 and 95% air to the rotameter **(I)**, which reduces the gas volume rate before the gas is filtered through the inline filter **(H)** and into the bioreactor flask **(G)**.

To find how an automated bioreactor system was developed to accommodate this finding. READ MORE

The system was further proposed for improvement as shown in Fig 3.

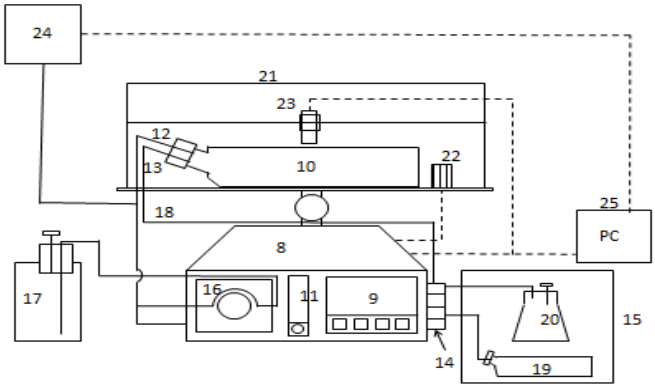

Fig. 4 Schematic diagram of automatic flask bioreactor

An integrated automatic T-flask bioreactor using a novel rocker and a modified T-75 flask was further proposed to improve the performance of the process shown above in FIG4. The system comprises an integrated rocker 8 with digital panel 9 mounted with a single T-flask (such as Corning T75) 10, a gas mixture system 11 to feed the gas mixture through inlet port 12 and exit from port 13 through the dispensing system 14 comprising several pinch valves to external designated containers 19, 20 with outlet air filters inside of a CO2 incubator or a refrigerator 15, a pumping system 16 to pump fluid (medium, seed, cell detaching solutions etc.) from the storage containers 17 through inlet port 12 for a fixed volume of 30 ml after the platform 18/vessels 10 return to the horizontal position, the content of flask 10 will be programmed based upon the glucose consumption rate to empty and/or harvest by tilting the platform 18 to an angle and through the dispensing system 14 to direct the harvest line to external designated containers such as T-flask 19 or bottle20 inside of a CO2 incubator or a refrigerator 15, the lid 21 covers the flask 10 and sits on platform 18, the temperature of the enclosure is controlled with a hot air heater 22, a portable image monitoring device 23 such as Lonza's CytoSmart placed on the platform remotely monitored, recorded and controlled by a PC 24.

The process will be started from a single monolayer growth of primary epithelial cells isolated from adult human epidermis or oral mucosa or ureters at T-flask 10 with intermittent replacement of standard volume of fresh medium (e.g. 15ml in T75 flask) every two to three days from the fresh medium bottle 17 automatically.

The spent medium will be discarded or saved for analysis. As the monolayer reaches greater than 80% growth, the T-flask will be replaced with 2x volume of fresh medium (e.g. 30 ml in T75 flask) automatically and production of e-PUK cells began. The gas mixture was regulated to a less oxygen tension (<21% O2, e.g. 5%) through 11 and fed to the system through 12 at constant gas flow rate to the system. Initially, the monolayer in the T-flask will be cultivated at horizontal position under static condition in the device for two 24 hour cycles and the content of e-PUK cells harvested and replaced with 30 ml of fresh medium in each cycle. The initial and end samples of each cycle will be analyzed for glucose concentration. Then the next cycle time will be calculated by the following equation and the process proceeded.

The frequency (cycle time) of medium replacement t_3 for the next cycle is calculated by the following equation (1):

$$t_3 = (C_0 - C_{min})/(dR + dC_2/t_2)$$

where t_1 and t_2 are the first and second cycle time of the most recent 2 cycles;

dC_2 is the difference of glucose concentration change during the second of the most recent 2 cycles;

C_0 is the concentration (mg/dl) of the fresh medium; C_{min} is the minimum concentration to be maintained in the culture;

$dR = dC_2/t_2 - dC_1/t_1$ is the change of glucose consumption rates between the two previous cycles, cycle 1 & 2, where dC_1 is the same as dC_2 but for cycle 1.

