Review

hMSC Production in Disposable Bioreactors with Regards to GMP and PAT

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Reactor concepts for human mesenchymal stem cell (hMSC) production are introduced. Thereby, special interest is laid on the realization of these concepts as disposables fulfilling the GMP and PAT requirements. The specialty of the hMSC production process is the cell itself being the product. This results in completely different process requirements compared to e.g. protein production in mammalian cells. Thus, great attention has to be given to the shear sensitivity of the cells. The cultivation and the harvest of the cells have to be very gentle to neither influence cell viability nor cell differentiability. Further, the production process should not cause any undesirable cell changes. For hMSC production, cell harvest is the main challenging process step. The reactor concepts should be suitable for hMSC production for clinical trials as ATMPs. Therefore, disposable systems are especially applicable. The review describes more detailed bone marrow-derived hMSC production in a disposable stirred tank reactor as promising reactor concept.

Keywords: Disposable reactor, Fixed-bed reactor, hMSC, Stem cell production, Stirred-tank reactor

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1 Introduction

Human mesenchymal stem cells (hMSC) are an interesting cell source for many applications. Besides their intensive use in research, they gain more and more importance for clinical applications. Especially in the field of regenerative medicine and more precisely, in cell therapy hMSC are of great interest. Clinical indications in which hMSC are pursuit include bone regeneration and cartilage repair, Crohn's disease as well as kidney or heart diseases [1–7]. Several characteristics make hMSC an ideal candidate for cell therapy approaches. Firstly, the ease of isolation and in vitro cultivation is a significant advantage compared to other cell types. Secondly, hMSC have been shown to control the inflammation process, cell death, fibrosis and tissue regeneration [8]. In addition, the cells release growth factors, cytokines and other signaling molecules which are often sufficient for a therapeutic effect [8]. A specialty of hMSC is immune modulation, which reduces immune response of the patient even after allogenic cell therapy [9].

For a widespread application of hMSC a decisive role is played by the cell quantity and quality. In vivo stem cells are found in low numbers while large numbers are required for clinical applications. The dosage of hMSC in therapeutic applications depends on the type of indication and is generally not well defined. In adults the minimal therapeutic hMSC dose is assessed with $1-2 \cdot 10^6$ hMSC per kg [10]. Furthermore, these cells have to be highly viable. In the last few years early phase studies have indicated the safety of autologous and allogenic hMSC. However, late-stage clinical trials are ongoing and final results are still pending. Contrary to this progress, the cellular product manufacturing including established and stable production processes, process monitoring and quality control is still immature. To commercialize hMSC products, the development of scalable manufacturing solutions needs to be expedited.

Disposable reactor systems seem to be the ideal basis for process development. The use of disposable bioreactor systems claims several advantages like simple and flexible process handling, an increase in process safety by reduced inci-

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dence of cross-contaminations and a cost reduction through lower personal demand and the disappearance of cleaning processes [11 – 13]. Furthermore, disposable systems simplify process admission by the authorities since the validation of cleaning in place (CIP) and sterilization in place (SIP) is not necessary. An effortless transfer of standard systems to geometrically similar disposable systems would be advantageous. Problems arise due to the lack of experience in disposable systems concerning material quality [14] or potential interactions with the products [15]. In addition, there is also an increase in costs concerning waste disposable [16]. In the following, available cultivation concepts for hMSC are briefly summarized and details on disposable reactor concepts are provided.

2 Process Requirements for Stem Cell Expansion

Clinical use of hMSC as advanced therapy medical products (ATMPs) underlies stringent quality control requirements. These include a validated measurement of e.g. purity, differentiability, and stability of the cells. Unfortunately, no available marker can fully define hMSC by itself today. The Mesenchymal and Tissue Stem Cell Committee of the International Society for Cellular Therapy has therefore defined minimal criteria to characterize hMSC. According to their definition hMSC are cells which are (i) plastic-adherent in standard culture conditions, (ii) positive for CD105, CD73, and CD90 and negative for CD45, CD34, CD14, or CD11b, CD79*a* or CD19, and HLA-DR expression, and (iii) able to differentiate into osteoblasts, adipo- and chondrocytes in vitro [17]. Therefore, during process development for hMSC expansion, product specifications must be maintained during the process.

Stem cell manufacturing is further complicated by the definition of the cell therapy approach. Therapies with autologous hMSC require other production facilities than allogenic cell products. Autologous therapy needs a small scale manufacturing plant that can handle multiple samples from individual patients without cross-contamination. Contrary, allogenic cell products are bulk productions requiring large-scale facilities. Furthermore, stem cell doses for many applications are not well defined. Currently cell number requirements for hMSC range from 1.5 up to $120 \cdot 10^6$ cells per dose dependent on the indication [18].

