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Efficient manufacturing of therapeutic mesenchymal stromal cells with the use of the Quantum Cell Expansion System

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Abstract

Background. The use of bone marrow–derived mesenchymal stromal cells (MSCs) as a cellular therapy for various diseases, such as graft-versus-host disease, diabetes, ischemic cardiomyopathy and Crohn's disease, has produced promising results in early-phase clinical trials. However, for widespread application and use in later phase studies, manufacture of these cells must be cost-effective, safe and reproducible. Current methods of manufacturing in flasks or cell factories are labor-intensive, involve a large number of open procedures and require prolonged culture times. *Methods.* We evaluated the Quantum Cell Expansion System for the expansion of large numbers of MSCs from unprocessed bone marrow in a functionally closed system and compared the results with a flask-based method currently in clinical trials. *Results.* After only two passages, we were able to expand a mean of 6.6×10^8 MSCs from 25 mL of bone marrow reproducibly. The mean expansion time was 21 days, and cells obtained were able to differentiate into all three lineages: chondrocytes, osteoblasts and adipocytes. The Quantum was able to generate the target cell number of 2.0×10^8 cells in an average of 9 fewer days and in half the number of passages required during flask-based expansion. We estimated that the Quantum would involve 133 open procedures versus 54,400 in flasks when manufacturing for a clinical trial. Quantum-expanded MSCs infused into an ischemic stroke rat model were therapeutically active. *Conclusions.* The Quantum is a novel method of generating high numbers of MSCs in less time and at lower passages when compared with flasks. In the Quantum, the risk of contamination is substantially reduced because of the substantial decrease in open procedures.

Key Words: *cell culture expansion, good manufacturing practices, mesenchymal stromal cells, Quantum, stroke*

Introduction

Mesenchymal stromal cells (MSCs) show promise in therapeutic applications, including inflammatory and immune-based diseases such as Crohn's disease or graft-versus-host disease, as well as in regenerative medicine treatments such as osteogenica imperfecta, burns, myocardial infarction and stroke (1–7).

MSCs can be enriched and expanded from numerous sources, including bone marrow, cord blood and adipose tissue and have the potential to differentiate into chondrocytes, osteoblasts and adipocytes (8–11). When grown under appropriate conditions, the tri-lineage potential of these cells is maintained. However, during expansion, the telomeres shorten and unbiased differentiation into the three lineages could become polarized (12). Therefore, for therapeutic applications, obtaining clinically relevant numbers of cells with a minimum number of cell passages and doublings is essential.

Current methods for generating large numbers of MSCs have usually involved traditional flask-based methods and cell factories. The use of hundreds of

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cell culture flasks to generate the required numbers of cells is extremely laborious and involves thousands of open events, which increase the possibility of contamination. Although cell factories overcome some of these issues (13,14), they can be technically challenging, even for experienced users (15). For example, visualizing cells is difficult because of the multiple layers, and, in our experience, a good cell recovery is challenging when these devices with MSCs are used. For these reasons, manufacture of MSCs is generally restricted to established cell therapy centers with considerable experience, resources, and Good Manufacturing Practices facilities (16,17).

Despite these limitations, there remains considerable interest in the use of MSCs for a diverse range of therapeutic applications. This interest is likely to continue because allogeneic MSCs may provide an "off-the-shelf" source of cells as the result of their lack of expression of human leukocyte antigen (HLA) class II and co-stimulatory molecules, which limits the immune response of the recipient to these cells (18,19). Therefore, large banks of MSCs can be prepared, making the cells rapidly available for use in early-stage clinical trials or eventually as a licensed drug. Generation of such cell banks through the use of the current flask-based technologies would be extremely labor-intensive and expensive.

One alternative could be the Quantum Cell Expansion System (henceforth referred to as Bioreactor) by Terumo BCT (Lakewood, CO, USA), a self-contained system including a hollow-fiber bioreactor. Although this system has been reported previously (20,21), large-scale production of MSCs ($>2.0 \times 10^8$) with the use of the Bioreactor and a head-to-head comparison of flasks versus the Bioreactor have not been done. Furthermore, MSCs expanded in the Bioreactor have not been tested for efficacy in an animal model. We report the use of the Bioreactor to generate large numbers of allogeneic MSCs that could be banked for multi-patient use. We demonstrate that these MSCs are functional in a rat model of ischemic stroke. In this study, we aim to compare the use of the Bioreactor with the traditional flask-based method for MSC production. The primary end point for this study is a comparison of MSCs expanded in the Bioreactor compared with flasks, and the secondary end point is the function of these cells *in vitro* and *in vivo*.

Methods

Preparation of D-5 medium

Expired apheresis platelets from eligible donors (Gulf Coast Regional Blood Service, Houston, TX, USA) were pooled and frozen in 30-mL aliquots at −80°C. Platelets were then thawed and centrifuged, and the supernatant was added (5% platelet lysate) to Dulbecco's Modified Eagle Medium (Lonza, Walkersville, MD, USA) containing 2.1 units/mL heparin (APP Pharmaceutical, Schaumburg, IL, USA), 2 mmol/L GlutaMax (Invitrogen, Carlsbad, CA, USA) and 10 mmol/L N-acetylcysteine (Sigma, St Louis, MO, USA). The complete medium was then filtered through a 0.2-μm filter (22).

Bone marrow processing

Twenty-five to 100 mL of bone marrow (BM) aspirate from normal donors was purchased from Lonza and was shipped on cold packs overnight. Twenty-five−milliliter tubes of BM were pooled in cell processing bags (Baxter, Deerfield, IL, USA). If the aspirate was intended for flask-based culture, the bone marrow mononuclear cell (BMMC) fraction was enriched with the use of a Ficoll density gradient (GE Healthcare, Pittsburgh, PA, USA) on a Sepax cell separation device (Biosafe, Geneva, Switzerland). In total, five BM aspirates were used; three were used to generate MSCs in both the Bioreactor and in flasks, and, of the remaining two, one was used for the Bioreactor only and one was used for flasks only.

Plating of BMMC in flasks and expansion of MSCs

BMMC were resuspended in D-5 medium and plated at 5×10^5 cells/cm^2 in T-175 cm^2 flasks (defined as passage 1) (15). On reaching 70−80% confluence, the monolayer was washed, harvested with the use of TrypLE Select (Gibco, Carlsbad, CA, USA) and the cells were split 1:4 into new T-175 flasks. Although variable, the typical seeding density was 2×10^3 to 2×10^4 cells/cm^2. Cells were maintained in incubators at 37°C and 5% CO_2 in air. After three to five passages, cells were harvested and frozen in cryopreservation medium containing a final concentration of 5% human serum albumin (Baxter), 10% dimethyl sulfoxide (Cryoserv, Bioniche Pharma, Lakewood, IL, USA) and 85% Plasmalyte (Baxter).

Bioreactor cell expansion device

The Bioreactor (Figure 1A) is a functionally closed system consisting of a disposable hollow-fiber bioreactor enclosed in a stand-alone incubator. It is fed through two circulation loops: the intracapillary loop and the extracapillary loop, both of which have inlets for media and reagents, or cells (intracapillary loop only). Waste is removed into a waste bag. The entire process is computerized and controlled by a