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Review

Ex vivo expansion of hematopoietic stem cells in bioreactors

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Abstract

The *in vitro* culture of human hematopoietic stem cells in different types of bioreactors is reviewed. After a brief description of the hematopoiesis and stem cells biology, the selection of growth medium for stem cells is evaluated as well as the parameters to be controlled for *ex vivo* expansion and differentiation of stem cells. Several types of bioreactors for *in vitro* stem cell culture are discussed and compared.

Introduction

Stem cells are considered as having both the capacity for unlimited or prolonged self-renewal and the ability to differentiate into highly distinctive cell lineages (Watt & Hogan 2000). Usually, between the stem cell and its terminally differentiated progeny there are intermediate populations of committed progenitors with a limited capacity for proliferation and a restricted differentiation potential. This is true for the hematopoietic system as well as for other existing tissue-specific stem cells in other organs. Tissue resident stem cells are found in many tissues, such as muscle, cartilage, bone, the nervous system and, probably the liver and pancreas. Recently, new insights in adult stem cell biology showing that cellular differentiation and lineage commitment are not irreversible processes has opened new fields in stem cell research.

Hematopoietic stem cell biology research has been directed towards the identification of cell populations that possess stem cell characteristics and the study of mechanisms that regulate stem cell self-renewal and differentiation (Heath 2000).

Because stem cells are rare, for example hematopoietic stem cells (HSC) account for about 1 per 10 000–100 000 nucleated marrow cells, one of the major focuses in experimental hematology is the *in vitro* manipulation of HSC with the ultimate goal of expanding long-term transplantable HSC. The delineation of strategies that allowed the successful *ex vivo* expansion of HSC would not only have a profound impact on the way HSC transplantation is thought out, but would also expand the limits of tumor cell purging and somatic cell gene therapy (Heath 2000).

Several studies have been performed to achieve *ex vivo* expansion of hematopoietic stem cells (HSC), thus demonstrating the feasibility of *in vitro* culture of these cells in the presence or absence of stroma (Emerson 1996).

Hematopoiesis and stem cells

The turnover of cells in the hematopoietic system in a man weighing 70 kg may be close to 10^{12} cells per day. This remarkable cell renewal process is supported by a small population of extremely rare cells found in the bone marrow and circulation of adults. These rare cells are the hematopoietic stem cells, or HSC. These cells comprise less than 0.1% of the total bone marrow cells. In general, the term HSC is used to refer to cells which are capable of long-term reconstitution of the hematopoietic system of a recipient animal (or human). These long-term repopulating cells have several characteristics that distinguish them from other classes of hematopoietic cells. Clearly, these cells

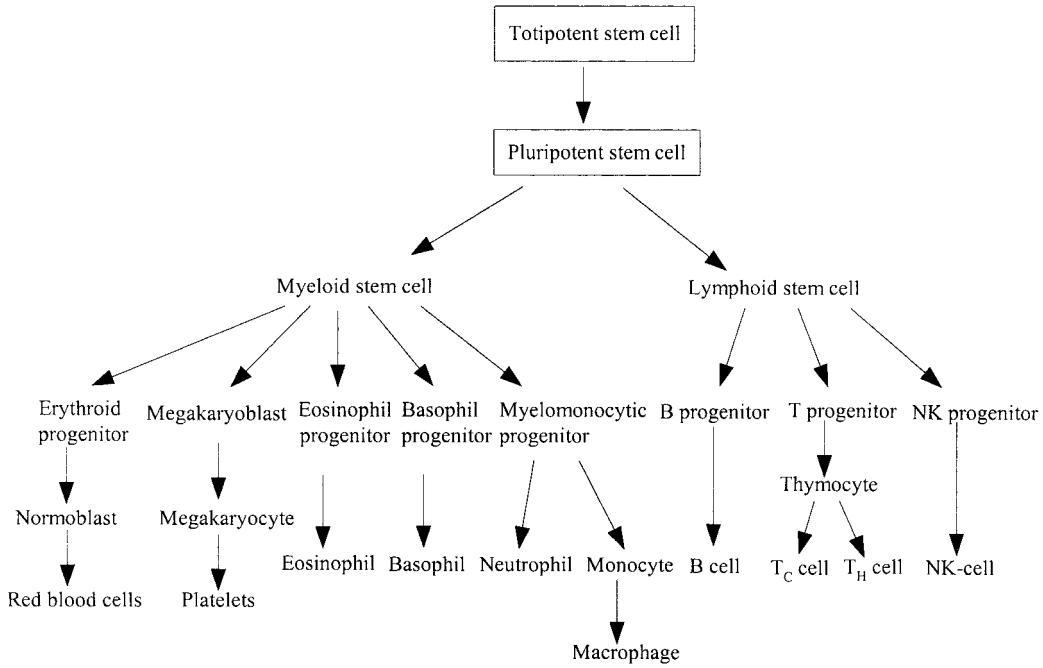

Fig. 1. The hematopoieitic system hierarchy.