The process development requires the identification of critical parameters and quality features as well as the parameter definition and their connection to the cell product. Afterwards, the process system can be designed, the strategy for quality assurance can be developed and the methods for process monitoring can be defined. Then the actual production process could be established by development of routine and reproducibility [19].

2.1 Suitable Growth Surfaces for hMSC

hMSC are strictly adherent cells, which need a suitable growth surface. In some studies hMSC were grown in aggregates in suspension [20]. However, hMSC change their phenotype in these cultivations. In conclusion hMSC growth as adherent cells on a cultivation surface is preferred. For cultivation in larger scale this growth surface is mainly provided by multiray cell culture systems or by carriers [14]. There are different kinds of microcarriers (porous, non-porous, coated or not-coated), on which hMSC have been shown to grow well and which enable the maintenance of multipotency of hMSC [21]. These consist of dextran, glass, gelatin, cellulose or other synthetic polymers. In addition, the microcarrier surface is often modified with e.g. collagen for better cell attachment. Besides cell growth, the surface has to assure hMSC adhesion but also guarantee a gentle cell detachment. These contradictory demands cannot be achieved by most commercially available carrier types. Almost all carriers are optimized for cell adhesion and growth but not for cell detachment since this is not needed in vaccine or protein production processes. For example, porous carriers have a high surface to volume ratio and protect the cells against shear forces but nutrient transfer and cell detachment are poor [22]. Therefore non-porous carriers are favored for hMSC expansion and have been described in the literature [23]. From these carriers high quality hMSC can be harvested [24].

2.2 Inoculation and Harvest Strategies for hMSC

The inoculation strategy is crucial for stem cell adhesion. Highest adhesion rates described in the literature were usually achieved by a cyclic procedure with transient stirring and resting phases [22, 25 – 28]. Inoculation densities ranging from 1 to $3 \cdot 10^4\,\mathrm{cm}^{-2}$ had only minor influence on the efficiency of cell adhesion [29]. Regarding a constant final cell density at the end of the process, the inoculation density has an influence on the process efficiency as the expansion factor is influenced. Therefore, the lowest inoculation density ($\approx 0.6 \cdot 10^3\,\mathrm{cells/cm}^2$) achieved the highest expansion factor ($\approx$ 7-fold) [25, 30].

The most challenging process part for high hMSC quality and quantity is the cell harvest. In standard cell culture, different mechanical and enzymatically methods as well as a combination of both have been described for cell detachment. Mechanical detachment is very shear-intensive and not suitable if the cell itself is the product. For enzymatic detachment the selection of the enzyme is critical. Some of the enzymes as the commonly used trypsin are aggressive and could damage the cell surface proteins [31]. Further, trypsin is available as cGMP grade but of costly nature. In clinical use, hMSC are considered as ATMPs. Thus, the use of animal derived products should be reduced due to safety

as well as ethical concerns. Up to now, no harvest protocol has been published for hMSC. The success of a harvest strategy strongly varies with the carrier type, culture medium and medium supplements, the hMSC source, and the reactor system used.

2.3 Process Monitoring

As hMSC are regarded as ATMPs, they must go through GMP regulations as well as requirements referred to process analytical technology (PAT). PAT is regarded as a tool for the design, analyses and control of production processes. The final product quality can be ensured through the measurement of process parameters and product characteristics [32]. This includes an extensive online process monitoring [33], which provides a useful tool for process characterization and the detection of process changes. However, basic knowledge of a link between various parameters and process procedure is sometimes missing. In addition, kinetics and balances are difficult to determine and describe. This means hMSC expansion processes are difficult to control and reproduce [34]. In this respect, a better process monitoring could accelerate the process development and improve efficiency of the production processes while ensuring high-quality products [35]. Furthermore, documentation of process data is necessary for regulatory approval and the validation of a biopharmaceutical process. Relevant parameters for the process characterization are cell growth, cell quality, medium conditions (temperature, pH, pO_2 and pCO_2) as well as metabolite concentrations (glucose, lactate, glutamine and ammonium) [36]. Temperature, pH, pO_2 and pCO_2 are routinely controlled online in cell culture processes and probes are available as disposables as well. Online monitoring of the other process parameters in dynamic systems is not routinely done today. For hMSC expansion in particular, the online monitoring of cell growth and viability should be satisfied. For this, the application of dielectric spectroscopy for biomass monitoring has been described in the literature [37].

