Research Article

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Modification and qualification of a stirred single-use bioreactor for the improved expansion of human mesenchymal stem cells at benchtop scale

Background: To improve cultivation conditions for human bone-marrow-derived mesenchymal stem cells, we redesigned the commercially available UniVessel[®] SU bioreactor using results obtained from computational fluid dynamics. The goal was to produce $\geq 1 \times 10^9$ cells and to achieve expansion factors ≥ 30 . Screening studies suggested that microcarrier solid fractions of at least 0.3% are required to reach the appropriate cell densities. **Results:** The fluid flow pattern found in the most promising modification (#2) was altered by increasing the impeller blade angle and lowering the off-bottom clearance. As a result, the maximum required specific power input was reduced by a factor of 2.2–4.6, depending on the microcarrier concentration, and peak cell densities were 3.4-times higher than in the standard version. **Conclusion:** The peak cell number of nearly 1.1 × 10⁹ cells (expansion factor = 35), which was achieved in our low-serum cultivations, indicates an improvement in the redesigned UniVessel[®] SU configuration for bone marrow-derived mesenchymal stem cell expansions.

Because of their high proliferation and differentiation capability, human mesenchymal stem cells (hMSCs), which are anchoragedependent, have a great deal of potential in human medicine [1-4]. So it comes as no surprise that they are used for a broad range of applications, as current pre- and clinical studies have shown, which are, for example, aimed at bone and cartilage regeneration, the treatment of myocardia, metachromatic leukodystrophy and Hurler syndrome [1,5-7]. However, to maintain the required number of cells at the desired consistent quality, an alternative to the planar cultivation systems that currently prevail is required [8].

Stirred bioreactors, in which adiposetissue- and bone-marrow-derived hMSCs are grown on solid or porous microcarriers (MCs), have been shown to be suitable at benchtop and pilot scales [8-13]. This is particularly true for single-use versions [10,12], whose main advantages are their ability to provide high process safety and flexibility. In their recently published paper, Schirmaier *et al.* [12] presented a successful approach for rapidly scaling up the expansion of adiposetissue-derived hMSCs from spinner flasks to two single-use stirred bioreactors: the Uni-Vessel® SU 2L and the CultiBag STR 50L (both from Sartorius Stedim Biotech, Göttingen, Germany). This approach required extensive screening studies in spinner flasks to define the optimal culture medium composition, MC type and operating conditions. Based on numerical simulations of the bioreactor types, model verification (particle image velocimetry [PIV]) and suspension investigations, optimum impeller speeds were predicted and main engineering parameters (e.g., local shear stress, turbulent dissipation rate, Kolmogorov microscale) for subsequent expansions were calculated for the different scales. The authors achieved the highest peak cell numbers and expansion factors reported to date for MC-based expansions of hMSCs in single-use stirred bioreactors. They point out the importance of the suspension criteria $N_{s_{11}}$ and N_{s_1} (which represent the lowest impeller speeds required for the MCs to become just-suspended) in bioreactor-based stem cell cultivations with MCs. It is worth mentioning that these suspension criteria had

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Key Terms

Particle image velocimetry: Contactless laser-based method for fluid velocity measurements.

Computational fluid dynamics: Numerical method for the prediction of fluid flow patterns and key engineering parameters.

 N_{s1u}/N_{s1} : Suspension criteria describing microcarrier distribution at just fully suspended conditions (N_{s1}) and below (N_{s1u}).

Human mesenchymal stem cell expansion: Propagation of bone-marrowderived human mesenchymal stem cells in a stirred single-use bioreactor system.

Stirred single-use bioreactor: Bioreactor type reported to be suitable for growing human mesenchymal stem cells on microcarriers.

already been applied by other authors [14-16] for this purpose.

The N_{S1} criterion represents the minimum impeller speed required to just fully suspend all MCs in the bioreactor (MC contact to bioreactor bottom <1 s) [17,18]. However, this does not necessarily mean that a homogeneous dispersion of all MCs can be achieved throughout the liquid medium [19]. By contrast, the N_{S1n} criterion displays the lower limit of N_{S1} , when the MCs are still located at the bottom of the bioreactor but none of them are at rest. In other words, there is a movement of MCs along the bioreactor bottom so that no MC is in the same position for longer than 1 s [18]. The stirrer speeds and $N_{_{S1u}}$ and $N_{_{S1}}$ and resulting fluid flow patterns are strongly dependent on MC concentration; increase ensures further increased cell numbers. However, in cultivations with adipose tissue- and bone marrow-derived hMSCs grown on solid and porous MCs in the UniVessel® SU in our lab, it was necessary to work below the N_{S1u} criterion for MC solid fractions ≥0.3% in order to facilitate good cell proliferation.