must be capable of self-renewal in order to successfully reconstitute hematopoiesis in a recipient animal and provide a lifelong supply of blood cells without exhaustion. Additionally, it is generally held that, in the steady state, the majority of HSC are dormant in the G_0 phase of the cell cycle, and only a few actively cycling HSC supply all of the hematopoietic cells at a given time. To supply these cells, HSC must also be multipotent, i.e., capable of differentiating into all of the blood cell lineages (Figure 1). To accomplish this, HSC, in the presence of the appropriate growth factors, begin by first differentiating into multipotent progenitor cells. These cells are distinct from HSC, because they no longer possess the ability to self-renew. From these early progenitors, differentiation proceeds, again in the presence of the appropriate growth factors, to yield lineage-committed progenitor cells, which, as their name suggests, are capable of giving rise to blood cells of a specific lineage. There are classes of lineage-committed lymphoid progenitors in the marrow that eventually give rise to all types of T and B and non-T and non-B lymphocytes involved in the immune defense. Likewise, there are erythroid progenitors that give rise to mature red blood cells and myeloid progenitors that ultimately generate neutrophils, eosinophils, basophils, mast cells, monocytes and platelets. The processes whereby stem cells self-renew and commit in different lineages, with the resultant progenitors giving rise to all of the differentiated mature blood cells that enter the circulation are collectively referred to as hematopoiesis.

The regulation of HSC self-renewal/multilineage differentiation is dependent upon intimate contact with the bone marrow microenvironment that is comprised of the so-called stromal cells (macrophages, adipocytes, endothelial cells and fibroblasts) and components of the extracellular matrix. The proliferation and maturation of both the highly primitive HSC and the more mature lineage committed bi- or uni-potent progenitor cells are influenced by a myriad of growth factors expressed on the surface of and/or secreted by stromal cells (Emerson 1996). At present all the factors required for hematopoiesis are unknown; stem cell factor (SCF), interleukin-3 (IL-3), granulocyte-macrophage colony-stimulating factor (GM-CSF) and thrombopoietin are known to be required for the maintenance/expansion of fairly primitive stem/progenitor cells, while other factors, like granulocyte colony-stimulating factor (G-CSF), macrophage colony-stimulating factor (M-CSF) and erythropoietin, act primarily on the more committed progenitors and their mature cellular progeny. In each case, it is presumed that these growth factors mediate their effects on survival, proliferation, and

differentiation by binding to specific receptors on the cell membrane of the target cells and activating the corresponding second messenger pathways.

The sources of hematopoietic stem cells for cellular therapy include bone marrow, peripheral blood and umbilical cord blood from the patient or from human leukocyte antigen (HLA)-compatible donors. HSC and progenitors are found at only low concentrations in the peripheral blood unless hematopoietic cytokines are administered to facilitate mobilization of the HSC from the marrow into the circulation. Umbilical cord blood represents an attractive alternative to bone marrow as a source of HSC because its collection is not invasive to either mother or child and it is rich in primitive stem and progenitor cells. However, due to the small volume that can be collected, the total cell number obtained is fairly small. As a result, the clinical uses of cord blood stem cells are currently limited to the treatment of infants and small children whose small body mass enables an adequate dose of cord blood-derived HSC to be administered. The future clinical potential for cord blood HSC is enormous if methods for successfully expanding HSC *ex vivo* without a loss of their engraftment ability could be developed. The widespread use of cord blood as a source of HSC for transplantation would clearly require that a large number of cord blood banks are established.

The source of hematopoietic cells is typically dictated by the graft setting under consideration (Shimizu *et al.* 1998, Zanjani *et al.* 1997, 1998). Stem cells and primitive progenitors are ultimately responsible for expansion. Accessory cells, however, play a role in conditioning the environment in which expansion takes place. In its natural environment, hematopoiesis resides in a well-defined microenvironment characterized by local geometry, by stromal cells (accessory cells of mixed origin) and by an extracellular matrix (ECM) composed of collagen-like molecules and proteoglycans.

Several studies have been performed to evaluate whether accessory cells in peripheral blood and umbilical cord blood can replace the active surface function of stromal cells and the ECM (Emerson 1996). Other studies have indicated that very high cytokine concentrations can facilitate protection and expansion of stem cells. Higher production of committed primitive progenitors was achieved by adding four cytokines, whereas addition of more cytokines was detrimental. These studies show that it may be possible to achieve stem cell retention and expansion in the absence of accessory cells and possibly even simultaneously induce differentiation of more committed progenitors present within the culture.

