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Short communication

Continuous production process of retroviral vector for adoptive T- cell therapy

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ABSTRACT

Adoptive T-cell therapy is being considered as a promising method for cancer treatment. In this approach, patient's T cells are isolated, modified, expanded, and administered back to the patient. Modifications may include adding specific T cell receptors (TCR) or chimeric antigen receptors (CAR) to the isolated cells by using retroviral vectors. PG13 cells, derivatives of NIH3T3 mouse fibroblasts, are being used to stably produce retroviral vectors that transduce the T cells. PG13 cells are anchorage-dependent cells that grow in roller bottles or cell factories and lately also in fixed bed bioreactors to produce the needed viral vector. To scale up viral vector production, PG13 cells were propagated on microcarriers in a stirred tank bioreactor utilizing an alternating tangential flow perfusion system. Microcarriers are 10 μm–0.5 mm beads that support the attachment of cells and are suspended in the bioreactor that provides controlled growth conditions. As a result, growth parameters, such as dissolved oxygen concentration, pH, and nutrients are monitored and continuously controlled. There were no detrimental effects on the specific viral vector titer or on the efficacy of the vector in transducing the T cells of several patients. Viral vector titer increased throughout the 11 days perfusion period, a total of 4.8×10^{11} transducing units (TU) were obtained with an average titer of 4.4×10^{7} TU/mL and average specific productivity of 10.3 (TU) per cell, suggesting that this method can be an efficient way to produce large quantities of active vector suitable for clinical use.

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1. Introduction

Adoptive T Cell Therapy is a rapidly growing field that uses the patient's immune system to battle cancer cells [1]. The patient's own T cells are modified by genetic engineering to enhance their interaction with the cancer cells and improve their capability to attack them [2]. One of the approaches for T cell modification is to add tumor-specific T cell receptors (TCR) or chimeric antigen receptors (CAR) to the patient T cells by transducing cells collected from the patient with the retroviral vector [3]. Following the transduction, the modified cells are administrated back to the patient [4].

Retroviral vector can be produced in PG13 packaging cell line derived from NIH3T3 mouse cells stably expressing the Moloney murine leukemia virus gag-pol proteins and the Gibbon ape leukemia virus envelope protein [5]. These cells are stably transfected with gammaretroviral backbone encoding TCR or CAR for constitutive production of secreted retroviral vector. In 1994, von Kalle et al. published an article describing the use of PG13-derived retroviral vector for the transduction of CD34+ cell [6]. In 1995, Bunnell et al. described the use of PG13-derived retroviral vector for transduction of human peripheral blood lymphocytes [7]. In 1997, Bunnell et al. used PG13-derived vector to assess persistence of gene-marked cells in non-human primate model [8]. In 2005, Cornetta et al. published the National Gene Vector Laboratory's (Indiana University) collective PG13 vector production experience [9], and the first clinical data reported was in 2006 by Morgan et al. [10]. Since 2006, many groups have published clinical results describing the introduction of T cell receptors and chimeric antigen receptors using PG13-derived vector products [11,12]. The PG13 cells are anchorage-dependent cells traditionally propagated in dishes, T flasks, roller bottles and cell factories [5,13] where the media can be harvested several times in a batch mode for viral

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vector production. Recently, these cells have been propagated in a packed-bed bioreactor which allows continuous media replacement and vector harvest increasing production efficiency [14].

A promising alternative production approach is the use of microcarriers support for growing anchorage-dependent PG13 cells. Microcarriers, first described in the 1960s by van Wezel [15], are small, approximately 10 μm–0.5 mm, charged, coated or porous beads that provide a surface for anchorage dependent cells suspended in a culture medium, have been utilized effectively for propagation of anchorage-dependent cells in bioreactors for production of different biologicals [16]. There has been significant amount of work associated with improving capabilities of cell culture using microcarriers [17,18]. Microcarriers have been utilized for cells and virus production for vaccines such as polio virus, as well as for antibodies and recombinant proteins [19]. Recently, attention has been directed towards utilizing microcarriers for growth and expansion of mesenchymal and pluripotent stem cells [20,21].

Microcarriers provide support for anchorage-dependent cell growth in bioreactor and, therefore, like other anchorage-dependent cell methodologies, the surface area is finite, e.g.3 g/L of Cytodex 1 provide surface area of 13.2 cm^2/mL. Since microcarriers are kept in suspension, it is possible to replace the media while maintaining the cells in the bioreactor without disrupting their growth, practically simulating suspension culture conditions, and, therefore, extending the production period [22].

This report proposes a procedure for continuous large-scale production of retroviral vector by using microcarriers in a bioreactor equipped with alternating tangential flow perfusion system.