Based on these results, the question was asked: is it possible to improve the suspension characteristics of the UniVessel® SU for hMSC expansions with MC solid fractions exceeding 0.3% through minor design modifications to the benchtop bioreactor? It was decided to focus on impeller modifications resulting in a reduction in specific power input and, thus, a reduction in shear stress, which influences cell numbers and cell quality. The goal of this study was to generate 1×10^9 human bone marrow-derived MSCs (hBM-MSCs) per batch in a modified bioreactor within 10 days. As a first step, nine different impeller designs were developed and analyzed using computational fluid dynamics (CFD) investigations. Prototypes of the most promising modifications were constructed and suspension criteria were determined experimentally. Improvements in fluid flow were validated using PIV measurements. Finally, the technological superiority of the modified UniVessel® SU for hBM-MSC expansions was demonstrated in comparative cultivation studies that were also performed in the standard version of UniVessel[®] SU and spinner flasks.

Materials & methods CFD as a tool for designing the new vessel

concept For all simulations, the fluid flows were modeled using the finite volume solver Fluent 14 (ANSYS Inc., PA, USA), for which the governing equation for mass and momentum of the single-phase flow can be described by Equations 1 & 2.

$$\frac{\partial \rho}{\partial t} + \nabla (\bar{u} \cdot \rho) = 0$$

$$\frac{\partial(\rho \cdot \bar{u})}{\partial t} + \nabla(\rho \cdot \bar{u} \cdot \bar{u}) = -\nabla p + \nabla \cdot \bar{\bar{\tau}} + \rho \cdot \bar{g} + \vec{F}$$

The symbols ρ , \bar{u} and p represent fluid density, velocity vector and static pressure, respectively. In addition, the terms ρ , \bar{g} and \bar{F} denote the volume-related gravitational and external body forces. The Reynolds stress tensor $\bar{\tau}$ was described by the k– ϵ turbulence model, where the turbulent kinetic energy k and the turbulent dissipation rate ϵ were calculated by two separate transport equations [20].

In addition to single-phase flow simulations, multiphase flow investigations, which take MC distributions into account, were performed at experimentally determined impeller speeds (N_{S1u} , N_{S1}). For this purpose, an Euler–Euler RANS (Reynolds-averaged Navier-Stokes) approach was used, which considered water as the continuous phase and the MC as the disperse phase. Here, the mass and momentum equations (Equations 3 & 4) can be described for the qth phase as follows.

$$\frac{\partial (\alpha_i \cdot \rho_i)}{\partial t} + \nabla (\alpha_i \cdot \bar{u}_i \cdot \rho_i) = 0$$

$$\frac{\partial (\alpha_i \cdot \rho_i \cdot \bar{u}_i)}{\partial t} + \nabla (\alpha_i \cdot \rho_i \cdot \bar{u}_i \cdot \bar{u}_i)$$

$$= -\alpha_i \cdot \nabla p + \nabla (\alpha_i \cdot \bar{\tau}_i) + \alpha_i \cdot \rho_i \cdot \vec{g}_i \cdot \vec{F}_i$$

The index i represents either the liquid (L) or the particular phase (P). Momentum exchange resulted in the coupling of the two phases and the drag force was modeled by Equation 5.

$$\vec{F} D = \frac{3}{4} \cdot \frac{\rho_{\scriptscriptstyle L}}{\rho_{\scriptscriptstyle P} \cdot d_{\scriptscriptstyle P}} \cdot m_{\scriptscriptstyle P} \cdot c_{\scriptscriptstyle D} \cdot (\vec{u}_{\scriptscriptstyle L} - \vec{u}_{\scriptscriptstyle P}) \cdot |\vec{u}_{\scriptscriptstyle L} - \vec{u}_{\scriptscriptstyle P}|$$

The drag coefficient c_D was modeled by the standard Schiller and Neumann model [21]. For the simulations, the MCs were considered to be spherical particles with a mean diameter of 255 μ m and a density of 1030 kg m⁻³. The maximum volume fraction of the disperse phase was restricted to 0.63. In all cases, the discretization

of the fluid domain was realized with an unstructured body-fitted mesh, consisting of approximately 770,000 tetrahedral elements (control volumes). The SIMPLE algorithm was used for pressure–velocity coupling, and convergence was assumed when the residuals decreased to below 10⁻⁵.