Regardless of the source, the HSC content of the initial hematopoietic product, be it from bone marrow, peripheral blood or cord blood collection, can be enriched by selecting for cells expressing the surface antigen CD34, which is found on many of the primitive HSC. The typical $CD34^+$ cell content of various sources is 1.7% in bone marrow, 0.15% in peripheral blood and 0.8% in cord blood (Nielsen 1999). However, it should be noted that CD34 expression alone is not indicative of HSC. Rather, combinations of antigens must be employed to accurately assess the HSC content of a hematopoietic graft. For example, despite the lower concentration of $CD34^+$ cells in umbilical cord blood compared to adult bone marrow, the cord blood still represents a better theoretical source of HSC, since a much higher percentage of the $CD34^+$ cells contained within the cord blood are also $CD38^-$, and the combined phenotype of $CD34^+CD38^-$ has proven to be a much better indicator of repopulating HSC content.

Selection of growth medium for stem cells

Ex vivo expansion initiates with a population widely distributed in terms of degree of differentiation. Depending on position in the differentiation pathway, each cell can respond to the expansion environment in different ways by: moving from quiescence into active cycle or reverse; going through the division cycle; entering apoptosis; and/or differentiating. Knowing what process is being affected and in what cells is important, yet most results are presented as merely increased or decreased expansion of a given subset of cells at the end of the culture. To tackle the complex kinetics of what processes are occurring and in which cell populations, it is necessary to obtain reliable data and to use advanced balance models.

The transient nature of most of subpopulations of interest is also another difficulty to correctly model the *ex vivo* expansion of these cells. They form and disappear as cells move through their position in the differentiation pathway. Depending on the culture conditions, the total number may be increasing, at peak, or decreasing at any given point. Many studies only compare the effect of culture parameters on expansion potential by comparing cultures at a single point, typically after 10–14 days. Experiments should be

carried out to compare the daily contents of desired precursors.

Traditional media for hematopoietic culture contains about 25% (v/v) horse or fetal calf serum (Nielsen 1999), however serum-free alternatives are available (Lebowski *et al.* 1995, Koller *et al.* 1998a, Shimizu *et al.* 1998). Serum-free media promote greater expansion of the erythroid and megakaryocytic lineages, primarily because serum contains the transforming growth factor-β. In contrast, serum-containing media are superior for expansion of the granulocyte and monocyte lineages.

The ability of particular growth factor supplements to stimulate an expansion of primitive hematopoietic cell populations *in vitro* has been extensively investigated. A summary of these studies is given by Zandstra *et al.* (1999).

The hematopoietic process is regulated, in part, by growth factors that influence the viability, proliferation, maturation, and function of cells at various stages of differentiation. In the absence of exogenous growth factors, CD34-enriched cells rapidly lose viability *in vitro*. A number of studies have shown that many growth factors must be added to CD34-enriched cell cultures at relatively high concentrations to achieve maximal proliferation. In contrast, perfused bone marrow mononucleated cell cultures support a high-density stromal and accessory cell population that endogenously produces a significant number of growth factors. Consequently, perfused bone marrow mononucleated cell cultures can be supported for at least 19 weeks in the absence of exogenous growth factors (Mandalam *et al.* 1999). However, the production of the greatest cell number within a clinically relevant time frame requires the addition of some exogenous growth factors at relatively low concentration.

The selection of optimal culture parameters for *ex vivo* expansion of stem cells is a major challenge (Nielsen 1999). Very few studies have thus far provided adequate information to assess all of the culture parameters (Collins *et al.* 1997, 1998a, Koller *et al.* 1995a, Zandstra *et al.* 1997). This is mainly due to the complex kinetics, the transient nature of stem cells and the use of assays that are primarily qualitative in nature, and as such, do not provide a measure of an invariant quantity and do not allow dissection of the complex interaction between parameters.

Unless controlled, hematopoietic cultures may experience pH variations of up to 0.5 pH unit (Nielsen 1999). Recent studies indicate that myeloid differentiation occurs optimally in the range of pH 7.2–7.4, with severe inhibition outside this range. The effect of pH can be very complex, as indicated by studies on a human promyelocytic leukaemia cell line showing a concomitant increase in metabolic rates for glucose consumption and lactate production with altered pH.

Dissolved O_2 tension is another parameter that varies substantially over the span of a culture (Nielsen 1999, Mandalam *et al.* 1999). The appropriate concentration of O_2 is a function of cell density, cell type, O_2 consumption rate and culture depth. Extreme levels (both high and low) of O_2 can be inhibitory to cell growth. In bone marrow, hematopoietic cells experience O_2 tension corresponding to 5% dissolved O_2 (DO) in culture. Initial studies indicated that low DO enhanced production of progenitors cells. However the enhanced expansion of erythroid progenitors could be mimicked by inclusion of erythropoietin. In general, low DO could stimulate complex intracellular interactions. These studies are limited however, as they are based on observations at only a few time points and the experiments controlled DO in the gas phase rather than in the liquid phase, making it impossible to assess what the true DO was. Therefore, to correctly address the effect of oxygen it is necessary to perform *ex vivo* expansion experiments in fully controlled bioreactors for optimizing the design and performance of hematopoietic bioreactors. Mathematical models are also needed to investigate issues associated with hematopoietic bioreactor design, including initial cell density, medium depth, reactor configuration and O_2 partial pressure.