2. Materials and methods

2.1. PG13Cells

A PG13 stable packaging clone was previously generated, constitutively expressing a gamma retroviral vector containing T cell receptor using PG13 gibbon ape leukemia virus packaging cell line (ATCC CRL-10686) and the human ecotropic packaging cell line Phoenix ECO (kindly provided by Dr. Gary Nolan, Stanford University, Stanford, CA) as previously described [13]. Cells were maintained in tissue culture flasks in a humidified incubator set at 5% carbon dioxide (CO_2) and 37 °C. The PG13 cells were grown in Dulbecco's Modified Eagle Medium (Thermo Fisher, Grand Island, NY) supplemented with 10% Fetal Bovine Serum (Atlanta Biologics, Flowery Branch, GA), Penicillin-Streptomycin (Thermo Fisher, Grand Island, NY) and 6 mM final concentration glutamine (Thermo Fisher, Grand Island, NY) abbreviated as DMEM10, both in the tissue culture flasks and the bioreactor.

2.2. Microcarriers

Cytodex 1 microcarriers (GE Healthcare Life Sciences, Uppsala, Sweeden) were used at a concentration of 3 g/L of culture plus 10% to account for transfer losses. The microcarriers were rehydrated in 75 mL/g Phosphate Buffered Saline (PBS, Lonza, Rockland, ME) for at least 3 h at room temperature in a siliconized glass bottle while being agitated on a rocking platform. The microcarriers were allowed to settle and the PBS removed. The microcarriers were then washed with 40 mL/g of PBS and resuspended in 40 mL/g PBS for autoclaving, with the bioreactor. After autoclaving, 30 mL/g of DMEM10 was used to wash the microcarriers. The microcarriers were then transferred to the bioreactor with some of the initial 500 mL DMEM10 described in Section 2.3.

2.3. Bioreactor

A one liter working volume univesel bioreactor with marine blades (Sartorius, Goettingen, Germany) equipped with 16 cm dissolved oxygen (Hamilton) and pH (Hamilton) electrodes, configured as shown in Fig. 1, was connected to a DCU touch controller (Sartorius, Goettingen, Germany). The growth was initiated at 37 °C, pH 7.5 and air flow of 0.3 L/min with 3.3 g of Cytodex 1 microcarriers, in 500 mL of DMEM10 and approximately 1.7×10^8 cells. For the first 4 h, the agitation was set at 100 rpm for 2 min followed by 5 rpm for 20 min to allow for cell attachment. After 4 h, the agitation was set at 100 rpm and additional 500 mL of DMEM10 was added. Dissolved oxygen, pH and temperature were continuously monitored and controlled (see next paragraph). Daily samples were collected for measurements of cell count nutrients, metabolite levels, and pH (Fig. 1).

Agitation and airflow increased as the dissolved oxygen decreased, and oxygen was added by cascade control at 1 L/min when the dissolved oxygen concentration reached 50%. Agitation was also increased to 110 rpm when the microcarriers started settling. When the glucose level reached 2 g/L and/or lactate increased to 2 g/L, the media was harvested and fresh DMEM10 was added. The harvest medium was used to measure viral vector titer as described in the next section.

2.4. Perfusion

An alternating tangential flow (ATF) unit (Repligen, Waltham, MA) specific for microcarriers culture (ATF2 MC) equipped with microcarrier screen filter module (73 μm pore, 162 $cm^{2)}$ operated by C24 controller was set up as shown in Fig. 1. On day two, the ATF was turned on with pressure setting of 0.9, and exhaust setting of 0.3 to prime the system for perfusion. The bioreactor was run as batch culture until day four when the feed and harvest pumps were turned on. The feed and harvest flow rates were set at 0.69 mL/min for a total bioreactor volume change in 24 h. The harvest was collected at 24 h intervals for measurements and samples were kept in the −80 °C freezer until further use.

2.5. Cell count and viability measurements

Samples were collected as described in Section 2.3. To measure the cell count and viability from the microcarriers, 1 mL of culture with microcarriers was allowed to settle in a 1.5 mL Eppendorf tube. The supernatant was removed and strained into a cell strainer tube. The cells were washed with 1 mL of PBS which was removed and strained in to the tube and 1 mL of trypsin (Thermo Fisher, Grand Island, NY) was added to the cells. After a 7-min incubation at 37 °C, this was then also strained into the mixture with media and PBS of which 300 μL was counted using the trypan blue exclusion method with the Cedex cell counter (Roche, Basel, Switzerland).

2.6. Nutrient and metabolite measurements

Daily samples were measured for nutrient and metabolite concentrations. Glucose and lactate concentrations were measured using YSI 2700 biochemistry analyzer (Yellow Springs Instrument Co., Yellow Springs, OH). Osmolality was measured with Vapro vapor pressure osmometer (Wescor, Logan, UT). Glutamine, glutamate, and ammonia concentrations were measured using the Cedex bioanalyzer (Roche, Basel, Switzerland). These measurements were in accordance with manufacturer instructions.