Validation of growth factors for serum-free manufacturing of human mesenchymal stem cells

Lonza
Pharma & Biotech

Schroeder J.[1], Bossie A.[2], Carmen J.[2], Schaefer S[1]., Spottke N.[1], Morie B.[2], Rowley J.[2] & Mueller-Hartmann H[1].
[1]Lonza Cologne GmbH, Nattermannallee 1, 50829 Cologne, Germany, [2]Lonza Walkersville, Inc., 8830 Biggs Ford Road, 21793 Walkersville, MD, USA

Abstract

Human mesenchymal stem cells (hMSC) are of tremendous interest for the field of regenerative medicine and cell therapy because of their tissue regenerative properties and potential to modulate the immune system. Many allogeneic and autologous clinical applications are based on hMSC and therefore the scalable expansion of hMSC according to GMP standards is an important task when moving through clinical phases towards commercialization.

In the past the majority of media used for the expansion of primary human cells contain whole serum like fetal bovine serum. Using serum-containing medium in cell culture adds both regulatory and supply chain risks as hMSCs are considered therapeutic drugs. There is a strong drive towards serum-free media for the manufacturing of cells for clinical purposes. In serum-free media, whole serum is replaced by a defined mixture of relevant factors, like vitamins and recombinant proteins (e.g. growth factors or cytokines). Serum-free media performance is highly dependent on the quality of recombinant proteins, and a consistent supply of proteins will be critical as hMSC-based therapies make it to market.

In order to find appropriate primary and secondary suppliers of high quality recombinant proteins for serum-free hMSC medium, recombinant human fibroblast growth factor basic (rhFGFb) from five different commercial suppliers was tested in combination with serum-free medium and compared to standard serum-containing medium.

Materials and methods

Cell culture: 6-well plates (Corning® Costar®) were coated over night with 5 µg/mL recombinant human fibronectin (R&D Systems). 2500 hMSC/cm^2 (Lonza, PT-2501) from three independent donors (Donor 1 – Donor 3) were cultured in duplicate in MSCGM™ Medium (Lonza, PT-3001) or TheraPEAK™ MSCGM™ Medium (BEBP18-936) with recombinant human fibroblast growth factor basic (rhFGFb) until ~90% confluency was reached. Cells were passaged after six days of culture according to standard procedures using ReagentPack™ Subculture Reagents (Lonza, CC-5034) and cultured for another five days. Cells were analyzed microscopically using the Zeiss AxioObserver Z1 microscope. Cells were harvested and counted using a Cedex XS device (Roche).

Flow cytometry: Cells were stained according to standard procedures against CD90 (mouse-anti-human CD90, IgG1, APC coupled, BD, 559869) and CD166 (mouse-anti-human CD166, IgG1, PE coupled, BD, 559263) and analyzed by flow cytometry in the presence of 250 ng/mL propidium iodide and Calibrite APC Beads (BD) using a FACScalibur device (BD) equipped with two lasers and CellQuestPro Software (BD).

IDO activity: After cell harvest on day 11, 30,000 cells/cm^2 were seeded in TheraPEAK™ MSCGM™ Medium and MSCGM™ Medium. Medium was changed after 24 hours to both media types containing no or 3 ng/mL recombinant human interferon gamma (rhIFN-γ). After another 48 hours supernatants were collected. 250 µL of supernatant were mixed with 250 µL of sodium phosphate solution, 50 mM, pH 6.0. Subsequently 100 µL of 2 M TCA were added to each sample. Samples were centrifuged for 10 minutes at 13,000 rpm at 4°C in a standard table top centrifuge (Heraeus). 180 µL of sample were transferred into a clear microtiter plate and 180 µL of Ehrlich's Reagent (80mg/mL p-dimethylaminobenzaldehyd in glacial acetic acid) were added to each well. After 10 minutes at room temperature the absorbance was read at 490 nm (FLUOstar Omega, BMG Labtech).

Summary

- Higher cell proliferation rates were observed in serum-free TheraPEAK™ MSCGM™ Medium compared cell culture medium containing serum. Comparable cell functionality was observed in both media types as analyzed by flow cytometry or IDO activity.
- Some variability between different hMSC lots was observed likely due to donor-to-donor variation. Therefore cells from different donors have to be used during a media optimization process in order to develop a largely donor-independent medium.
- Highest cell proliferation rates and low experimental variability were observed with rhFGFb from supplier A. rhFGFb from the suppliers B, C, and D are also suitable for the expansion of hMSC even though higher concentrations of the recombinant protein might be required for optimal performance.
- For the selection of appropriate primary and secondary rhFGFb suppliers further factors will be considered, like the price per rhFGFb quantity required and the lot-to-lot variability of different rhFGFb batches from a single supplier. This way there is a consistent production of an optimal, cost-effective and robust serum-free hMSC medium.
- This report is an example of a best practice for qualifying suppliers of critical raw materials for cell expansion media. Similar procedures should be applied when initiating each cell manufacturing process development campaign.