Stem cell bioreactor design and operation

A major difficulty for the *ex vivo* expansion of primary human hematopoietic cells, stem cells as well as more differentiated cells is that donor derived cell samples consist of only small numbers of cells despite the fact that large amounts of cells are needed for cell therapies. Therefore it is very important to develop adequate culture systems in an effort to obtain clinically relevant cell numbers from a small initial patient sample.

Bone marrow cells have traditionally been cultured on the flat, two-dimensional surface of tissue culture dishes. Dexter *et al.* (1977) were the first to develop a long-term murine bone marrow culture system, which primarily produced granulocytes. During the first 7–14 days, an adherent layer of cells (stromal cells) grows

to confluence. After the establishment of the stromal layer, progenitor cells begin to expand forming cell colonies within 14–21 days.

Static culture systems such as well-plates, T-flasks or gas-permeable blood bags are the most widely used options for expanding hematopoietic cells (Collins *et al.* 1998b). However, despite their widespread usage, static systems have serious limitations leading namely to concentration gradients (pH, dissolved O_2, metabolites, etc.) in the culture medium. Additionally, well-plate and T-flask environmental conditions are not readily monitored or controlled on-line. These systems also require repeated handling in order to feed cultures or obtain data on culture performance and are limited in their productivity by the number of cells that can be supported by a given surface area.

The use of bioreactor systems is an alternative approach to standard flasks cultures of *in vitro* tissue. Advanced bioreactors are required when: (i) a large number of cells are required; (ii) acessory cells are used; or (iii) high cell densities are desired (Nielsen 1999). Furthermore, a bioreactor based system can be automated to perform the necessary medium exchange and avoid a daily culture maintenance which is labor intensive and susceptible to culture variation and to microbial contamination.

Perfusion chambers

Palsson and collaborators (Koller & Palsson 1999, Koller *et al.* 1993, 1995a,b, 1998a,b, Oh *et al.* 1994, Palsson *et al.* 1993, Peng & Palsson 1996) have developed a perfusion culture system based on small-scale cell culture chambers for the expansion of human hematopoietic cells (Figure 2). These chambers were inoculated with bone marrow mononucleated cells (e.g., 3×10^7 cells) and were thereafter maintained at 37 °C. After 24 h without perfusion to facilitate stromal layer attachment, medium flow was initiated, typical values being 22.5 ml d^{-1}. The 20- to 25-fold *ex vivo* expansion of a population of unselected human mononuclear bone marrow cells over a two-week period was obtained (Palsson *et al.* 1993). A stromal layer developed from this inoculum and thus no preformed stroma was required. Colony-forming units of granulocyte-macrophage (CFU-GM) progenitor cells expanded 10- to 30-fold. This expansion was dependent on the gas phase oxygen concentration (optimal results being obtained with 20% v/v), the seeding density and time of cell harvest. The seeding density mainly influenced the expansion of CFU-GM cells. The CFU-GM progenitor cell density increased with time and reached a maximum at day 10.

Koller *et al.* (1993) reported the expansion of human stem and progenitor cells from bone marrow mononuclear cell populations. Bioreactors containing three cell populations were analysed separately: non-adherent cells; cells that were loosely adherent to the endogenously formed stromal layer; and an adherent cell layer that required trypsinization for removal. Total cell numbers increased continuously up to 10-fold by day 14. The adherent stromal layer significantly expanded but remained less than 6% of the total cell population. CFU-GM cells expanded 21-fold by day 14, whereas burst-forming unit-erythroid (BFU-E) cells peaked earlier with a 12-fold expansion on day 8. In contrast to CFU-GM, which were predominantly non-adherent, BFU-E were more evenly distributed between the three cell populations. Importantly, a 7.5-fold expansion of the long-term initiating cell (LTC-IC) was obtained. More than 3 billion cells, containing 12 million CFU-GM were reproducibly generated from the equivalent of a 10 to 15 ml bone marrow aspirate.

The factors that constrain the expansion of cell numbers in perfusion bioreactors are not known at present. Growth factor or O_2 delivery could be limited. Another possible constraint of the perfusion-based bioreactor systems is the size of the growth surface area required. This constraint can be partially overcome by harvesting half the cell population periodically (e.g. every 3 to 4 days, beginning on day 11 of culture) (Oh *et al.* 1994).

