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Performance evaluation of a proliferation chamber with external stirred conditioning tank for expansion of a suspendable stem cell model

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ABSTRACT

Performance of a proliferation chamber with external stirred conditioning tank (PC-ESCT) was evaluated to expand a cell line as a model of suspendable stem cells. A hematopoietic cell line (U937) showed considerable cell proliferation in the PC-ESCT at seeding densities of 10^4 and 10^5 cells/mL (97.5 ± 1.7 and 24.5 ± 1.9-fold), as compared to the cultures in T-flask (80 ± 2.8 and 21.5 ± 1.4-fold) and stirred tank bioreactor (STB) (no detectable increase and 2.85 ± 0.8-fold) after 8 days of culture. The round-shaped cells appeared dominantly in the T-flask and PC-ESCT cultures, while few cells could maintain their original phenotype or survive in the STB system during the culture period. The cell surface marker analysis of CD14 for testing self-renewing proliferation of the cells also coincide with the cell appearance and quantitative morphological analysis based on the roundness parameter. The present study showed the potential of the PC-ESCT for the expansion of suspendable stem cells *in vitro*.

1. Introduction

Stem cells and their derived products offer great promise for new medical treatments. The most important potential application of human stem cells is the generation of cells and tissues that can be used for cell-based therapies [1]. Stem cells can be roughly categorized as adherent (anchorage-dependent) and suspendable (non-anchorage-dependent) cells which differ markedly in their culturing requirements. Adherent cells require attachment to a solid substrate for viability, while suspendable ones can proliferate in suspension cultures [2].

In addition to biopharmaceutical applications, suspendable stem cells (e.g. hematopoietic stem cells) have been recently used to treat a wide range of diseases successfully [1,3]. However, the initial number of the stem cells obtained from their sources is not sufficient for treating patients [4,5]. For example, one of the most important suspendable stem cells which has many applications in treatment of life-threatening diseases is hematopoietic stem cell (HSC). HSC transplantation is the transplantation of multipotent hematopoietic stem cells, which may be autologous (the patient's own stem cells) or allogeneic (the stem cells from a donor) to treat patients with certain cancers of the blood or bone marrow, such as multiple myeloma or leukemia. Indeed, high-dose chemotherapy (HDCT) followed by transplantation of autologous blood stem cells is widely used in the treatment of various cancers such as breast cancer, ovarian cancer as well as solid tumors and non-Hodgkin's lymphoma [6,7]. One report showed that autografting of at least 2.5×10^6 $CD34^+$ cells/kg could rapidly restore hematopoiesis after high-dose conditioning therapy in malignant lymphoma [8]. Another valuable report from 433 patients indicated a 2×10^6 $CD34^+$ cells/kg threshold for hematopoietic reconstitution after HDCT for treatment of multiple myeloma as well as breast

Abbreviations: PC-ESCT, proliferation chamber with external stirred conditioning tank; STB, stirred tank bioreactor; HSCs, hematopoietic stem cells; DO, dissolved oxygen; PLC, programmable logic control; PBS, phosphate-buffered saline; EPC, endothelial progenitor cell; ECs, embryonic stem cells; A_c, cell area; l_c, peripheral length of cell; R_c, roundness.

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cancer, ovarian cancer, solid tumors and non-Hodgkin's lymphoma [9]. Recently, use of autologous nonmyeloablative HSC transplantation (5×10^6 $CD34^+$ cells/kg) could induce prolonged and significant increases of C-peptide levels associated with reduction of daily insulin doses in a small group of patients [10]. The literature survey reveals at least 2×10^6 $CD34^+$ cells/kg is needed for HSC transplantation for various treatment purposes. Therefore, it is necessary to develop adequate culture systems to expand the stem cells for providing the clinically relevant cell numbers for different therapies.

Although T-flasks and well-plates have been the most widely used static culture systems for expanding suspendable stem cells, these systems have several drawbacks such as lack of mixing, resulting in critical concentration gradients of pH and dissolved oxygen (DO). Static systems also suffer from difficult online monitoring and control, low process reproducibility, repeated handling required to feed cultures or obtain data on culture performance as well as productivity limitation [11,12].

Use of bioreactor is as an alternative approach to static flask cultures for stem cell expansion when a large number of cells are required. A bioreactor-based system can be automated to perform the necessary medium exchanges and reduce culture variations and microbial contamination by avoiding the daily culture maintenance.

Several studies have been performed with different types of bioreactors for the expansion of suspendable stem cells. Bioreactors such as stirred tank [13–16], fixed bed and air-lift [17] as well as perfusion chamber [18,19], rotating wall vessel [20] and hollow fiber bioreactors [21] have been recently studied for suspendable stem cell expansion.

Although it is somewhat difficult to compare performance of different bioreactors for the cultivation of suspendable stem cells based on expansion rates and/or achievable cell densities, results obtained from the few studies reveal that the development of bioreactors is very important step for the suitable cultivation of suspendable stem cells. Novel systems and approaches, therefore, are still needed to offer suitable strategies to expand suspendable stem cells *in vitro* [22,23].