The process will be continued in the same manner for substantially extended time (for months). Each cycle of e-PUK cells will be harvested for immediate use at bottle 20 or at another T-flask 19 in a CO_2 incubator 15. The cells collected at T-flask 19 will be subsequently further attached, cultivated, harvested, cryopreserved using traditional method for later use.

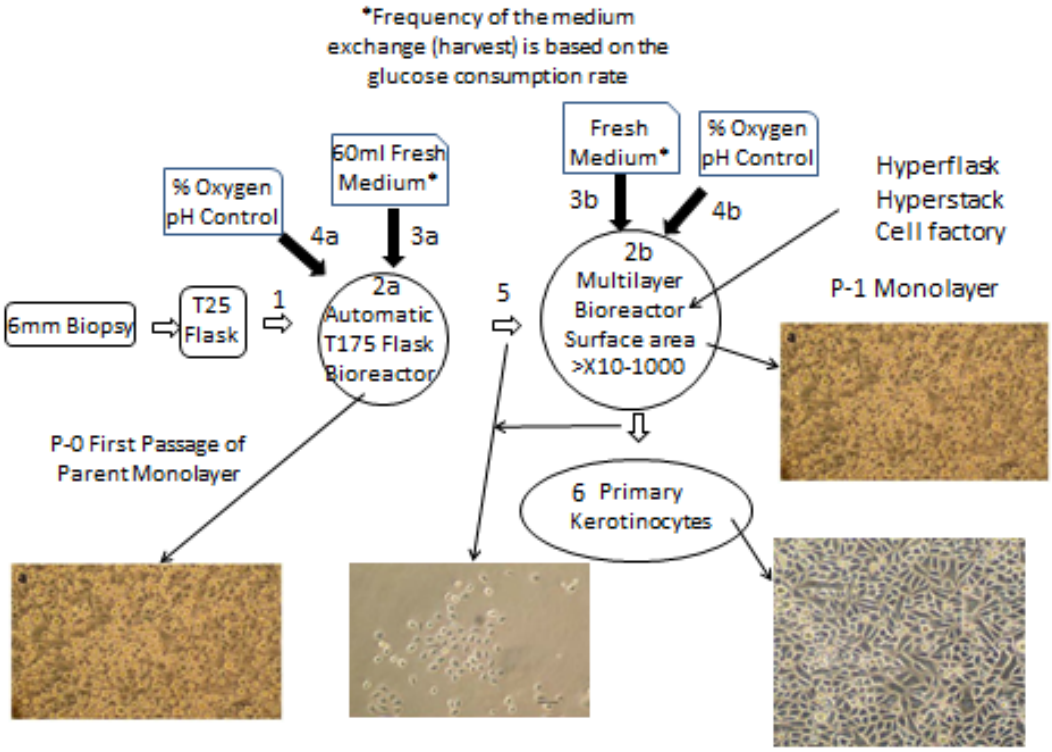

Fig. 5 Schematic diagram of large scale of the continuous ePUK production

FIG. 5 further illustrates a production bioreactor using modified vessel with multi-layer of surface plates, such as Corning's Hyperflask, Cellstack or Thermo's Cell Factory, to perform the semi-continuous process as established above using T-75 flask. The multi-plate structures are comprised of multiple layer of surface plates to increase cell growth surface area (> 25280 cm^2) compared to the small surface area of 75 cm^2 available in the T-75 flask bioreactor shown in FIG. 4. The process begins with a T175 flask bioreactor 2a using the same protocol with optimal control of nutrient 3a and oxygen 4a as shown in FIG.2 and FIG.3 to continuously produce the e-PUK daughter cells 5 from the P-0 monolayer which are directly used to seed the production bioreactor 2b. The e-PUK cells quickly attach to the multi-plates of 2D surface carrier in the bioreactor 2b and continue the growth and production process using the same protocol applying the same optimal control of nutrient 3b and oxygen 4b as that in the seed bioreactor 2a. During the seeding and growing process the production of e-PUK cells from the bioreactor 2b is also self-seeding to the available open surfaces along with the e-PUK cells from the seed T175 bioreactor 2a until all complete surfaces are fully occupied. Then the full production process begins. The production bioreactor 2b continues to apply the same optimal control of nutrient 3b and oxygen 4b and produce e-PUK

cells 5 and keratinocyte cells 6 from the same P-1 monolayer throughout the entire production process for a greatly expanded time.