2.4 Balancing Nutrient Supply, System Homogeneity and Shear Forces

The adherent growing hMSC should be expanded and harvested in a preferably simple homogenous system with low shear. In addition, the supply of the cells with nutrients and oxygen must be incorporated into the system. While unproblematic in static systems, this will be crucial for dynamic systems especially at larger scales. Using a stirred tank reactor, the maintenance and mixing of the medium and the associated nutrient supply is realized by the suitable stirrer speed. Stirring should be high enough to distribute the hMSC on the microcarrier homogenously during cell adhesion. Later in hMSC expansion process stirring has to be optimized to avoid formation of large cell-carrier aggregates. The formation of aggregates is disadvantageous because of the aggravation at the point of cell harvest and a loss of cell viability. On the other hand, the stirring speed should not have any negative influence on the cells. In a perfused system (fixed-bed bioreactor), shear forces are much lower and are determined mainly by the superficial velocity.

hMSC are sensitive to high shear and respond with cell detachment from the growth surface, reduced growth rate, loss of viability and changes in expression [38]. Therefore, oxygen supply should be realized with low shear. In static systems, oxygen is supplied by surface aeration without shear to the cells. In perfused systems, oxygenation is performed outside of the reactor and therefore no shear is imposed to the cells. In stirred systems, however, aeration can cause shear forces in addition to the stirring itself. Direct sparging via air bubbles can damage cells [39]. External or membrane aeration is more cell-preserving but requires extended technical expenditure. A reduction of shear in stirred systems can further be realized by pulsed aeration based on an oxygen set point. This might be sufficient for the oxygen supply of cell cultures depending on cell line.

2.5 Economic Process Requirements

Besides the aforementioned concerns, economic reasons need to be considered. The limited expandability of primary hMSC demands an efficient process. To reduce expansion process failures the process handling should be kept simple and the risk of contamination should be minimized. The number of vessels should be reduced to a minimum, resulting in a high surface to volume ratio. Preferably, production should be performed in an automated closed reactor system. Especially for expansion of autologous cells in clinical use disposable reactor systems are favorable. These closed systems minimize cross-contamination between autologous cells from different patients providing the cells for one patient in one vessel. They are ready-to-use solutions without any further preparation steps such as sterilization. For clinical use reactor size for hMSC expansion is low (L-scale). On the other hand, the safety and hygiene requirements for therapeutic hMSC products are high. For the production of allogenic hMSC, which is more like a bulk production setup, disposables can be also preferable, because depended on production frequency and scale the overall costs for disposable setup can be lower compared to conventional production in stainless steel or glass reactors.

3 hMSC Expansion in Disposable Bioreactor Systems

3.1 Expansion of hMSC in Static Systems

The cultivation of hMSC is typically performed statically in standard systems like tissue culture flasks, culture trays, roller bottles, gas permeable blood bags or multi-well plates [19, 40, 41]. These mostly sterile disposable systems contain standard plastic surfaces. Static cultivation is widely used in research labs and many therapies and clinical trials have been developed with cells expanded this way [42].

However, these culture vessels offer a limited scale-up potential and little possibilities for process control and cannot be automated easily. Furthermore, the process monitoring of these systems requires sampling, which endangers the system sterility [19]. In terms of cell characterization, these systems enable an easy determination of growth rate and metabolite kinetics because of simple sampling of media and cell detachment for cell count measurements. These cultivation systems realize oxygen and nutrient supply by diffusion through the culture media causing mass transport limitations of nutrients, which can result in low cell densities. The low volume/surface ratio of these 2D systems is a further disadvantage resulting in low time-space yield and therefore a low process efficiency. Furthermore, the contamination risk and high personal demand decrease the process efficiency. Even though this production type might be appropriate for autologous cell production due to the comparatively small number of cells required per patient, the need of different efficient cultivation systems for mass production of hMSC is obvious.

3.2 Expansion of hMSC in Dynamic Systems – State of the Art

To overcome the limitations described in static cultivations of hMSC, dynamic carrier based systems are being used, but still limited to 200 mL volumes in non-disposable spinner flasks in most cases [24, 26, 30, 43 – 47].

Currently, different disposable dynamic bioreactor systems are commercially available, e.g., carrier-based spinners, wave-mixed, orbitally shaken and stirred reactors, as well as fixed-bed systems. As decades of knowledge in scale-up and process development is available for these systems, they are predestinated to being transferred to stem cell production. These systems can be used with disposable and/or standard online sensors for process monitoring. But compared to the reusable systems, the commercial availability of sensors or other reactor components is restricted [16]. In contrast to the static systems, cells in these systems reach much higher surface-to-volume ratios. A constant circulation (perfusion) and mixing is ensured by stirring, wave movement or rotation of the culture [40] enabling process monitoring as demanded by PAT.