This type of bioreactor has been also used for expansion of CD34-enriched cells and compared to the expansion of unprocessed whole bone marrow cells (Koller *et al.* 1995b). After 14 days, an average 13-fold expansion, 12-fold, 10-fold and 1300-fold were obtained from bioreactors inoculated with whole unprocessed bone marrow cells, bone marrow mononuclear cells, CD34-depleted, and CD34-enriched cells on stroma, respectively. These cultures also expanded CFU-GM numbers by 28-fold, 14-fold, 13-fold and 41-fold respectively. The unprocessed whole bone marrow cells generated 3.76×10^6 CFU-GM per ml bone marrow aspirate, whereas mononuclear cells resulted in 1.42×10^6 CFU-GM. CD34-enriched cells gave 7×10^5 CFU-GM per ml bone marrow aspirate, whereas CD34-depleted cells generated 4.97×10^5 CFU-GM. Unprocesssed whole bone marrow cell cultures had high concentrations of endogenous epidermal growth factor (EGF) and platelet-derived growth

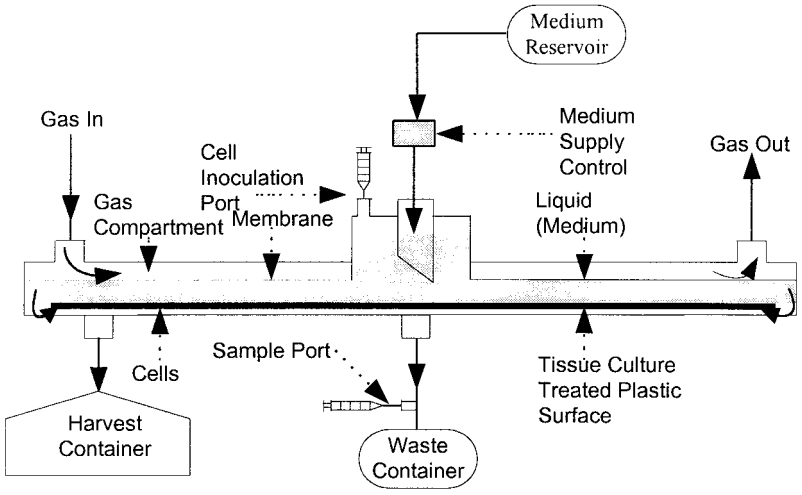

Fig. 2. Schematic diagram of the Aastrom perfusion bioreactor (adapted from Mandalam *et al.* 1999).

factor (PDGF) production, which may have been responsible for the more extensive stromal development observed.

Perfusion bioreactors were used to analyse the consumption and production of growth factors and their relation with specific cell production (Koller *et al.* 1995a). The exogenously added interleukin-3 (IL-3), granulocyte-macrophage colony-stimulating factor (GM-CSF), stem cell factor (SCF) and erythropoetin (Epo) each exhibited different consumption kinetics. Epo and IL-3 were consumed slowly for the first 5–7 days, and then the consumption rate of both increased. Epo consumption reached a plateau by day 10, whereas IL-3 consumption continued to increase. Consumption of SCF was similar to that of Epo but began 2–3 days earlier. GM-CSF was consumed throughout the culture period in an accelerating manner. Consumption of SCF and Epo were related, as omission of Epo from the growth medium reduced SCF consumption by 53% and omission of SCF reduced Epo consumption by 82%. Measurement of growth factor consumption rates may be useful for quantifying the types of cells present in culture. The results of this work suggest that perfusion supports accessory and hematopoietic elements which interact and therefore represent a partially functional tissue *ex vivo*. This system provides a useful model for studying relationships within growth factor networks and for elucidating approaches that result in primitive cell expansion *ex vivo*.

Single pass perfusion systems composed of parallel plates have proven to be an appropriate configuration for growth of human tissues *ex vivo* for cellular therapy and tissue engineering. Peng & Palsson (1996) showed that bioreactor geometry affect cell growth and differentiation. Four geometries (slab, gondola, diamond and radial shapes) for the parallel-plate bioreactor were analysed. Among these geometries the radial-flow type bioreactor provided the most uniform environment in which parenchymal cells could grow and differentiate *ex vivo* due to the absence of walls that are parallel to the flow paths creating slow flowing regions.

Koller *et al.* (1998b) showed that tissue culture surface chemistry and texture influence human bone marrow mononuclear and CD34-enriched cell cultures. T flasks modified for perfusion culture were used in this study. Tissue culture treated plastic significantly increased mononuclear cell culture output as compared with non-treated plastic.

A clinical pilot study was conducted in women with a poor prognosis of breast cancer (Chabannon *et al.* 1999) with the goal of evaluating the feasibility and safety of producing hematopoietic progenitors and cells from a small bone marrow sample, for clinical use after high-dose cyclophosphomide. Cells were cryopreserved and later thawed to inoculate perfused bioreactors leading to *ex vivo* expanded cells which were reinfused after the second chemotherapy cycle. Patients recovered neutrophils and platelets at similar times after the first and second chemotherapy cycles, and showed comparable clinical events.