PIV measurements for CFD data validation

All PIV measurements were carried out using a Flow-Master PIV system (LaVision, Göttingen, Germany), in which the illumination of the field of investigation was performed by a double-pulsed Nd:YAG laser generating a 1-mm-thick laser sheet ($\lambda = 532$ nm; litron lasers, Rugby, UK). The laser sheet was vertically oriented through the impeller shaft (mid-bioreactor plane) to capture the axial velocities at four different heights in a vertical direction. An Imager Pro X4 CCD camera (LaVision) with a resolution of 2048 × 2048 pixels was used for image capturing and was positioned at a 90° angle relative to the laser field. DaVis® 7.2 software (LaVision) was used to control the camera, the traverse system and the laser, as well as for image acquisition and flow-field prediction. Rhodamincoated fluorescence particles with a density of 1.19 kg/m³ (LaVision) were added into the reactor to allow the flow field to be seen. A set of 1000 images per position, based on an interrogation window of 32×32 pixels with an overlap of 50%, were recorded in order to obtain statistically significant results. The velocity components were determined by deducing the instantaneous displacement of the illuminated particles at two instants in time using cross-correlation. Phase-locked measurements were recorded by means of a photoelectric barrier focused on the reactor shaft.

Determination of optimal process parameters using suspension investigations

In order to avoid MC sedimentation and resulting gradient formation in the UniVessel[®] SU systems, experimental investigations were carried out to evaluate the MC suspension characteristics. Suspension experiments were performed for working volumes of 2 l to determine the N_{S1u} and N_{S1} criteria. All suspension experiments in the standard UniVessel[®] SU as well as the modifications were carried out in the rigid, transparent plastic vessels. A mirror was placed below the vessels for better optical accessibility of the bioreactor bottoms. The investigations were performed for MC solid fractions of 0.1, 0.2, 0.3, 0.4 and 0.5%, which provided sufficient growth area for the hBM-MSCs.

hBM-MSC expansions in the standard & modified UniVessel® SU

Cryopreserved hBM-MSCs from the first passage were used for the cultivations. The cells were obtained

from a single, informed, consenting donor and were provided by Lonza Cologne (Germany). A standard UniVessel[®] SU as well as a modified version was inoculated with 30×10^6 cells for an MC solid fraction of 0.3% in a 2 l working volume of a specially developed serum-reduced medium (Lonza, MD, USA).

Before inoculation, 0.7 l of the MC suspension was equilibrated overnight in the bioreactor at 37°C and a pH of 7.2–7.4. During the equilibration phase, the stirrer speed was set to the N_{slu} criterion. After inoculation with thawed cells, only the temperature was controlled during the 16 h attachment phase. Afterward, the reactor was topped up to the final volume (2 l) with preheated medium and the closed loop controls for O₂ and pH were started. The hBM-MSCs were cultivated at 37°C, pH 7.2 and 0.1 vvm (head space aeration) for 9 days. Cascade-link aeration was performed via a sparger for oxygen saturation <20%.

Daily offline sampling was performed to determine the cell density, substrate and metabolic profiles (glucose, lactate, glutamine, ammonia) as well as for the 4',6-diamidin-2-phenylindol staining. The sampling was done at 10% higher stirrer speeds. Cell density, substrate and metabolite concentrations were measured using a NucleoCounter NC-100 (chemometec, Allerod, Denmark) and a Cedex Bio (Roche, Risch, Switzerland).

In addition to the experiments in the standard and modified UniVessel[®] SU, control spinner experiments (Corning, MA, USA) with analogous conditions (1.5×10^6 cells for a MC solid fraction of 0.3%) were carried out for 100 ml working volume.

Flow cytometric investigations were performed by random sampling. For this purpose, MC-free, purified hBM-MSC samples (centrifuged, washed and frozen) were thawed and stained with fluorochrome-conjugated antihuman CD34, CD45, CD73, CD90 and CD105 antibodies (eBioscience, CA, USA or BD Bioscience, Heidelberg, Germany) in accordance with the minimum criteria recommended by the International Society for Cellular Therapy.