The simplest dynamic disposable systems described are spinner flasks, usually used for process optimization because of their simple sampling procedure. However, in spinner flasks a satisfactory online monitoring is not possible and volumes of more than 1 L represent major exceptions [30]. A different, but also simple system is the wave-induced motion. First experiments of hMSC expansion on carrier in a rocket-motion bioreactor (WAVE) have been described. However, hMSC strongly agglomerated during the expansion process in this interesting reactor concept [48].

Perfusion systems for 3-dimensional cultivation of hMSC are fixed and fluidized bed reactors as well as wall-rotating vessels. The reactors were partially filled with microcarriers and perfused with media. These systems apply low shear, which is advantageous when cultivating sensitive adherent cells. Cell concentration is mostly measured indirectly by the determination of glucose or oxygen consumption. Cell sampling during the expansion process is not possible. Problematic is the formation of a metabolite gradient over the reactor height. As well as the suspension systems, perfusion systems can be automated. As an example an easy to automate and low shear fixed-bed reactor system for genetically modified hMSC-TERT was described by Weber at al. [23, 49 – 53]. The small scale of 20 mL was realized as disposable system in syringes. Weber also described greater volumes (up to 300-mL glass systems, offering 5500 cm^2 growth surface), which are an ideal candidate for a new disposable reactor system that is not commercially available yet. In this process, key metabolites and the final cell number at the end of cultivation were used for offline monitoring, resulting in a successful expansion process. Oxygen, pH and temperature were used for online process monitoring. Furthermore, the system has the advantage of being one single system for the inoculation, cultivation and harvest to ensure sterility and a high surface to volume ratio. The decisive disadvantage, however, is the inhomogeneity of the fixed-bed system at larger scale. A strong variability was observed concerning the cell growth and especially the harvest for cultivations in 300-mL fixed-bed volumes in standard configuration. This inhomogeneity makes an online monitoring of cell growth directly in the fixed-bed difficult. Another disadvantage is the performance of the flow rate in the system. The flow rate has to be very low ($\nu = 3 \cdot 10^4\ m\ s^{-1}$) to maintain the cell quality which limits scale-up options.

Small stirred tank reactors with a larger volume than spinner reactors are also available on the market as rigid systems by two different companies: Sartorius Stedim (UniVessel® SU) [54], and Merck-Millipore (Mobius™ CellReady) [55]. Both systems are compatible with various process control units due to different motor adapters. The UniVessel is equipped with two impellers, macrosparger, and disposable sensors for pH and DO, whereas the Mobius™ CellReady 3L bioreactor carries one marine impeller, micro- and macrosparger, no sensors, but a harvest port to the reactor bottom. Cell growth conditions under low shear stress can be enabled by the combination of bubble-free aeration and the use of

appropriate impeller design. The ultimate benefit of the stirred tank reactor is the homogeneity of the system. This simplifies the online monitoring of cell growth during the whole process and allows an easy scale-up. A balance between homogenous mixing, sufficient oxygen supply and gentle cell handling must therefore be achieved.

3.3 Expansion of hMSCs in Dynamic Systems – New Concepts

Based on an established process in a 3-L glass stirred tank reactor with a model cell line (hMSC-TERT) the transfer of the expansion process to disposables and primary hMSC is introduced. hMSC-TERT cells (passage 68 – 84) are bone marrow-derived, gene-modified cells, carrying the telomerase reverse transcriptase (TERT) gene. This increases the geno- and phenotypic stability of the cells without affecting their differentiation potential [56]. In clinical trials, these cells were used for allogenic cell therapy approaches [57]. For the established process, the transfer to disposable is likely, as a geometric similar stirred system is available on the market (Mobius™ CellReady 3L bioreactor). Especially with regards to cell harvest and online monitoring of the production process, the stirred tank reactor represents a promising solution [58 – 61].

The process in the glass stirred tank reactor was inoculated with a density of 3000 – 7000 cells per cm^2 growth surface, which were grown before in T-flasks. The inoculation strategy was performed in four adhesion cycles with stirring (80 – 160 rpm) and subsequent resting phase. Key metabolites and the final cell number, and also microscopic analysis of the cells on the microcarriers were used for offline monitoring because of the possibility of sampling during the production process. Oxygen, pH, temperature and the integrated permittivity measurement via the dielectric spectroscopy were used for online monitoring. Cells were cultivated in 1.7 L of DMEM with high glucose, which avoids an exchange of culture medium and the associated influence of the permittivity signal. For the cultivation, a growth surface of 13 500 cm^2 and a cultivation time of six days were used. The growth rate varied from 0.55 to 0.59 per day. At the end of the cultivation, a cell density of around 50 000 cells per cm^2 growth surface, a total cells number up to $4 \cdot 10^8$ cells per L of production volume, an expansion factor of 7.2 and a cell yield after harvest of over 95 % was determined. Quality control of the harvested hMSC-TERT showed high viabilities and differentiability [60].

