REVIEW ARTICLE

Bioreactors for Hematopoietic Stem Cell and Progenitor Expansion

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ABSTRACT

Objective: **To describe the use of bioreactors for hematopoeitic stem cell and progenitor cell culture and comparing the various bioreactors to know which one is the best.**

Materials and Method: **We conducted a data search on PubMed in December 18, 2021 and January 18, 2022. We used "Bioreactor" AND "Hematopoietic stem cell" OR "progenitor" as keywords. Data collection procedure: search results were filtered and selected so that only articles, which contained data about the type of bioreactor, mechanism of bioreactor, and cell source of HSC and progenitors, were used.**

Result and Discussion: **The search yielded nine articles that were used to compose this narrative review. There are two main bioreactor classification, perfusion and stirred tank bioreactor. There are various models, which can be adapted easily according to research needs, such as microfluidic, hollow fibre, continuous perfusion and micro-cavity array bioreactors.**

Conclusion: **Comparison between those bioreactors showed that perfusion bioreactor is more advantageous and widely used for research purposes that are related to HSCs and progenitors. This fact is due to the possibility of a simpler type and more benefits than stirred types.**

KEY WORDS

bioreactor, hematopoietic stem cell, progenitor

INTRODUCTION

Hematopoietic and progenitor cell culture, which is also known as hematopoietic ex vivo expansion, is a cell culture technique that has numerous uses in gene therapy, immunotherapy, bone marrow transplantation, and blood product manufacturing. Hematopoietic cell culture is extremely complicated, with numerous cell types at various stages and stages of growth existing at any given moment and never in a constant state. Furthermore, through adhesion molecules, cytokines, and growth factors, as well as their metabolism, the cells interact strongly with each other in their environment[1].

Hematopoietic stem cells (HSC) and progenitors are self-renewing cells that can develop into eight or more different cell types. Hematopoiesis, which is the process of HSC differentiation, culminates in the ongoing creation of mature red and white blood cells. HSCs finally mature into erythrocytes, granulocytes, macrophages, megakaryocytes, and lymphocytes. HSC differentiation is controlled by cytokines that consist of cell-to-cell communication peptides, proteins, and glycoproteins, which are secreted and regulated by stromal cells. Fibroblasts, adipocytes, endothelial cells, and macrophages are stromal cells that reside in the bone marrow extracellular matrix. Stromal cells of bone marrow play a role in the secretion of extracellular matrix and cytokines that support and manage stem cell commitment, self-renewal, proliferation and differentiation. In vivo, the relation between HSCs, stromal cells, and their cytokines show the complexities of HSC development, proliferation, and differentiation. Ex vivo factors including serum intake, oxygen content, hydrodynamic shear stress, and pH also influence HSC expansion and differentiation[2].

In recent years, bioreactors have become one of the alternative

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Table 1: Bioreactor for HSCs and Progenitors[3-13)]

Reference	Type of Bioreactor	Mechanism	Cell - sources
3)	Microfluidic Perfusion	3D-KITChip inserted into bioreactor housing, a medium reservoir, a gas mixing station and cassette pump	BM-MSCs
6)	Microfluidic Perfusion	Fibronectin-coated microfluidic bioreactor compared with conventional method	UCB - CD133+
7)	Perfusion	Vascularization 3D osteoblastic bone marrow niche engineered in perfusion bioreactor	UCB - HSPCs
8)	Perfusion	Multi compartment hollow fibre membrane based 3D perfusion bioreactor	BM-MNCs
9)	Perfusion	Human osteoblastic niche engineered by cultivating mesenchymal stromal cell with 3D porous scaffolds	CD34+
10)	Perfusion	Hydroxy-apatite scaffolds were seeded directly in perfusion bioreactor	UCB CD34+
11)	Perfusion (hollow fibre)	Fibroblasts were seeded on a hollow fibre micro-bioreactor. The cells were attached on the surface of the extra-luminal membrane	BM cells
12)	Stirred	Immobilised Delta-Like 1 ligand in a stirred bioreactor	UCB - MNCs CD34+
13)	Stirred	Bioprocess based on stirred tank bioreactor with a sequencing batch aeration system for one-step clinical mass production of suspended stem cells	Cell Line U937

3D-KIT = 3 dimensions Karlsruhe Institute of Technology, BM = bone marrow, MSCs = mesenchymal stem cells, UCB = umbilical cord blood, HSPCs = hematopoietic stem progenitor cells, MNCs = mononuclear cells