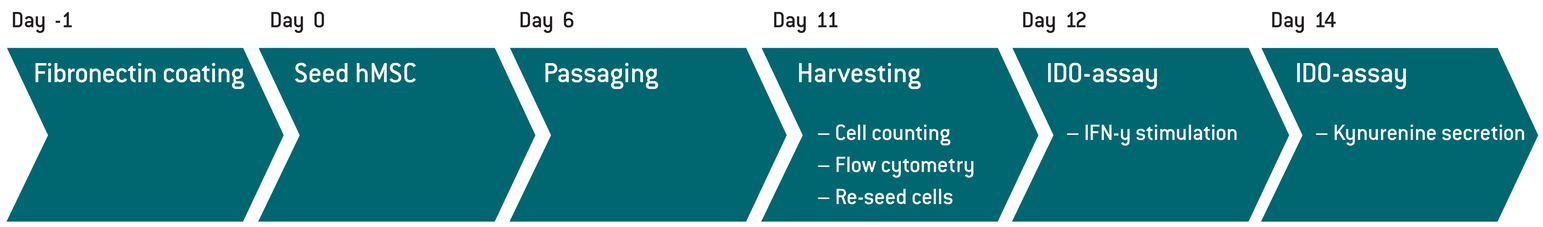

Figure 1. **Experimental set-up.** Details see Materials and Methods.

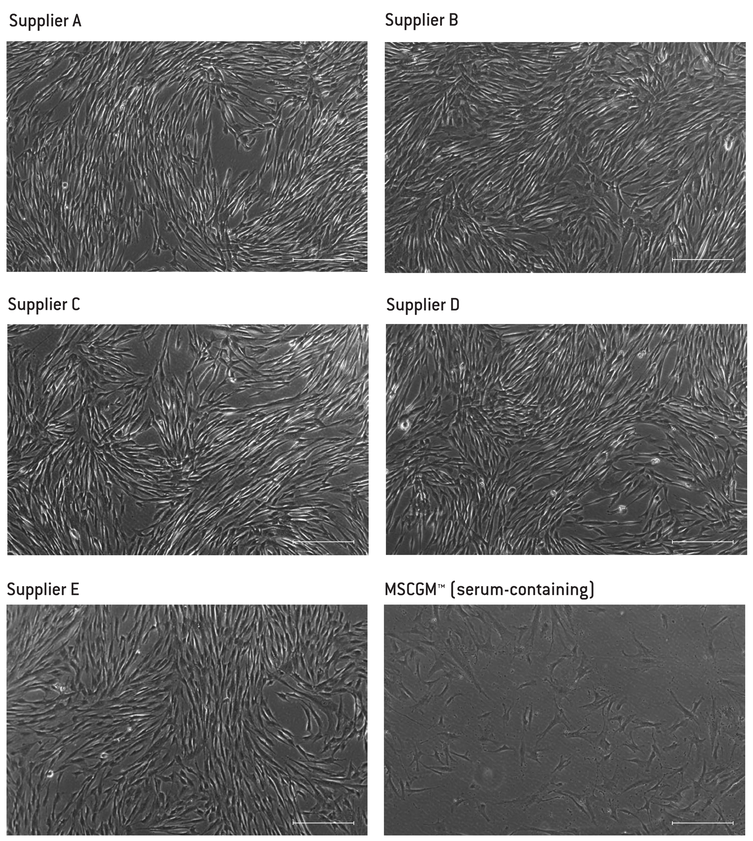

Figure 2. **Proliferation of hMSC in TheraPEAK™ MSCGM™ Medium** with rhFGFb from five different suppliers (A-E) or MSCGM™ (serum-containing). Cells were passaged on day 6 and pictures taken on day 11 of culture. Independent from the source of rhFGFb the cells are well attached and confluent after 11 days of culture in TheraPEAK™ MSCGM™ Medium. Cells were less dense, but more spread out, when cultured in serum-containing MSCGM™. (Scale bar: 300 µm)

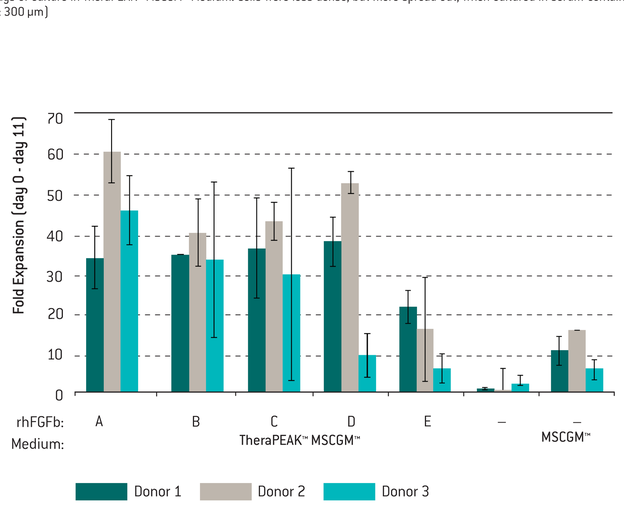

Figure 3. **Proliferation of hMSC in TheraPEAK™ MSCGM™ Medium** without rhFGFb or with rhFGFb from five different suppliers (A-E) or MSCGM™ (serum-containing). Cells were passaged on day 6 and harvested on day 11. The number of viable cells was determined and cell proliferation is shown as fold expansion of cells during 11 days of culture. Higher cell proliferation rates were observed in rhFGFb supplemented, TheraPEAK™ MSCGM™ compared to standard MSCGM™. rhFGFb from supplier E supported less cell proliferation compared to the other suppliers.