Aspects to be considered for the carrier-based process transfer were the rotation speed, the aeration, the carrier concentration, the reaction volume and according to PAT, the possibility of process monitoring and control.

3.3.1 Transfer of hMSC-TERT Production Process in a Disposable System

Cultivation parameters of the established cultivation process of hMSC-TERT in a glass stirred tank reactor have been used for the transfer to the geometric similar disposable reactor (Mobius™ CellReady 3L bioreactor, Merck-Millipore). All parameters were kept constant except the stirrer speed, which was reduced to 60 rpm because of the change in stirrer diameter based on tip-speed calculation. The data of an expansion are shown in Fig. 1. A cell density of 3000 – 7000 cells/cm^2 (growth surface = 14 500 cm^2) was used to inoculate the disposable reactor, also performed in cyclic mode. Compared to an expansion process performed with allogeneic cells, the inoculation density used is higher because of the availability of a sufficient number of cells (autologous) and the possibility to minimize process time. During 6 days of cultivation, the stirrer speed was stepwise increased to 90 rpm to prevent cell agglomeration. Nevertheless, cell agglomeration occurred (Fig. 1a) indicating non-optimal culture conditions. The growth rates of 0.53 d^{-1} were comparable to the hMSC-TERT expansion in the glass tank. At the end of cultivation, cell harvest was performed by the use of an external sieve and a cell density of 48 500 cells/cm^2 growth surface, a total cells number of $4.1 \cdot 10^8$ cells per L of production volume, an expansion factor of 6.9 and a cell yield after harvest of over 99 % was determined. Thus, a successful transfer of hMSC-TERT production process into the disposable system could be shown. Further optimization of the process is needed to reduce the

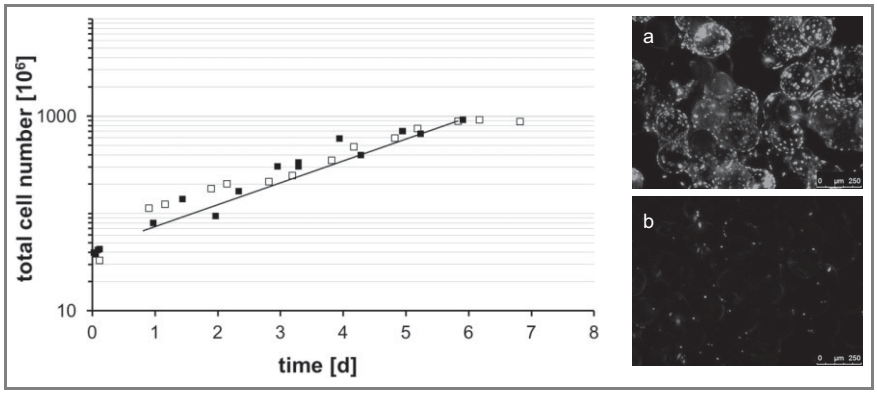

Figure 1. Expansion of hMSC-TERT in a disposable stirred tank reactor. The process was performed in a Mobius™ CellReady 3L bioreactor (Millipore) filled with RapidCell® carrier (MP Biomedicals). As cultivation medium DMEM-HG with 2 mM glutamine was used. Temperature was set to 37 °C, pH to 7.2. hMSC-TERT were inoculated with 3000 – 7000 cells/cm^2 and grown for 6 days under continuous stirring (60 – 90 rpm). Two different cultivations are exemplary shown (□ and ■). Cell growth was determined offline via a florescence assay [37]. At the end of the cultivation, cells were harvested with an external device using trypsin. a) SYBR green staining of carrier grown with hMSC-TERT after 6d cultivation; b) SYBR green staining of carrier after harvest of hMSC-TERT.

aggregate formation to guarantee an equal quality of the harvested cells.

3.3.2 Production Process for Primary hMSC in a Disposable System

After successfully realizing the production process for the model cell line hMSC-TERT in a disposable reactor, expansion of primary bone marrow-derived hMSC was investigated. It is assumed that primary cells react differently compared to continuous cell lines [14]. To evaluate this for primary hMSC, the impact of the cultivation conditions was determined by gene expression analysis. Therefore, hMSC, which were grown before on cell culture surfaces, were cultivated on collagen-coated SoloHill microcarriers either statically in T-flask or dynamically in the Mobius™ CellReady 3L bioreactor and compared to a standard cultivation of hMSC as monolayer in T-flasks. Prelimitary tests showed that best growth and harvest efficiency of primary hMSC was investigated by collagen-coated Solohill microcarriers. The agitation of the microcarriers (Fig. 2b) had the smallest effect compared to shearing cells in a device (data not shown) or the change from microcarrier culture to flask culture (Fig. 2a). The difference between flask culture and microcarrier generated 17 genes that were differentially regulated five times into three independent cell lines. To prove the functionality of the hMSC, dynamically cultivated cells were analyzed. hMSC taken from dynamic culture showed similar surface markers and differentiation capacity when compared to hMSC taken from the static culture.

