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Microcarrier choice and bead-to-bead transfer for human mesenchymal stem cells in serum-containing and chemically defined media

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ABSTRACT

The production of stem cells for clinical applications requires a suitable mass expansion and harvest process, which is implemented in a microcarrier-based bioreactor. Important parameters to consider include the choice of microcarriers for cell expansion, the growth medium and the scale-up strategy. We investigated six different microcarriers to determine whether they can support the growth and harvest of primary human mesenchymal stromal/stem cells (hMSCs) derived from bone marrow, and the immortalized cell line hMSC-TERT. A serum-containing medium (SCM) and a new chemically defined medium (CDM) were compared under dynamic culture conditions. We also investigated bead-to-bead transfer from spinner flasks to a stirred tank reactor as a scale-up strategy. We observed cell type-dependent differences in growth rate and attachment behavior on each microcarrier. Missing serum components in the CDM led to slower cell attachment and growth, whereas microcarriers suitable for the SCM were also suitable for CDM. Glass-coated microcarriers supported hMSC-TERT growth and bead-to-bead transfer in SCM, whereas plasma-treated plastic surfaces promoted cell growth in CDM. We demonstrated that stem cell cultures can be scaled up by bead-to-bead transfer, avoiding the need for pre-cultures in tissue flasks. The replacement of pre-cultures with a monitored bioprocess could therefore facilitate the development of conditions suitable for large-scale stem cell production.

1. Introduction

Human mesenchymal stromal/stem cells (hMSCs), primary hMSCs and hMSC lines, are an important source of cells for clinical cell therapy applications. Depending on the proposed treatment, between 5×10^7 and 1×10^8 cells are required per patient and dose [1–3]. Typically, healthy and undifferentiated cells are required, and it is challenging to manufacture enough cells of sufficient quality according to good manufacturing practice (GMP). Therefore, it is necessary to scale-up the manufacturing process and to use a controlled bioreactor expansion system to ensure a consistent cell product quality [4] and quantity. Regulatory demands imposed by the EMA or the FDA must also be met [5].

MSC growth is strictly anchorage-dependent, which means the cells need to attach to a growth surface to proliferate. In bioreactors, microcarriers are typically used to provide this growth surface. Porous and non-porous microcarriers are available, but only the non-porous variety is suitable for hMSC expansion, because the cells are difficult to detach from porous surfaces. It is therefore necessary to choose the most appropriate microcarrier, which not only supports attachment and growth but also allows efficient detachment without losing hMSC viability. The attachment and proliferation of anchorage-dependent hMSCs is strongly influenced by the interaction between the carrier surface and the cell. This interaction is driven by non-specific forces arising from the physicochemical characteristics of the surface material, as well as specific interactions involving adhesive proteins, peptides or other factors on the material surface and cell surface receptors [6,7]. A number of commercial microcarriers with different surface properties are available for hMSC expansion. Some have been developed to improve cell attachment using new synthetic or natural

Abbreviations: bFGF, basic fibroblast growth factor; DMEM, Dulbecco's modified eagle's medium; ECM, extracellular matrix; FBS, fetal bovine serum; HG, high glucose; LG, low glucose; RGD, arginyl–glycyl–aspartic acid sequence; SCM, serum containing medium; CDM, chemically defined medium; TERT, telomerase reverse transcriptase; GC, glass-coated; EA, enhanced attachment; SM, synthemax; PP, plastic plus; PN, pPronectin

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materials, whereas others are optimized for the specific harvest requirements of hMSCs.

Three main types of non-porous carriers can be distinguished: (a) plastic or treated-plastic microcarriers, (b) glass-coated microcarriers, and (c) protein-coated microcarriers. Plastic microcarriers mimic the surface to which hMSCs adhere in static tissue-culture flasks and often consist of polystyrene, which simplifies the transfer of cells from static to dynamic cultivation environments. Some plastic surfaces are further treated with plasma (corona-gas treatment), which increases the number of oxygen-containing functional groups in the polystyrene backbone [8] and hence the hydrophilicity and wettability of the surface, which is known to encourage cell attachment. Glass has been used as a surface in static cell cultures for decades, and the corresponding microcarriers comprise a copolymer plastic core with a high-silica glass coating [9,10]. Protein-coated microcarriers mimic the natural extracellular matrix (ECM) by incorporating common ECM proteins on the carrier surface, e.g. collagen [11] or synthetic peptides similar to vitronectin [12]. This encourages cell attachment and growth *in vitro* by providing adhesion ligands. Such defined coatings are reproducible with high purity and are therefore suitable for applications requiring defined, xeno-free conditions.

The expansion of hMSCs requires not only a suitable growth surface, but also an appropriate culture environment to provide optimal nutrition. For clinical applications, hMSC production falls under the classification of advanced therapeutic medicinal products (ATMPs) [13], for which the regulatory agencies strongly recommend the avoidance of animal-derived raw materials to reduce the risk of contamination and to simplify downstream processing. Therefore, serum-free medium or even better chemically defined medium (CDM) should be used for hMSC expansion. Whereas serum-free medium has been described for stem cell growth in static [14,15] and dynamic [12] cultures, we tested a complete chemically defined medium for hMSC expansion. This basal medium is defined, meaning that each component has a Chemical Abstracts Service (CAS) registration number, and it has a protein-free formulation [16]. The absence of attachment promoting factors is likely to affect attachment behavior of hMSCs in CDM and an attachment-promoting growth surface would therefore be advantageous.