Results & discussion

New vessel design & flow-field improvements

Since axial velocities have been shown by various authors to have a significant impact on the suspension of MCs, improving these axial velocities was the main objective of the exercise to define the dimensions for the bioreactor modifications [22,23]. Taking this into consideration, nine UniVessel[®] SU designs with different impeller diameters (d), blade angles (β) and off-bottom clearances (c) were investigated in advance using numerical fluid flow simulations. The geometrical dimensions of the designs, which were investigated at constant impel-

Research Article Jossen, Kaiser, Schirmaier et al.

Table 1. Geometrical dimensions, specific power inputs, local normal and shear stress levels as well as recirculation numbers for the standard and modified UniVessels® SU.											
Characteristics		Standard	Modified UniVessel								
			#1	#2	#3	#4	#5	#6	#7	#8	#9
D	[mm]	130	130	130	130	130	130	130	130	130	130
H	[mm]	179	179	179	179	179	179	179	179	179	179
d	[mm]	54	54	54	72	72	72	54	54	72	72
с	[mm]	54	35	35	35	54	54	54	35	54	35
s	[mm]	70	73	73	73	64	64	64	73	64	73
β	[°]	30	30	45	30	30	45	20	20	20	20
d/D	[-]	0.41	0.41	0.41	0.55	0.55	0.55	0.41	0.41	0.55	0.55
c/D	[-]	0.41	0.26	0.26	0.26	0.41	0.41	0.41	0.26	0.41	0.26
s/d	[-]	1.29	1.35	1.35	1.01	0.88	0.88	1.18	1.35	0.88	1.01
P/V	[W m ⁻³]	1.6	0.6	2.4	0.9	0.9	1.5	1.3	1.3	0.8	4.0
$\tau_{nn}^{\ t}$	[10 ⁻³ N∙m ⁻²]	2.63/656	2.20/806	2.08/554	6.27/1454	5.66/1401	5.39/1463	1.58/697	1.73/664	5.45/1651	2.72/1724
$\tau_{nt}^{\ \ *}$	[10⁻³ N∙m⁻²]	0.81/482	0.93/798	0.65/429	3.12/1015	2.64/1078	2.28/659	0.53/618	0.82/661	2.55/1193	1.52/3177
Fl _{z,p}	[-]	0.29	0.28	0.87	0.52	0.49	0.7	0.67	0.60	0.45	0.39
The specific power inputs, local normal and shear stress levels as well as the recirculation numbers are given for constant impeller speeds of 100 rpm.											

*LSS: local shear stress given with volume-weighted mean/maximum values.

shown for the standard UniVessel® SU (Figure 1). Based

ler speeds of 100 rpm, are summarized in Table 1 and on the findings of the theoretical investigations, modification number 2 showed the most favorable properties







Figure 2. Dimensionless fluid magnitude (A1, B1) and axial (A2, B2) velocities in the standard (A) and modified (B) UniVessel® SU. Contour plots are given for the mid-reactor and mid-impeller planes.

for the cultivation of the hBM-MSCs, with regard to the dimensionless primary axial recirculation number ($Fl_{z,p}$) as well as local normal and shear stress levels ($_{n}$) (Table 1). It should be noted that just decreasing the offbottom clearance, as present in modification number 1, was expected to be insufficient to improve MC suspension, even though data from the literature indicates such an effect [17]. With modification number 1, flow partitioning at the reactor wall was still evident, which may hamper the swirling up of the MCs. For this reason, it was decided to focus on modification number 2 in subsequent investigations.

As shown in Figure 2A1 & B1, the fluid flow is primarily axial in both systems and agrees well with other computational investigations [24]. In accordance with our expectations, fluid velocities were highest at the tips of the impellers, correlating well with the theoretical tip speed ($u_{tip} = \pi \cdot d \cdot N$). A strong inclination of the flow toward the reactor wall was found in the standard Uni-Vessel[®] SU, resulting in two flow loops and weak axial fluid velocities near the reactor bottom (Figure 2A2). This strong flow inclination occurs as a result of the relatively low impeller blade angle (30°) as well as the high off-bottom clearance (0.41), and significantly hampered the suspension of the MCs. By contrast, flow partition was avoided in modification number 2 (Figure 2B2). This can be explained by the higher impeller blade angle of 45° and the 35% lower off-bottom clearance, which is, at c/D = 0.26, within the typical range for suspension processes when compared with literature data (c/D = 0.1–0.3) [18]. As a result, approximately 20% higher axial fluid velocities were found in the discharge from modification number 2 for comparable suspension conditions (N_{s1u}, N_{s1}). In addition, the axial fluid velocity impinged on the reactor bottom and allowed the MCs to swirl up at lower impeller speeds, resulting in lower specific power inputs (see Table 2).