The gene expression and functional analysis indicated that an expansion of primary hMSC in a disposable stirred tank reactor could give viable cells in a comparable quality as the standard static cultivations. The data of an expansion are shown in Fig. 3. A Mobius™ CellReady 3L bioreactor was inoculated with 800 primary hMSC per cm^2. In this case a low cell number is used for inoculation associated with an extended cultivation time because of the limitation of available autologous cells for expansion process. Growth surface in the reactor was provided by a collagen-coated carrier (SoloHill). At the beginning of the expansion process the growth surface was 5400 cm^2. During expansion the surface was increased to 10 800 cm^2 at day 7 and 12 960 cm^2 until process end. No special inoculation was performed; cells adhered well during slow stirring (35 rpm). During 12 days of cultivation, the stirrer speed was increased to 55 rpm and 75rpm subsequently. The increase in stirrer speed was combined with a medium feed (1 L DMEM-LG after 7 d, 0.4 L DMEM-HG after 11 d). This fed-batch strategy gave a 6-time higher expansion factor when compared to batch cultivations (data not shown).

Although the cell distribution on the carrier was quite inhomogeneous especially at the cultivation beginning (Fig. 3a), the primary bone marrow-derived hMSC grew fast and did not agglomerate very strong. The growth rates of the hMSC fed-batch expansion were 0.45 to 0.53 d^{-1}. Compared to the hMSC-TERT, expansion growth rates were comparable. Final cell density at the end of the expansion was 49 750 cells/cm^2 growth surface with a total cell number of $2.7 \cdot 10^8$ cells per L reaction volume (Fig. 3) and an expansion factor of 62. The expanded primary hMSC maintained their differentiability (Fig. 4). These results showed that mass of primary hMSC can be successful produced in a dynamic disposable reactor system.

3.3.3 System Modification for Process Optimization

The described disposable bioreactor requires an additional unit to allow cell harvest. To simplify the harvesting procedure and to minimize the contamination risk, the disposable reactor was modified. A stainless steel sieve in a polycarbonate construction was inserted into the bioreactor system (Fig. 5). This modification allows the separation of the cells

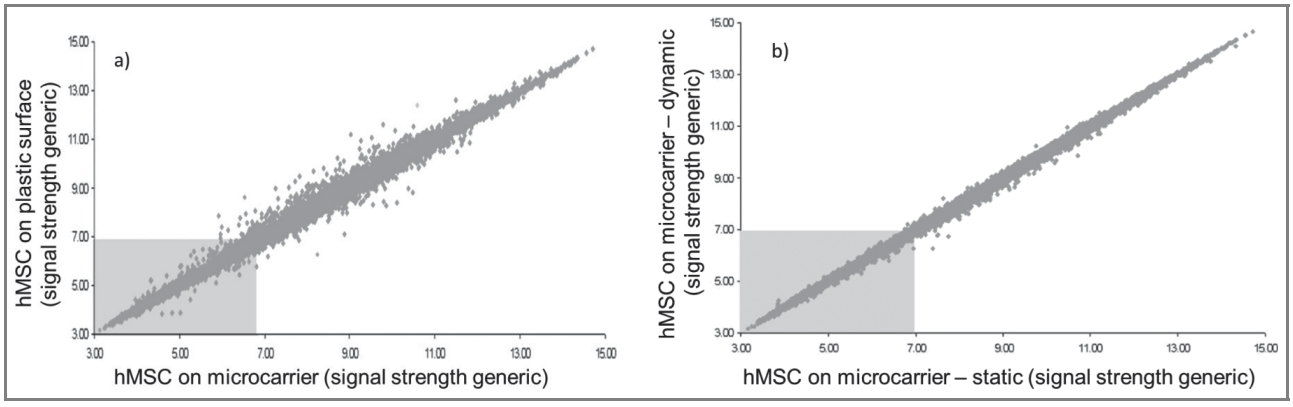

Figure 2. Impact of growth surface and cultivation system on hMSC gene expression. For gene expression analysis, hMSC were cultivated, harvested and frozen. mRNA und microarray analysis (Affymetrix Gene Chip Human Gene 1.0 ST) of the cells were performed by Precision Biotech (Illinois). a) Comparison of different growth surfaces. Primary hMSC were cultivated on collagen-coated plastic surfaces in T-flasks or on collagen-coated SoloHill microcarriers for 2 days in DMEM-LG containing 10 % MSC-qualified FBS (LifeTech), 2 mM glutamine und 8 ng rFGF2. b) Comparison of non-agitated and agitated cultivation. Primary hMSC were cultivated on SoloHill microcarriers for 2 days in DMEM-LG containing 10 % MSC-qualified FBS (LifeTech), 2 mM glutamine und 8 ng rFGF2. Cultivation was done in low-adhesion petri dishes (static) or in a Mobius™ CellReady 3L bioreactor (dynamic).