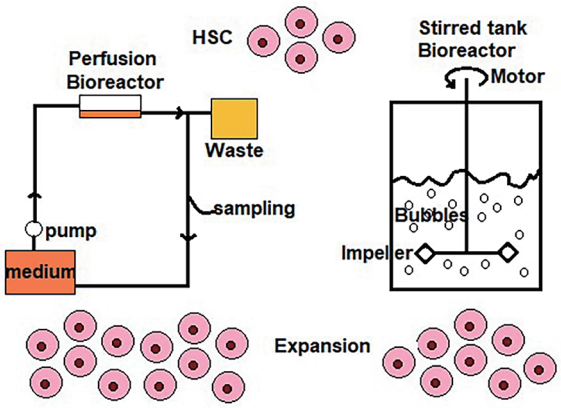

Figure 1: Perfusion and stirred tank bioreactor

media for HSC expansion. This is because the use of bioreactors has more advantages than conventional culture methods. Conventional culture methods are two-dimensional (2D) or static culture vessels, such as T-flasks and wells. The use of bioreactors is carried out to overcome problems that occur during the use of static or 2D methods. The limitations of the static method include the absence of a mixing process resulting in a high concentration gradient of oxygen, and frequent replacement of potentially contaminated culture media. Then there are obstacles in controlling and supervising the culture parameters and the last one is the limited surface area of the culture vessels. All these obstacles can be overcome by using existing bioreactors[3-5)]. Unfortunately, after expansion, mostly there are problems of unwanted cell differentiation[4)]. So, the aim for this review was to describe the use of bioreactors for HSC and progenitor cell culture and comparing the various bioreactors to know which one is the best.

MATERIALS AND METHOD

This review article described and analyzed the developments and challenges in the use of Bioreactors for HSC andprogenitor expansion in the past 5 years. For writing this review we conducted a data search on PubMed in December 18, 2021 and January 18, 2022. "Bioreactor" AND "Hematopoietic stem cell" OR "progenitor" were used as keywords for the search. Inclusion criteria were articles that were related to bioreactor that were used for expansion of HSC and/or progenitors. The exclusion criteria were non-English articles. Data collection procedure: search results were filtered and selected so that only articles, which contained data about the type of bioreactor, mechanism of bioreactor, and cell source of HSC and progenitors, were used.

RESULTS AND DISCUSION

The search yielded nine articles that were used to compose this narrative review. The type of bioreactor, mechanism of bioreactor and cell source of HSC and progenitors are listed in Table 1[3,6-13)].

Recently, umbilical cord blood hematopoietic stem cells (UCB-HSCs) and progenitors are very attractive due to their promising results in clinical hematopoietic stem and progenitor cell (HSPC) transplantation. A variety of methods can be used to increase HSC population ex vivo, particularly UCB-HSCs. In conjunction with this, HSC ex vivo expansions have been explored using cytokines and various factors that regulate cell proliferation[5)].

Previously, the most often culture technologies that are used for increasing hematopoietic cells are static culture methods, such as gas-permeable blood bags and TC-flasks. However, static cultures have several disadvantages, including (1) no mixing ability, which results in heterogeneity, lack of critical gradients for cytokines, which have high molecular weight, and low level of dissolved oxygen, (2) no control on pH and dissolved oxygen level; and (3) no frequent feeding. Therefore, these disadvantages cause static cultures to be only practical for low-density cultures that yield low number of total cells, such as stem cell production for gene therapy using stem cells, or dendritic cell production to be used as cellular vaccines. When more abundant or high cell numbers are required, such as in blood cell factories, T-cell therapy, or neutrophil precursor synthesis, then advanced bioreactors are needed. Advanced bioreactors are also necessary to culture accessory cells. For those purposes, hematopoietic bioreactor design depends on cell sensitivity to mixing and shear, as well as compatible material availability[1)].

From the review of several articles above, it turns out that bioreactors are an attractive alternative for HSC and progenitor expansion. From the various bioreactors (Table 1)[3,6-13)], the majority used the perfusion method. Moreover, an automated bioreactor can be used to operate the required exchange of medium and to avoid the labour-intensive manual daily maintenance that might cause microbial contamination and culture fluctuation.