The improved axial discharge of the modified impeller was also confirmed by the dimensionless primary axial recirculation or pumping number ($Fl_{z,p}$), which is described by Equation 6 [23]. The integral was evaluated for both systems on a plane as close as possible to the impeller, in the pumping direction.

$$Fl_{z,p} = \frac{2\pi}{N \cdot d^3} \int_{r=0}^{r=\frac{d}{2}} r \cdot v_z(r) dr$$

Table 2. Summary of CFD-predicted specific power inputs, shear stress levels, turbulent dissipation rates and Kolmogorov microscales of turbulence for the N_{s1u} suspension criterion of different microcarrier solid fractions.

UV	MC [%]	Impeller speed N _{s1u} [rpm]	Tip speed u _{tip} [m s ⁻¹]	Reynolds Nr Re [-]	Specific power input P/V [W m ⁻³]	LSS τ _{nt} [10 ⁻³ N m ⁻²]	TEDR ε [10 ⁻³ m ² s ⁻³]	KMST l _σ [μm]
Standard	0.1	107	0.30	5200	2.02	1.01/585	0.45/528	29/168
	0.2	110	0.31	5346	2.08	1.03/645	0.48/585	28/163
	0.3	115	0.32	5589	2.39	1.05/655	0.53/642	27/159
	0.4	118	0.33	5735	2.68	1.08/660	0.59/661	27/156
	0.5	122	0.34	5929	2.90	1.10/668	0.67/696	26/151
Modification	0.1	55	0.16	2662	0.48	0.42/212	0.18/165	40/252
(#2)	0.2	63	0.18	3050	0.70	0.50/248	0.28/237	36/228
	0.3	70	0.20	3388	0.95	0.64/288	0.38/301	33/210
	0.4	75	0.21	3630	1.16	0.67/315	0.49/367	31/198
	0.5	77	0.22	3727	1.24	0.73/325	0.52/396	31/195

KMST: Kolmogorov's microscale of turbulence (volume-weighted minimum/mean values); LSS: Local shear stress (volume-weighted mean/maximum values); MC: Microcarrier solid fraction; TEDR: Turbulent energy dissipation rate (volume-weighted mean/maximum values); UV: UniVessel® SU.

The primary axial pumping number in the modified UniVessel[®] SU ($\text{Fl}_{z,p}$ 0.87) was approximately threefold higher than for the standard version, showing that a fluid element (or even an MC) passes over the same area more frequently, guaranteeing sufficient mixing. Various authors obtained primary pumping numbers in the range of 0.17 to 1.27 for downward pumping stirrers [22,23,25,26], indicating that the modified Uni-Vessel[®] SU ensures sufficient axial flow for hBM-MSC cultivations on MCs.

The CFD-predicted MC distribution in the standard and modified UniVessel® SU was compared at 70 rpm for a solid fraction of 0.3% (Figure 3). Not entirely surprisingly, a relatively high volume fraction was found in the standard configuration under the stirrer shaft (orange-red zones; contour plot), showing an insufficient swirling of the MCs for this stirrer speed. This effect arose from the relatively weak axial velocities and the recirculation loop in the lower part of the reactor. A relatively narrow distribution of the MC volume fraction was achieved in the modified UniVessel® SU, indicating a more homogeneous suspension throughout the reactor volume than in the standard version. The highest volume ratios for MC volume fractions were found to be 2.9×10^{-3} for both the standard and modified bioreactor versions, which is similar to the volume-averaged MC volume fraction. A heavy-tailed distribution was identified in the standard UniVessel® SU as a result of the deposited MCs under the stirrer shaft. The spatial MC distribution observed at the reactor bottom showed good agreement in terms of position and shape to the simulated data. Thus, the MC distribution was accurately predicted by the multiphase CFD models.