Continuous perfusion cultures were used in an *ex vivo* expansion of stem/progenitor cell populations

from umbilical cord blood and mobilized peripheral blood (Van Zant *et al.* 1994). Under the best conditions tested with mobilized peripheral blodd (MPB), total cell numbers, CFU-GM, and long-term culture-initiating cell (LTC-IC) populations were expanded by about 50-, 80- and 20-fold, respectively over 14 days. At low cell inoculum (1 million), the presence of stroma enhanced the expansion of total cells and CFU-GM but not of LTC-IC. When stem cell factor (SCF) was not included in the medium, both total cells and CFU-GM expanded to a much lesser extent, but the expansion of LTC-IC was not affected. At the higher cell inoculum (10 million), expansions of total cells and CFU-GM were equivalent with or without stroma. With UCB an average 40- to 60-fold expansion of CFU-GM was obtained. In the absence of SCF, cell expansion averaged 1.5- to 2-fold, and CFU-GM were expanded only 10- to 14-fold by day 14. As before, the presence of preformed stroma did not affect either total cell or CFU-GM yields, provided the inoculum was at least 4.5 million cells. In summary, small, easily obtainable amounts of UCB and MPB cells are sufficient for expansion into adult-sized grafts using perfusion bioreactors. These results also corroborate the previous results with bone marrow cell expansion obtained by the same group, which showed that a single marrow aspirate of <10 ml is sufficient for expansion into a full-sized graft (Koller *et al.* 1993).

Perfusion and static cultures of peripheral blood and marrow mononuclear cells were supplemented with IL-3, IL-6, granulocyte colony-stimulating factor (G-CSF) and SCF and compared with or without a preformed irradiated allogeneic bone marrow stromal layer (Sandstrom *et al.* 1996). Stroma-containing and stroma free perfusion cultures were conducted at 37 °C in three parallel polycarbonate culture chambers, each loading 15 ml of culture medium under flow conditions, which were each perfused at 2.5 ml min^{-1} with medium equilibrated with ambient O_2 and with the pH controlled at 7.35. There is no gas phase in the culture chambers. For stroma-free cultures a culture chamber was used with a modified bottom surface with grooves perpendicular to the direction of flow. Perfusion enhanced CFU-GM expansion and LTC-IC maintenance more for the stroma-free cultures than for stroma-containing cultures.

Horner *et al.* (1998) studied the transport of O_2 and lactate in a flat-bed perfusion chamber modified to retain cells through the addition of grooves, perpendicular to the direction of flow, at the chamber bottom (Figure 3). Comparison of grooved vs non-grooved

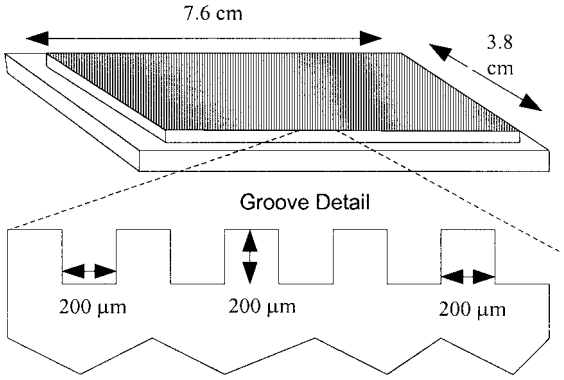

Fig. 3. Schematic diagram of a perfusion grooved culture chamber (adapted from Horner *et al.* 1998).

chambers revealed that the presence of grooves only affected solute transport on a local scale. This type of bioreactor has been used for the *ex vivo* expansion and maintenance of CFU-GM and LTC-IC using the cytokines IL-3, IL-6, G-CSF and SCF in the absence of stroma.

Stirred reactors

Stirred bioreactors, which are well characterized for the culture of both microbial and animal cells, have been used to overcome the limitations of static culture systems. This type of bioreactors (Figure 4) provides a homogeneous environment and is easy to operate allowing sampling, data collection and control of medium conditions.

Zandstra *et al.* (1994) investigated the potential of stirred suspension cultures to support hematopoiesis from starting inocula of human bone marrow cells. Their studies showed that the short term maintenance of both colony-forming cell (CFC) numbers and their precursors, detected as LTC-IC, could be achieved in both stirred suspension and static cultures. Supplementation of the medium with 10 ng SCF ml^{-1} and 2 ng IL-3 ml^{-1} resulted in a significant expansion of LTC-IC, CFC and total cell numbers in stirred cultures. After 4 weeks the number of LTC-IC and CFC present in stirred cultures initiated with the highest starting cell concentration (1 million cells) reflected average increases of 7- and 22-fold, respectively. Stirred suspension cultures offer the combined advantages of homogeneity and lack of dependence on the formation and maintenance of an adherent cell layer.