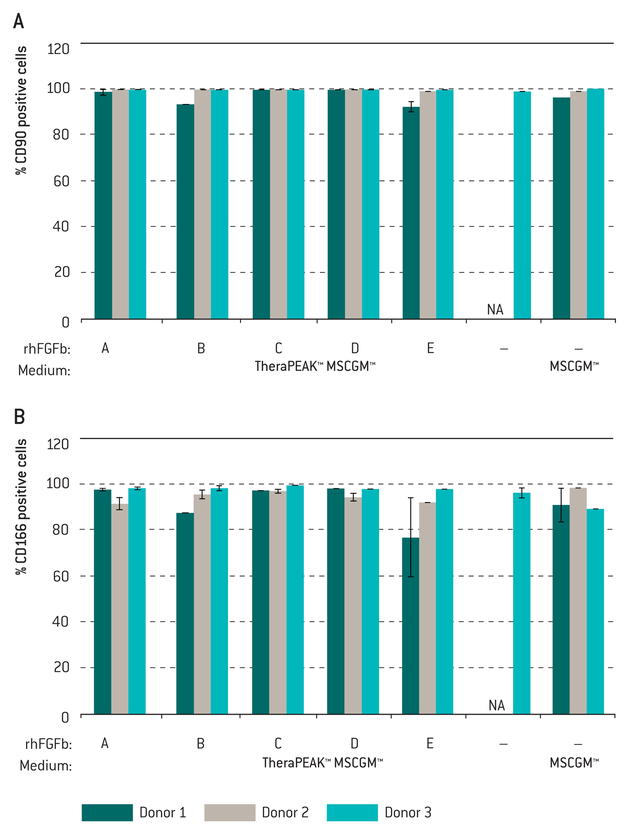

Figure 4. **Expression of the mesenchymal markers CD90 (A) and CD166 (B)** on hMSC from three independent donors cultured in TheraPEAK™ MSCGM™ Medium without rhFGFb or with rhFGFb from five different suppliers (A-E) or MSCGM™ (serum-containing). Cells were passaged on day 6 and harvested on day 11 followed by flow cytometric analysis. A high percentage of hMSC were positive for CD90 and CD166 after 11 days of culture – independent from both, the type of medium and the rhFGFb used.

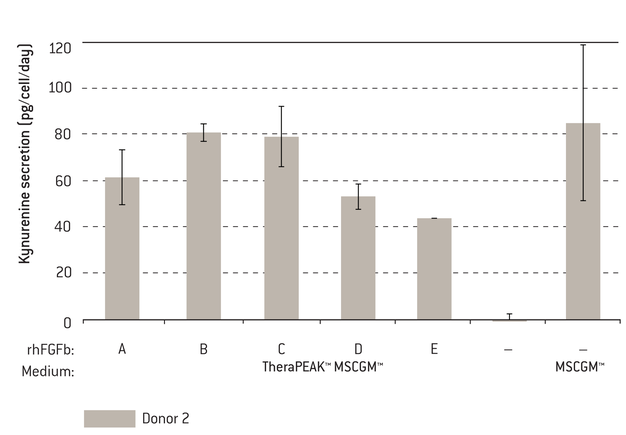

Figure 5. **IDO activity.** The enzyme IDO (Indolamin-2, 3-Dioxygenase) is induced by IFN-γ in different human cell types. The enzyme catalyzes the degradation of L-tryptophan to N-formyl-kynurenine, which becomes L-kynurenine. This leads to local L-tryptophan depletion, which is a limiting factor for pathogens and T-cells. In addition, L-kynurenine induces apoptosis of T-cells. This results in a local immune suppression and might enhance e.g. the survival of implanted hMSC within the human body. Therefore detection of IDO activity by measuring the secretion of kynurenine from hMSC *in vitro* could be used as a marker for the potency of implanted hMSC.
hMSC were cultured in TheraPEAK™ MSCGM™ Medium without rhFGFb or with rhFGFb from five different suppliers (A-E) or MSCGM™ (serum containing). After 11 days of culture, cells were re-seeded in MSCGM™ and stimulated for 48 hours with 3 ng/mL rhIFN-γ. Cell culture supernatants were analyzed for kynurenine secretion. No clear differences in kynurenine levels were detected among the samples, indicating that the IDO can be induced equally well in cells that were expanded in both types of media - serum-free as well as serum-containing.