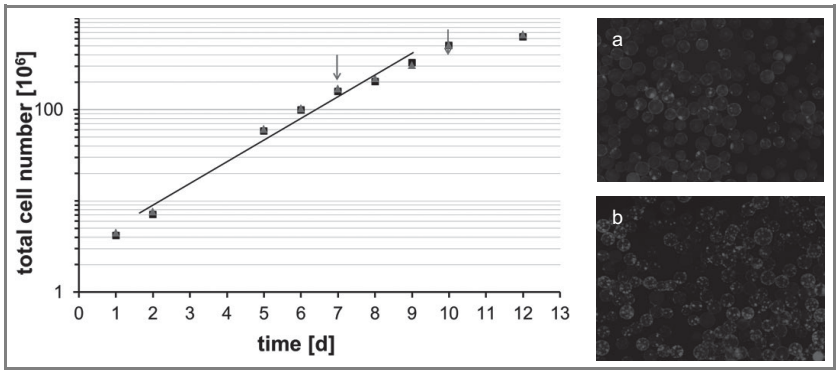

Figure 3. Expansion of primary hMSC in a disposable stirred tank reactor. The process was performed in a Mobius™ CellReady 3L bioreactor (Millipore) filled collagen-coated carrier (SoloHill). As cultivation medium DMEM-LG with 2 mM glutamine, 10 % MSC-qualified FBS (LifeTech) and 8 ng rFGF2 was used. The temperature was set to 37 °C, pH to 7.2. hMSC were inoculated with 800 cells/cm^2 and grown for 12 d under continuous stirring (35 – 75 rpm). Two different cultivations are exemplary shown (▲ and ■). The arrows indicate the feed with medium and carrier (1 L medium and 15 g carrier at day 7, 0.4 L medium and 6 g carrier at day 10). Cell growth was determined offline. DAPI staining of carrier grown with primary hMSC after a) 2 days cultivation; b) 8 days cultivation.

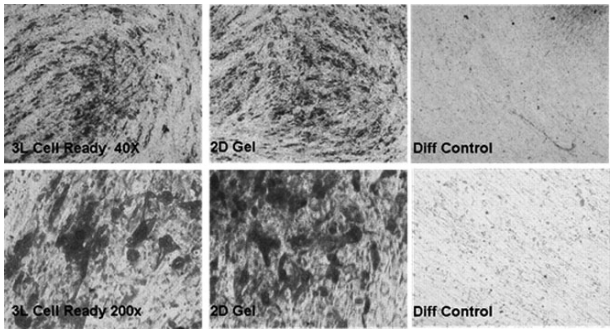

Figure 4. Differentiation potential of cultivated hMSC. Primary hMSC were cultivated dynamically in the Mobius™ CellReady 3L bioreactor or statically in T-flasks for 7d in DMEM-LG containing 10 % MSC-qualified FBS (LifeTech), 2 mM glutamine und 8 ng rFGF2. After cultivation, the cells were harvested and differentiated into adipocytes. Lipid droplets in the cells were stained with oil-red-o and analyzed via microscopy. Undifferentiated primary hMSC served as controls.

from the carriers after cell harvest. So cell detachment can be performed in the sterile reactor containment and the harvested cells can be pumped out of the reactor. At the end, the cell inoculation, expansion and harvest could be combined in one single disposable system [62]. Furthermore, the cell harvest within the reactor would allow the monitoring and control of the harvesting process according to PAT by dielectric measurement. Thereby, the enzymatic incubation time could be reduced to a minimum to avoid cell damage.

4 Conclusion

Over the last few years, it has been noticed that a commercial realization of cell therapy cannot succeed without a parallel development of manufacturing strategies for the therapeutic cells. The current expansion methods used in research or clinical trials are unsuitable for a reproducible, safe and controlled hMSC expansion with reasonable effort. Many academic and industrial groups work on process strategies and reactor concepts for hMSC expansion. Especially the use of disposable technologies seems to be promising for this application. Even though the advantages of disposable are convincing, long-term tests to proof material quality and exclude unwanted interactions with the product have to be done. Also availability of probes for online process control as disposables has to be ensured.