In another study, Zandstra *et al.* (1997) studied the parameters that may limit the cytokine-mediated

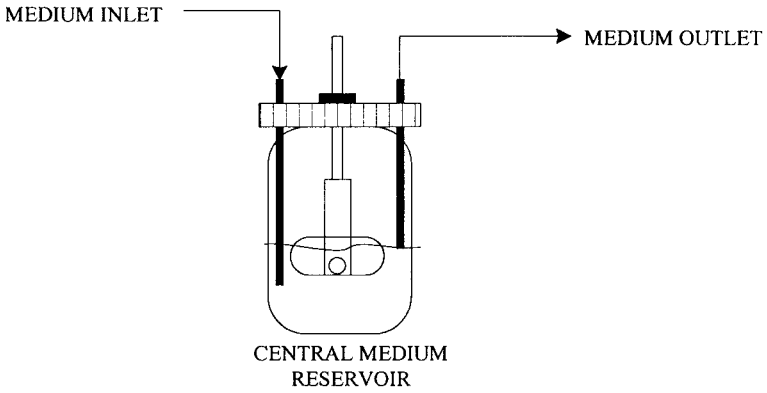

Fig. 4. Schematic diagram of a stirred bioreactor system.

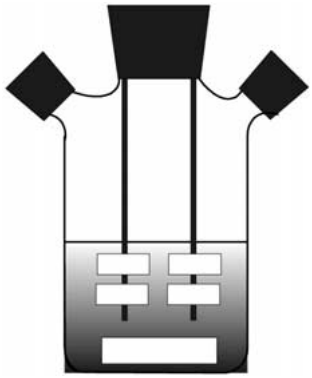

Fig. 5. Schematic diagram of a spinner flask.

expansion of primitive hematopoietic cells in stirred suspension cultures. Measurements of the rate and extent of progenitor expansion and cytokine depletion from the medium were made for cultures in which the cells were exposed to different cytokine concentrations. Supplementation of the media with IL-3, IL-6 and IL-11 at 2 ng ml^{-1} plus Flt-3 ligand and SCF at 10 ng ml^{-1} allow a 45-fold expansion of CFC numbers within 2 weeks along with a 2.5-fold expansion of their precursors, detectable as LTC-IC. Additional experiments with highly purified marrow cell fractions showed that the rate of cytokine depletion varied according to the type of responding cell as well as the specific cytokine. $CD34^+CD38^-$ cells exhibited the highest average cell-specific cytokine depletion rates (35-fold higher than unseparated bone marrow cells). This study suggests that cytokine depletion may provide a feedback control mechanism *in vivo*, which would contribute to the control of primitive hematopoietic cell proliferation and differentiation.

Collins *et al.* (1997) reported a kinetic study in which glucose and lactate metabolic rates were evaluated for cultures of umbilical cord blood mononuclear cell (MNC), peripheral blood MNC, and peripheral blood $CD34^+$ cell cultures in spinner flasks (Figure 5) and in T-flasks in both serum-containing and serum-free media. Specific glucose uptake rates and lactate generation rates correlated with the percentage of CFC present in culture. A two population model was developed to describe lactate production which could be used to predict the harvest time that corresponded to the maximum number of CFC in culture.

The proliferation and differentiation characteristics of peripheral blood MNC, umbilical cord blood MNC and peripheral blood $CD34^+$ were also examined in spinner flasks and (control) T-flasks cultures by the same authors (Collins *et al.* 1998b). Culture proliferation in spinner flasks was dependent on both agitator design and agitation rate as well as on the establishment of critical inoculum densities in both serum-containing (2×10^5 MNC ml^{-1}) and serum-free (3×10^5 MNC ml^{-1}) media.

Collins *et al.* (1998a) describe the characterization of hematopoietic cell expansion of umbilical cord blood and peripheral blood MNC, oxygen uptake and glycolysis in a controlled, stirred-tank bioreactor system. Expansion of total cells and CFU-GM was greatly enhanced by the use of a cell-dilution feeding protocol. The specific oxygen consumption rate (qO_2) for these cultures ranged from 1.7×10^{-8} to 1.2×10^{-7} μmol $cell^{-1}$ h^{-1}. The maximum in qO_2 for each culture closely corresponded with the maximum percentage of progenitor or CFC present in the culture. Examination of the ratio of lactate production to oxygen consumption in these cultures suggests that post-progenitor cells of the granulomonocytic lineage

