Cytotherapy, 2013; 15: 416–422

International Society for Cellular Therapy
ISCT®

PROTOCOLS IN CYTOTHERAPY

Manufacturing mesenchymal stromal cells for phase I clinical trials

PATRICK J. HANLEY[1], ZHUYONG MEI[1], MARIA DA GRACA CABREIRA-HANSEN[2], MARIOLA KLIS[1], WEI LI[1], YALI ZHAO[1], APRIL G. DURETT[1], XINGWU ZHENG[3], YONGPING WANG[3,4], ADRIAN P. GEE[1] & EDWIN M. HORWITZ[3,4]

[1]*Center for Cell and Gene Therapy, Baylor College of Medicine, Texas Children's Hospital and The Methodist Hospital, Houston, Texas, USA,* [2]*The Texas Heart Institute, St Luke's Episcopal Hospital, Houston, Texas, USA,* [3]*The Children's Hospital of Philadelphia, Philadelphia, Pennsylvania, USA, and* [4]*The University of Pennsylvania Perelman School of Medicine, Philadelphia, Pennsylvania, USA*

Abstract

Mesenchymal stromal cells (MSCs) are multipotent progenitor cells capable of differentiating into adipocytes, osteoblasts and chondroblasts as well as secreting a vast array of soluble mediators. This potentially makes MSCs important mediators of a variety of therapeutic applications. They are actively under evaluation for immunomodulatory purposes such as graft-versus-host disease and Crohn's disease as well as regenerative applications such as stroke and congestive heart failure. We report our method of generating clinical-grade MSCs together with suggestions gathered from manufacturing experience in our Good Manufacturing Practices facility.

Key Words: *clinical trials, Good Manufacturing Practices, mesenchymal stromal cells, phase I trials*

Introduction

First identified by Friedenstein *et al.* (1) more than three decades ago, mesenchymal stromal cells (MSCs) are multipotent cells of the non-hematopoietic lineage that reside in bone marrow and the stroma. They are capable of differentiating into adipocytes, chondroblasts and osteoblasts *in vitro* (2,3). In bone marrow, MSCs are very rare, composing 0.01–0.001% of all cells. The highest frequency is in newborns, with a decrease in frequency with age (4,5). MSCs are routinely generated from bone marrow (BM), but they can also be expanded from adipose tissue, cord blood, amniotic fluid and placenta as well as fetal liver, blood, lung and spleen (6–9).

The manufacture of MSCs is a routine and relatively simple procedure that involves culturing whole adherent BM cells or isolated bone marrow mononuclear cells (BM MNC). This heterogeneous cell population is initially plated in tissue culture flasks, and the adherent cells—containing the MSC progenitors—are passaged to produce a homogenous population of MSCs that have a morphology similar to that of fibroblasts (10,11).

Given the physical properties of MSCs (large size, adherence), expanding clinically applicable numbers of MSCs can be difficult with the use of conventional methods because this may require many hundreds of culture flasks and a large number of open procedures. Alternatively, MSCs can be plated in cell factories and bioreactors for large-scale expansion; however, the optimal culture conditions are still under investigation (10). One concern when expanding large numbers of MSCs is the number of passages (P) required to meet dose requirements (4). Many groups limit their passage number to retain differentiation potential but ignore the number of cell doublings occurring during each passage. In light of reports of telomere shortening and reduced proliferation *in vivo* correlating with reduced telomere length, the number of cell doublings should be minimized (12).

Another variable to consider during the expansion is the protein source (fetal bovine serum versus human

Correspondence: Dr **Patrick J. Hanley**, Texas Children's Hospital, The Methodist Hospital and Baylor College of Medicine, Center for Cell and Gene Therapy, 1102 Bates Street, Suite 1630, Houston, TX, USA. E-mail: pxhanley@txch.org

(Received 13 September 2012; accepted 14 September 2012)

http://dx.doi.org/10.1016/j.jcyt.2012.09.007

platelet lysate versus serum-free media). All of these conditions are able to support MSC growth; however, they also contribute to variability between MSC lots (13). Moreover, the use of fetal bovine serum may lead to immunologic reactions in patients (14).