Stirred bioreactors which are relatively simple and readily scalable provide a homogeneous environment and are easy to operate, allowing sampling, monitoring and control of culture conditions. Suspendable stem cells do not require surface attachment for growth and may be successfully cultured in stirred bioreactors with improved performance, due to homogeneous nature of culture system. However, these cells are sensitive to shear, and agitation as well as aeration is thought to affect the cell behaviors such as proliferation and differentiation [24–26].

In this study, cell proliferation place was separated from a STB system and the stirred tank was considered as a conditioning vessel to adjust pH and DO as well as automated medium change. The PC-ESCT system which can reduce shear stress on the cells and monitor the system easily was used for expanding a U937 cell line as a model of suspendable stem cells. The U937 cell line is a human hematopoietic cell line established from a generalized histiocytic lymphoma, displaying several properties of immature monocytic cells [27,28]. The cell line has been extensively utilized as a powerful *in vitro* model for the study of suspendable stem cells.

Furthermore, the cell line does not need additional or specific growth factors for expansion, and consequently is suitable for use in the initial performance tests of bioreactors for suspendable stem cell expansion [29].

The objective of the research was to assess the performance of the PC-ESCT system in comparison with the STB system and static T-flask culture for self-renewing expansion of suspendable stem cells *in vitro*.

2. Material and methods

2.1. Cell cultures and experimental procedures

A hematopoietic cell line (U937, Invitrogen) was used to inoculate static and dynamic culture systems. Roswell Park Memorial Institute medium (RPMI 1640, Invitrogen) supplemented with 10% (v/v) fetal bovine serum (FBS, Invitrogen) and 100 U/mL antibiotics (penicillin and streptomycin obtained from Sigma-Aldrich) were used as a culture medium. Experiments were carried out at seeding densities of 10^4 and 10^5 cells/mL both in T-flasks and bioreactors for a period of 8 days.

The T-flask cultures (TPP Switzerland, 25 cm^2 surface area, 10 mL) were incubated at 37 °C under an atmosphere of 5% CO_2 (n-biotek NB-203XL), with medium exchange every 2 days after centrifuging at 1500 rpm for 10 min (Sigma 3-30k).

Stirred tank bioreactor (STB) and proliferation chamber with external stirred conditioning tank (PC-ESCT) were applied as dynamic systems. A 60 mL water-jacketed contactor was used as a STB (ID: 40 mm, H: 70 mm) which was equipped by water circulator having temperature controller (UC5000, Sahand Azar Co.), a pH controller (3611, Jenco Instrument Inc.) and a dissolved oxygen controller (M400 Mettler Toledo AG) with temperature sensor. The STB was placed on a magnetic stirrer (IKA) for the mixing at 30 rpm with 1 cm length of magnetic bar.

The inlet gas having 5% CO_2 and 95% air was provided by mixing the gases of CO_2, N_2, O_2 and air using mass flow controllers (Seven star, D07 series) and introduced into the bioreactor at an aeration rate of 0.1 vvm after passing through a humidifier. Using the dissolved oxygen (DO) controller system, the concentration of dissolved oxygen inside the STB was controlled at 5 mg/L.

Temperature was under control at 37 °C by using the water circulator having temperature controller. The culture medium was changed automatically after giving 2 h rest to the system by replacing 50% (v/v) of the medium every 24 h using a programmable logic control (PLC) unit (SUT-001) and a set of peristaltic pumps (SUT-P01). 30 μL of antifoam (PDM3263) was used in the bioreactors to avoid foam formation. pH was controlled by using CO_2 as a part of gas flow as well as the medium exchange strategy. The pH control strategy used could maintain pH at 7.5 with a slight fluctuation (±0.2).

To set up the PC-ESCT system, as illustrated in Fig. 1, cell proliferation place was separated from conditioning vessel. The proliferation chamber was a water-jacketed column with 30 mL capacity (ID: 20 mm and H: 80 mm) having DO probe. The conditioning vessel was a 30 mL water-jacketed stirred tank (ID: 30 mm, H: 50 mm) equipped with the water circulator and pH probe, connected to the proliferation chamber. The total volume of the PC-ESCT system was, therefore, 60 mL equal to the STB system. The stirred conditioning tank was placed on the magnetic stirrer for the mixing at 30 rpm and the inlet gas was introduced into the unit through a diffuser after humidification in the humidifier.

The medium was circulated between the proliferation chamber and conditioning vessel by using two peristaltic pumps (SUT-P01). A filter with 8 μm mesh size (Filter lab, Barcelona) was used in the exit of the proliferation chamber to prevent the entrance of the cells to the conditioning vessel. The medium exchange in the PC-ESCT system was carried out by replacing 50% (v/v) of the medium every 24 h in the conditioning vessel by using the PLC unit and peristaltic pumps. Temperature, pH and DO values were controlled the same as the STB system.

Prior to starting the experiments, all parts of the bioreactors were carefully washed twice with deionized water and autoclaved