Perfusion Bioreactors

Perfusion bioreactor is perfusion culture system based on small-scale cell culture chambers with continuous culture media change for the production of human hematopoietic cells (Figure 1). Continuous perfusion of recycled or fresh media has been proven to improve oxygen delivery to cells, which results in increased cell proliferation and

growth. The design of a perfusion bioreactor with continuous perfusion is based on this principle. Another benefit of this bioreactor is in its simplicity in vitro that does not require a stromal-cell or feeder layer. The use of highly porous collagen microspheres in a continuous-perfusion bioreactor could give increased scaffolding area for cell expansion while also allowing biochemical and physical parameters to be adjusted[4]. Perfusion bioreactors are microfluidic, continuous perfusion, hollow fibre and microcavity array perfusion.

According to a study by Moghadasi *et al*, UCB-HSCs could be expanded ex vivo in a microfluidic bioreactor. The microfluidic bioreactor was made up of micro-cavities that were coated with fibronectin to mimic bone marrow niches. In comparison to standard 2D culture, this bioreactor was employed to generate HSCs with a better capability of colony-forming, which was expected to retain more CXCR4 expression, in order to increase total expansion[6]. CXCR4 is a critical component in the capacity of these cells to home and hence to resume haematopoiesis. CXCR4 messenger RNA (mRNA) and CXCR4 surface expressions of CD133+ cells that were cultured in different systems revealed microfluidic system role in CXCR4 expression of hematopoietic cell expansion. UCB-HSC in vitro expansion, which is required for HSPC transplantation, greatly reduced CXCR4 expression to yield a small percentage of CXCR4 positive HSCs when was cultured in a microfluidic system[6].

Perfusion bioreactors with two compartments, i.e. the niche that was located in the bioreactor chamber, and a liquid phase (supernatant), which mimic the vascularized bone marrow (BM) niches were generated by Born *et al*. HSPCs could freely travel between the two compartments since they were connected, which imitate the in vivo situation in which HSPCs circulate between the circulation and BM niches[7].

A hollow fibre bioreactor's culture chamber contains thousands of hollow fibres, where each of them is perfused with culture media for cell development. Cells adhere to the hollow fibres outer wall and can take nutrients from the inner chamber, which is the space inside the hollow fibre. The metabolites from the growing cells enter the inner chamber through the wall, and then are removed via general export. Therefore, the metabolites do not harm the growing cells in the external chamber, which is the space between the hollow fibres[4].

Schmelzer *et al*. used hollow fibres with multiple compartments containing perfusion bioreactor over a 6-week period, to successfully expand human BM mononuclear cells (MNCs). In addition, they studied bioreactors that contained open-porous hydroxyapatite scaffolds, which were placed between the fibre layers to mimic trabecular bone architecture and chemistry[8].

Garcia-Garcia *et al*. revealed that cellular niches might be constructed in a 3 dimension (3D) scaffold and perfusion flow-based bioreactor system and used for ex vivo maintenance, growth, and phenotypic and functional modulation of patient-derived malignant HSPCs. The fully humanized model was used to study human leukemogenesis in the presence of tailored niche components (e.g., osteoblastic vs. stromal-vascular elements) and to assess chemotherapeutic responsiveness[9].

In a work by Bourgine *et al*., bone marrow (BM)-like tissues were successfully engineered in vitro in a perfusion bioreactor system. The niches were biologically complex, embodying structural, organizational and compositional aspects of a natural human osteoblastic environment, which allowed HSPC function to be supported[10].

Because of the rising gap between HSC supply and clinical need, scalable bioengineering solutions are needed to address the proliferation of critical hematopoietic subpopulations, the culture periods efficiency, and the necessity for minimum supplementation of cytokine. Khong et al. discovered a preliminary evidence that whole bone marrow cell (BMC) viability can be sustained for up to 48 hours in 3T3 fibroblast-seeded hollow fibre micro-reactors, while enriching for the LinSca-1 + c-Kit + (LSK) population, which provided a method for HSC manufacturing and engineering in clinical settings[11].