The absence of co-stimulatory molecules and human leukocyte antigen (HLA) class II molecules, as well as low HLA class I expression on MSCs, make them ideal cells for allogeneic, or "off-the-shelf" use in both regenerative medicine and immunomodulatory applications (15,16). Work is still in progress to characterize the optimal profile of MSC lots used in specific applications and cultured by means of different techniques, media and passage numbers. Although the Food and Drug Administration (FDA) has not issued standard release criteria for investigational new drug applications that involve MSCs, the International Society of Cellular Therapy (ISCT) has established the minimum criteria that should be used to define MSCs, and these and other regulatory issues are discussed elsewhere (10,11,17,18).

The unique immunosuppressive properties of MSCs—as well as their ability to function in the allogeneic setting—has sparked interest in the clinical use of these cells (19). The release of immunosuppressive factors such as prostaglandins (20) and Factor H (21) inhibit the innate immune system, whereas the release of transforming growth factors (TGF), prostaglandins, indoleamine 2,3-dioxygenase (IDO) and interleukin-10, as well as the expression of PDL-1 and HLA-G, modulate the adaptive immune system (20). This makes MSCs an attractive therapy for autoimmune or inflammatory diseases such as graft-versus-host disease (GvHD), diabetes and inflammatory bowel disease (9). There are currently more than 40 clinical trials listed under "Mesenchymal Stromal Cells" on www.clinicaltrials.gov, ranging from treatments for acute GvHD to therapies for Crohn's disease, emphysema, congestive heart failure and stroke. Allogeneic MSCs are also being produced by biotechnology companies and pursued in phase III clinical trials for acute GvHD and Crohn's disease (22). In light of their relative ease of manufacture, their potential to be used allogeneically and their long-standing record of safety, we anticipate that the number of applications will increase rapidly in the next 5 years.

Methods

In this report, we describe our current method of generating MSCs from human bone marrow (for the manufacturing standard operating procedure [SOP], see Supplemental data Figure S1). To address some of the concerns mentioned above, we limit our expansions to four passages and attempt to reach required cell numbers with less than 30 cell doublings. In these cultures, we uniformly used platelet lysate as the protein source. We are also investigating other cell culture devices to reduce manufacturing time to obtain clinical doses and to limit the passage number in a cost-effective manner.

Manufacturing BM-derived MSCs

All cell culture manipulations, quality and release testing and flow cytometry are performed by the Center for Cell and Gene Therapy Good Manufacturing Practice (GMP) facilities in Houston, Texas, USA.

BM MNC isolation

Upon receiving BM, aliquots are sent for phenotyping, cytogenetics and sterility testing. The marrow is then passed through a 200-μm filter (Pall, Port Washington, New York, USA) to remove residual bone fragments. To enrich the MSC-containing BM population, MNCs are isolated with the use of a Sepax device (Biosafe, Geneva, Switzerland). Briefly, cells are loaded into the Sepax device, which layers them onto a Ficoll density cushion. The cells are then centrifuged and the BM MNC are harvested and washed by the device. Other systems, such as the Bone Marrow MSC Separation Device (Kaneka Corp, Osaka, Japan), also offer closed systems that may more efficiently enrich MSCs directly from fresh marrow, leading to improved final cell yields. Manual MNC separation can be substituted but is an open system and increases the likelihood of contamination.

Plating MNCs

After isolating MNCs, cells are again sent for phenotyping and sterility and a viable count performed by Trypan blue exclusion and 7AAD staining. MNCs are then plated in T-175 cm^2 flasks at approximately 5×10^5 cells per cm^2 (Table I). The medium used is D-5 containing 5% human platelet lysate (see D-5 media SOP in Supplemental data Figure S2). Many groups seed cells at a much lower density, and there are reports showing that such cells reach target cell numbers faster, on average, than those seeded at a higher density, probably because of the delay in contact inhibition. We chose to seed at 5×10^5 cells/cm^2 to reduce the number of flasks.

Passaging MSCs

MSCs should be fed with fresh medium every 3–4 days. When the cells are 70–80% confluent, they can be split at a 1:4 flask ratio (Figure 1). We typically pool the cells into one common vessel and split the cells