In order to achieve the performance of a production bioreactor using the modified vessel with multi-layer of surface plates, such as Corning's Hyperflask, Cellstack or Thermo's Cell Factory indicated above, the AMTBR (automated multi-tray bioreactor) based on our patented HRBR technology for adherent cell cultures using 2D multi-tray culture vessel was established and patented (US10590374 B2). See Fig. 5&6 and view the following YouTube video. **https://www.youtube.com/watch?v=mZUk695xURQ&feature=emb_title**

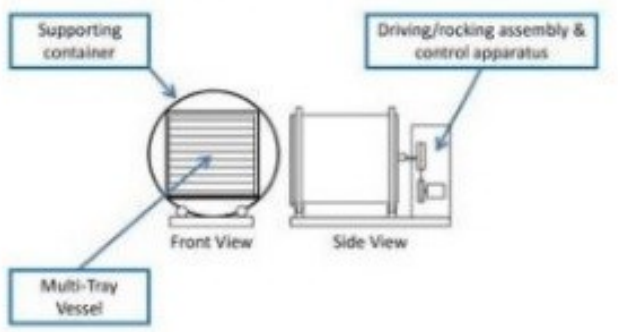

Fig 4 Schematic diagram of AMTBR

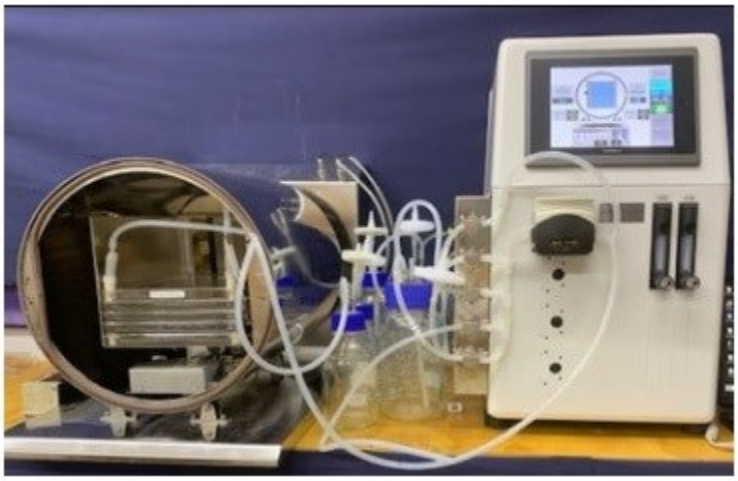

Fig 5 Photo of AMTBR (for 10 tray Cell Factory)

The fully automated AMTBR resolves limitations of current 2D flat culture surface technology by applying the active gassing and automatically performing the entire

culture functions including filling, seeding, culturing, medium exchange, emptying, infection/transfection, sampling, cell detachment, and harvesting all in one closed system and in one click.. The AMTBR possesses increased functionality and control-ability, while also substantially simplifying/decreasing the number of devices and thus greatly reducing the physical footprint and operating cost compared to the commercial automatic Cell Factory system (AMCF). Lastly, it costs only fraction of the complex AMCF system. The system can periodically monitor the glucose concentration, pH, etc. by off-line analysis with samples; or optionally by continuously monitoring and control (of pH and DO) by a non-invasive optical pH/DO monitoring system such as PreSens' Featured pH Monitoring System for Bioprocess Development.

References

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- US Patent Number 10590374 B2 Automatic multi-tray and multi-plate bioreactor systems for adherent cultures; Lewis and Timothy Ho (2020)

Learn more about the patented bioreactor

Your comments are highly appreciated