Flow-field validation

To validate the numerical models, the post-processed data from the simulations (single and multiphase) were compared with the experimental PIV measurements along the radial profile in the lower part of the reactor ($H_1/D = 0.12$). Only slight differences in the axial velocity profiles were predicted between the single-phase and multiphase CFD models. These findings agree well with recent investigations in small-scale bioreactor systems (Figure 4) [15]. Therefore, it can be assumed that the MCs follow the fluid flow in an almost slip-free fashion in both systems, without significant momentum exchange between the two phases. This can be explained by the low-density difference and the small particle sizes. Only minor differences in the mean relative deviation (δ), of less than 10%, were found between the predicted CFD and measured PIV data for the standard UniVessel® SU using Equation 7.

$$\delta_{r} = \frac{\sqrt{\frac{1}{X} \sum_{i=1}^{X} (X_{exp_{i}} - X_{sim_{i}})^{2}}}{\sqrt{\frac{1}{X} \sum_{i=1}^{X} X_{exp_{i}}^{2}}}$$

Furthermore, the relatively low axial velocities in the lower part of the reactor were also recognizable in



Figure 3. Volume-weighted frequency distributions and contour plots of the microcarrier volume fractions α for the (A) standard and modified UniVessel[®] SU at an impeller speed of 70 rpm (0.3% microcarrier solid fraction). The distribution was discretized into 200 bins and logarithmically scaled.

experimental investigations of the standard UniVessel[®] SU. Therefore, it can be postulated that the established single-phase and multiphase CFD models provide reliable fluid flow predictions. The axial velocities in the modified UniVessel[®] SU were slightly over- or underestimated by approximately 0.1 m s⁻¹ (r/R 0.2–0.5; 0.8–0.85), which resulted in a mean relative deviation of 18%. The differences may be explained by the strong curvature of the reactor surface, which may have introduced inaccuracies into the optical measurements. Nevertheless, the CFD velocity profiles were well captured, and the overall agreement of PIV and CFD was satisfactory.

Determination of suspension characteristics

After determining fluid flow, the MC suspension characteristics (N_{S1u} , N_{S1}) were investigated in the standard and modified UniVessel[®] SU for their dependence on MC solid fractions. For all solid fractions investigated, it was found that N_{S1u} was fulfilled at approximately 20% (average) lower impeller speeds than N_{S1} (Figure 5). This result agrees well with those found in previous suspension investigations in other benchtop scale reactor systems [10,15]. Independent of the MC concentration, the increase in the impeller speed required to achieve suspension characteristics with increasing solid fractions was higher than the experimental errors of 5% described by Bohnet *et al.* [27].

In accordance with our expectations from CFD investigations, significantly lower impeller speeds of 70–90 rpm (depending on MC solid fraction) were required to just-suspend the MCs (N_{s1}) in the modi-



Figure 4. Comparison of dimensionless axial fluid velocities predicted by computational fluid dynamics (single phase: solid line; multiphase: dashed line) and measured by particle image velocimetry (dotted line) for the standard (A) and modified (B) UniVessel[®] SU. The results are compared for coordinates along a line over the vessel radius in the lower part of the reactor (H,/D = 0.12).

fied UniVessel® SU. Owing to the lower off-bottom clearance, the impeller speeds were reduced by approximately 40%. These findings again correspond well with observations from other groups [26]. Owing to these lower impeller speeds, 2.2–4.6-times lower maximum power inputs (0.47–1.24 W m⁻³) were necessary to just-suspend the MCs in the modified UniVessel® SU, resulting in a gentler mixing of the MCs. Furthermore, the required specific power inputs, which agreed well with experimental torque measurements (data not shown), were in a range comparable to those observed for Corning spinner flasks, which are frequently applied in MC-based hMSC cultivations (Figure 6) [10,15].

Reduction of shear stress & turbulence levels

Based on the experimentally determined impeller speeds for the N_{S1u} criterion, the shear stress distributions in the bioreactors were computed. For this purpose, the shear stresses were calculated in accordance with Wollny *et al.*'s method [28] (Equation 8), which obtains logarithmical normal distributions similar to values obtained for other conventional and single-use bioreactors [24].

$$\tau_{nt} = \sqrt{\left(\frac{\partial \tilde{w}_x}{\partial \tilde{y}} + \frac{\partial \tilde{w}_y}{\partial \tilde{x}}\right)^2 + \left(\frac{\partial \tilde{w}_x}{\partial \tilde{z}} + \frac{\partial \tilde{w}_z}{\partial \tilde{x}}\right)^2} \cdot \eta$$

As expected, the shear stress levels in both systems increased as stirrer speeds increased (Table 2), and the

highest shear stress levels were found to be located near the impeller tips. However, in both cases the predicted shear stresses in the bulk of the suspension were approximately two orders of magnitude lower than in the stirrer region. The mean $(0.42-40.73 \times 10^{-3} \text{ Pa})$ and maximum $(212-325 \times 10^{-3} \text{ Pa})$ shear stress levels were reduced by approximately 50% by the adaptations to the UniVessel® SU design and the resulting lower stirrer speeds. Since investigations by Yeatts *et al.* [29] have shown that high shear stress can cause inadvertent differentiation of hMSCs, a reduction in the shear stress is not only important for hMSC growth but may also contribute to preventing undesired cell differentiation.