Expansion systems for hMSCs have recently been reviewed [17] with a particular focus on microcarriers [18,19]. However, traditional cell expansion in static systems remains the most common method to generate a sufficient cell number for cell therapy. This is suitable for products where a small number of cells are isolated from the patient and only 10^7–10^8 cells are needed at the end of the expansion for a treatment [20]. But static expansion strategies become more challenging when the number of patients increases and several parallel batches have to be handled and controlled. Although not specified, roughly a hundred tissue flasks for a phase III clinical trial with a dose of 1.2×10^8 cells per patient were needed [21]. In such cases, a multistage bioreactor process would facilitate the production of stem cells for clinical applications for ten patients. This process would include bead-to-bead transfer, i.e. batch feeding of fresh microcarriers (beads) and media to the final volume of the bioreactor [22]. Allogenic stem cell production, e.g. products of the immortalized hMSC-TERT cell line [23], involves much larger cell numbers, and here it is worthwhile to produce one large batch at a several hundred liter scale because quality and potency testing must be performed per batch. Bead-to-bead transfer is a GMP-compatible strategy suitable for a 100-L scale bioreactor using microcarriers. Bead-to-bead transfer is a well-known procedure for the large-scale microcarrier-based expansion of anchorage-dependent mammalian cells [24–26].

Here we investigated the attachment, growth, metabolite consumption and detachment of hMSC-TERT and primary bone marrow-derived hMSCs on different microcarriers to identify differences in the behavior of these two cell types. We also investigated differences in cell behavior when the cells were cultivated in CDM instead of serum-containing medium (SCM). Finally, we investigated the suitability of the hMSC-TERT cell line for dynamic expansion based on bead-to-bead transfer in both SCM and CDM.

2. Materials and methods

2.1. Cell lines

Primary hMSCs from bone marrow (passages 3–10), kindly provided by EMD Millipore, USA, and the immortalized cell line hMSC-TERT [27,28] (passages 72–80), kindly provided by M. Kassem, University of Southern Denmark, were used.

2.2. Media

Three different media were used (unless otherwise specified, all components were purchased from Biochrom, Germany): DMEM high glucose (HG) supplemented with 2 mM L-glutamine and 10% standardized fetal bovine serum (FBS; article no. S0615); DMEM low glucose (LG), with 2 mM L-glutamine and 10% FBS and 8 ng/mL basic fibroblast growth factor (bFGF; article no. W1370950050); and chemically defined medium (CDM) Stem Cell 1 (Cell Culture Technologies, Switzerland) supplemented with 2 mM L-glutamine and 8 ng/mL bFGF.

2.3. Static cultures in tissue flasks

Cryoconserved hMSC-TERT cells (passage 72, 10% DMSO, 90% FBS) were thawed and cultivated in tissue flasks (Sarstedt, Germany) containing DMEM-HG with seeding densities between 5×10^3 and 1×10^4 cells/cm^2 at 37°C, in a 5% CO_2 humidified atmosphere. Passaging was carried out at 80–90% confluence using TrypZean solution (T3449; Sigma-Aldrich, USA). CDM adaptation after the first passage was carried out using a mixture of 50% DMEM and 50% CDM, and subsequently 100% CDM was used in Advanced TC™ tissue flasks (Greiner Bio-One, Austria). All subsequent passaging was carried out using 25% conditioned medium from earlier cultures. The medium was replaced with 50% fresh medium every 3–4 days. Cells were passaged at least twice in CDM alone before starting the microcarrier experiments. Cryconserved primary hMSCs (passage 2) were cultivated in DMEM-LG containing 2 mM L-glutamine, 10% FBS and 8 ng/mL bFGF with 1×10^4 cells/cm^2 seeding density at 37°C, in a 5% CO_2 humidified atmosphere.

2.4. Microcarriers

All microcarriers were non-porous spheres with a diameter of 125–212 µm and a specific surface area of 360 cm^2/g (Table 1).

2.5. Spinner cultures

Human MSC-TERT cells were cultured in 100-mL spinner flasks (pendulum design, Integra Biosciences, Switzerland). For our SCM, we used 70 mL DMEM-HG medium and 12.5 g/L microcarriers (4.5 cm^2/mL). For CDM, we used 40 mL medium and 15 g/L microcarriers (5.4 cm^2/mL). The spinner speed was initially 30 rpm and increased after 48 h to 55 rpm, and after 72 h to 75 rpm to prevent aggregation of the microcarriers. Spinner flasks with microcarriers were autoclaved at 121 °C for 20 min. After cooling, the flasks were filled with 80–90% of the final volume of medium and preheated (37 °C) in an incubator. Cells from tissue flasks were harvested with TrypZean (3 mL per T-175 flask). After centrifugation (250 × *g*, 5 min), the cells were suspended in medium, cell suspension was added to the prefilled spinner flasks to reach final culture volume, and spinner expansion was started on a stirring plate in a humidified CO_2 incubator at 37 °C. Attachment was evaluated by counting the cells in the supernatant. Cells were grown in spinner flasks at a 5% CO_2 humidified atmosphere. Primary hMSCs