Wuchter *et al*. developed a novel 3D stem cell niche model system by co-culturing primary human progenitor cells (HPCs) and human BM mesenchymal stem cells (MSCs) in a perfused microcavity array to better mimic the in vivo structure of BM. They used the Karlsruhe Institute of Technology created 3D chip (3D-KITChip) system. This chip is a special microchip with micro-well chambers for 3D high-density cell culture. In the 3D-KITChip, MSCs grew in several layers, producing a cellular network where HPCs could proliferate. All 3D-KITChip studies were compared to a 2D co-culture system reference, which consisted of a single layer of MSCs on top of which HPCs grew. The result showed that after 14 and 21 days, increased expression of specific stem cell markers was seen when compared to reference 2D co-culture settings[3].

Stirred Bioreactors

The first and most common bioreactor is the bioreactor with stirred tank. The twirling of the stirrer may ensure uniform nutrients and cell distribution, which allow the cells to fully utilize these nutrients, enhance interface of gas-liquid to provide adequate oxygen for cell proliferation, and maintain stem cells in a normal metabolic condition (Figure 1)[4].

The internal structure of this device is simple, so that it can be used for both adherent and suspension cell culture. Various types of stirrers with different styles were improved, such as the Celli Gen bioreactor (New Brunswick Scientific) with Cell-lift double-screen impeller, as well as the BIOSTAT B bioreactor (Sartorius, Germany) with a bubble-free aeration system and two blade impellers. In addition, EMD Millipore and the Centre for Commercialization of Regenerative Medicine (CCRM) collaborated on the Mobius Cell Ready stirred tank bioreactor to optimize stem cell manufacturing system[4].

Various stem cells (SCs), including adherent and suspension SCs, could be expanded in stirring bioreactors. Suspension SCs, like HPCs, were successfully cultivated in stirring bioreactors without the need for surface attachment. Adherent SCs, such as MSCs, iPSCs, and ESCs, might be cultivated in stirring bioreactors after being attached to micro-carriers for suspension. Because of the homogeneous nature of the culture system, the growth efficiency and homogeneity of cultivated SCs in stirring bioreactors were increased. SCs, on the other hand, are sensitive to shear stress, aeration, and agitation that might have an impact on cell behaviours like proliferation and differentiation[4].

Although stirring bioreactors provide a homogenous environment and are easy to use for scaling-up, which allow for monitoring, sampling, and culture parameters control, their force of shear stress may cause injury and non-specific differentiation in the SC growth process[4].

Moore *et.al* has provided a method for immobilizing the Notch ligand DL1 on the surface of magnetic microparticles. Notch ligand DL1 is one of the various factors that boosts the progenitor cells for quick engraftment and establishes a population with long-term repopulating capacities. It plays a role in Notch pathway, which was demonstrated to modulate stem cell differentiation, as well as proliferation and commitment of HSCs and HPCs. Binding of membrane-bound ligands and their trans-membrane receptors, such as Delta like (DL1, DL3, DL4) group, mediate Notch signalling. In comparison to the control group, culture of HPCs in the presence of DL1 that was immobilized on magnetic micro-particles (iDL1) resulted in a 100-fold increase in CD34+ cells. These cells have excellent repopulation capacity in sub-lethal irradiated mice, which showed considerably higher and faster levels of lymphoid and myeloid cell engraftment. Scalable stirred culture platforms as well as static culture can both be used to adjust the DL1 surface concentration, which can be detached using simple magnetic methods[12].

Kaleybar *et al*. developed a stirred tank bioreactor in combination with aeration in consecutive batches, which was used for suspension culture of U937 stem cells. The result showed that logarithmic cell growth could be sustained for up to 168 hours that yielded a 30 fold expansions, which is essential for transplantation[13].

Comparison of various bioreactors

The stirred-tank bioreactor solves the static culture system's many shortcomings, including heterogeneity, concentration gradients, and intensive handling that could lead to inability to properly take samples, monitor and control culture conditions as well as increased risk of contamination. HSC development and differentiation are supported by this platform that provides homogeneous environment. However, stirring the culture causes shear stress, which might disrupt the HSCs' surface-marker expression and thus have negative impacts on differentiation and growth because HSCs are shear-sensitive. Because of these negative impacts, stirred-tank bioreactors produce less cell expansion than other types of bioreactors[14].

From the above review, it shows that the perfusion method bioreactor is more advantageous and widely used. Various models such as microfluidic, hollow fibre, continuous perfusion and microcavity array show that this method can be adapted easily according to research needs.

CONCLUSION

The perfusion bioreactor is currently the most widely used type of bioreactor for research purposes that are related to HSCs and progenitors. This fact is due to the possibility of a simpler type and more benefits than stirred types.

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