First studies have shown that both autologous and allogenic hMSC can be grown under dynamic and controlled conditions on microcarriers without losing viability and potency. Cultivations of hMSC-TERT in the glass bioreactor and the disposable system were comparable in cell yield. A higher expansion factor of cells compared to literature has been investigated [63]. However, in literature the cultivation was implemented with primary hMSC, a different inocula-

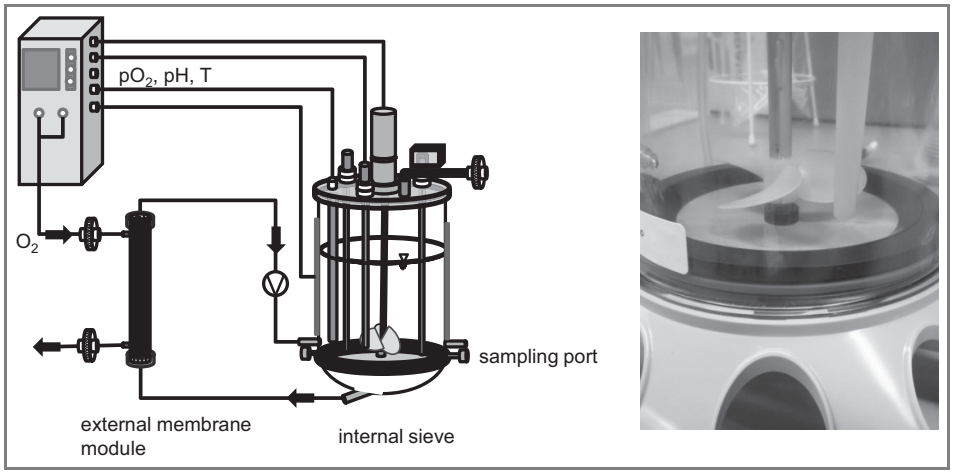

Figure 5. Modification of the Mobius™ CellReady 3L bioreactor to simplify cell harvest. The reactor was modified with an internal sieve to separate the carriers from the cells after cell harvest. Further, in addition to surface and microsparger aeration was performed bubble-free aeration via an external membrane module which decreased shear forces in the reactor.

tion density and a cultivation time of five days. As shown in [62] for autologous hMSC expansion the fed-batch-strategy combined with a low inoculation density gave the highest expansion factor. This extremely high expansion factor depended on the low inoculation density and was paid with a long process time. Total cell yield and cell number per cm^2 at the end of the primary hMSC expansion process was similar to allogenic hMSC-TERT expansion. So the superiority of an expansion strategy cannot only be discussed with the expansion factor but also with the process time, total cell yield and process costs. The disposable stirred tank system seems to be very suitable for the GMP production of high quality hMSC in high yield. It could be shown that the high process requirements for hMSC expansion have been complied in a disposable reactor system.

Nevertheless, besides the reactor system itself, further improvement needs to be done concerning basic cultivation tools for hMSC. For example, commercially available carriers are missing, which are exclusively optimized for cell adhesion. The possibility of cell harvest, which is imperative in hMSC expansion, is thereby not considered. Cell harvest from carriers is of little relevance for mammalian cell culture processes providing only few harvest strategies that are possibly adaptable to hMSC expansion process. Further, the optimal culture conditions for hMSC are hardly defined. Due to a lack in an absolute determination of hMSC quality it is almost impossible to truly investigate the impact of the cultivation parameters.

This means that research concerning hMSC production strategies and quality control urgently need to be further intensified. Unfortunately, in the past, these fields were mostly neglected. At the end, the reliability of hMSC manufacturing is significantly involved in the success of cell therapy. Without an effective manufacturing strategy cell therapy cannot be profitable.

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Symbols used

A	[cm^2]	surface area
c	[mmol L^{-1}]	concentration
l	[mm]	length
m	[ng, kg]	weight
n	[rpm]	rotation spped
t	[d]	time
V	[mL, L]	volume
$\bar{v}$	[m s^{-1}]	superficial velocity
pCO_2	[bar]	partial pressure of carbon dioxide
pO_2	[bar]	partial pressure of oxygen
T	[°C]	Temperature
$\bar{\mu}$	[d^{-1}]	specific average growth rate
μ_{max}	[d^{-1}]	specific maximal growth rate

Abbreviations

ATMP	advanced therapy medicinal product
cGMP	current good manufacturing practice
CIP	cleaning in place
DMEM	Dulbecco's modified eagle medium
DO	dissolved oxygen
FBS	fetal bovine serum
GMP	good manufacturing practice
HG	high glucose
hMSC	human mesenchymal stem cells
LG	low glucose
PAT	process analytical technology
rFGF2	recombinant fibroblast growth factor 2
SIP	sterilization in place
TERT	telomerase reverse transcriptase

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