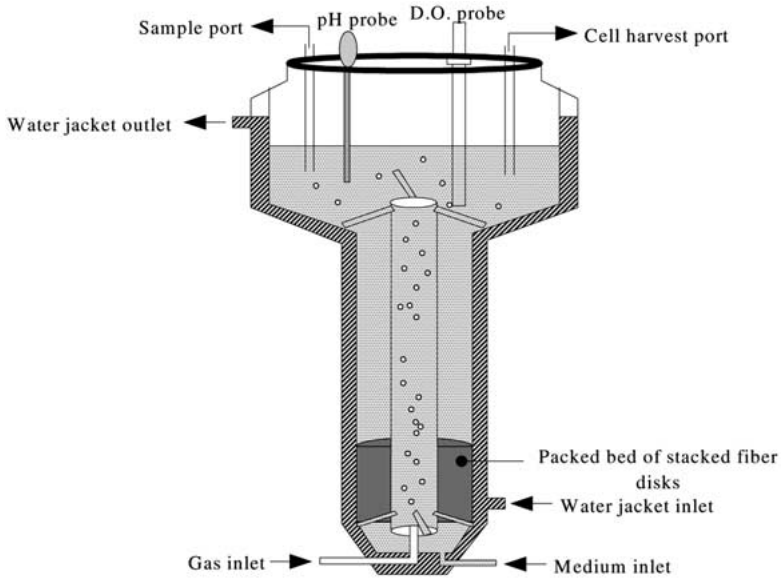

Fig. 6. Schematic diagram of an airlift packed bed bioreactor (adapted from Highfill *et al.* 1996).

obtain a greater portion of their energy from glycolysis than do CFCs. The different metabolic profiles of CFC and more mature cells suggest that monitoring the uptake or production of oxygen, lactate and other metabolites may predict the content of several cell types in culture.

Stirred suspension bioreactors have also been used for the expansion of neural stem cells (Kallos *et al.* 1999). The cells were grown for over 35 days in suspension with an overall expansion ratio of over 10^7 with no decrease in growth rate, maximum cell density or viability. The cells also remained karyo-typically normal through 25 doublings and retained their ability to be differentiated into all major cell types of the central nervous system – neurons, astrocytes and oligodendrocytes.

Hollow fiber reactors

Cell culture systems used to evaluate the scale-up of marrow cultures included suspension, microcarrier, airlift and hollow fiber bioreactors (Sardinini & Wu 1993). Using identical media, cytokines and feed schedules, MNC in the suspension bioreactor expanded to a value of 1.6 compared to a normalized value of 1.0 for static cultures. Expansion results for microcarrier culture averaged 0.75 when compared to static cultures. A cell number increase in the airlift bioreactor resulted in an expansion which was 0.70 of the control static culture. The experiment in the hollow fiber system demonstrated no observable expansion of hematopoietic cells when compared to control static cultures.

Packed bed reactors

The results of a comparative study of large-scale culture of human bone marrow using medium supplemented with IL-3, GM-CSF and SCF in spinner flasks, hollow fiber cartridges and airlift packed bed bioreactors showed that suspension cultures in spinner flasks were the most productive system (Sardinini & Wu 1993). However, no cell-cell or cell-matrix interactions are permitted in suspension systems, and the fluid dynamics are not characteristic of the *in vivo* environment. A three-dimensional system for the culture of murine bone marrow cells was described, but this was confined to a very small volume. Thus attempts to scale up bone marrow cultures within a three-dimensional growth environment have been very limited.

Large-scale cultivation of murine bone marrow was accomplished in an airlift packed bed bioreactor system (Figure 6) designed to mimic the *in vivo* bone marrow environment (Highfill *et al.* 1996). The attachment-dependent stromal cell population was grown on the fiber glass matrix packed in the annular region of the bioreactor. Once the stromal cell layer was established, fresh bone marrow cells were inoculated to initiate hematopoiesis. A 500 ml perfusion

culture experiment resulted in the production and harvest of 3.6×10^8 suspended bone marrow cells over the course of 11 weeks.

Mantalaris *et al.* (1998) developed a three-dimensional bone marrow culture system in which marrow cells were cultured in a bioreactor packed with porous microspheres. *Ex vivo* human erythropoiesis was studied in this three-dimensional culture system. The system sustained extensive erythropoiesis at low concentrations of Epo plus SCF, IL-3, GM-CSF and insulin-like growth factor-I. Erythroid cell production lasted for more than 5 weeks and the percentage of erythroid cells in the nonadherent cell population was approximately 60%. When compared to the three-dimensional culture, the traditional flask cultures failed to support extensive erythropoiesis under the same conditions.

Recently, by co-culturing $CD34^+$ cells from umbilical cord blood with an irradiated stromal cell line a stable *ex vivo* hematopoietic system was established, demonstrated by a 114-fold expansion of the hematopoietic progenitor cell CFU-GM and a 6-fold expansion of the even more immature cobblestone-area-forming cells (Jelinek *et al.* 2000).

Conclusions

The results obtained indicate that the development of bioreactors is very important for the suitable cultivation of hematopoietic cells. Novel systems and designs are needed to offer a broad spectrum of possibilities for different culture strategies and the cultivation of various cell types – from stem cells to differentiated cells for gene, cellular and tissue therapies.

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