In addition to shear stress, the Kolmogorov theory of isotropic turbulence was also taken into account when evaluating hydrodynamic stress in the two UniVessel® SU configurations (Equation 8). This presupposes that the flow is highly turbulent (Re < 10^4 ; see Table 2). In this study, the flow was in a transient region between laminar and fully turbulent conditions. However, the maximum dissipation rates in the impeller swept volume were significantly higher than in the bulk of the suspension, which agreed well with numerical investigations in other stirred systems [30,31].

$$l_{\lambda} = \left(\frac{\eta^3}{\rho^3 \varepsilon}\right)^{0.25}$$



Figure 5. Experimentally determined microcarrier suspension characteristics (N_{stu} = dark gray bar, N_{st} = shaded bar) for the standard (left) and modified (right) UniVessel® SU. A theoretical deviation of 5% was taken into account for all determined stirrer speeds in accordance with Bohnet *et al.* [27].

Investigations by Abranches et al. and Kehoe et al. have shown that stem cells that are attached to MCs appear more sensitive to turbulent eddies than suspension cells [32,33]. This phenomenon may be explained by the higher probability of MC collisions that might damage the cells [34]. It was found that cell damage became significant when the Kolmogorov microscale was approximately two-thirds of the MC size [35]. As expected, the volume-weighted mean (151–168 µm) Kolmogorov microscales determined for the standard UniVessel® SU were closer to the critical values of two-thirds of the MC size (170 µm). Because of the higher stirrer speeds required, higher energy dissipations occurred, which were inversely proportional to the Kolmogorov microscale. Considering the MC size of approximately 255 µm, the CFD results indicated that turbulent eddies with sizes comparable to and smaller than the MCs were present. The volumeweighted mean values of the Kolmogorov microscales were 29-49% higher in the modification, indicating that the volume fraction of the turbulent eddies of comparable size was reduced. However, it should be emphasized that no predictions were made for the Kolmogorov microscale under fully turbulent conditions, for which the applied $k-\varepsilon$ turbulence model has been developed. Thus, the shear stress levels were considered to be more appropriate than the Kolmogorov microscale in the present study.

hBM-MSC cultivation in the standard & modified UniVessel® SU

In order to biologically verify the simulation results, hBM-MSCs were cultivated in modified and standard UniVessel® SUs as well as in Corning® spinner flasks. In the modified UniVessel® SU the cell density $(5.3 \times 10^5 \text{ cells ml}^{-1})$ obtained after 9 days of cultivation was three-times higher than in the standard version, resulting in a total cell number of 1.06 × 10⁹ cells (Figure 7A). The expansion factor for hBM-MSCs (which was 10 for the standard UniVessel® SU) could be increased by approximately 3.4-fold through the adaptation of the UniVessel® SU design. A slower increase in cell concentration (μ_{max} = 22.2 × 10⁻³ h⁻¹ vs 31.0×10^{-3} h⁻¹) was found in the standard UniVessel® SU, most likely due to higher cell stress. The cell density in the Corning® spinner was slightly lower $(4.4 \times 10^5 \text{ cells ml}^{-1})$ than in the modified UniVessel® SU. Nevertheless, comparable growth was recognizable for the hBM-MSCs in both cases, which can be ascribed to the comparable specific power input and shear stress levels. In fact, the reduced mean and maximum shear stress levels of $0.64-288 \times 10^{-3}$ Pa observed in the modified UniVessel® SU appear to be beneficial for the expansion of the hBM-MSCs.



Figure 6. Specific power inputs in the modified and standard UniVessel® SU configurations dependent on microcarrier solid fraction. The specific power inputs in the modified UniVessel® SU are reduced by a factor ranging between 2.2 and 4.6 when compared with the standard UniVessel® SU. The adaption of the impeller and taking the N_{s1} and N_{s10} criteria into consideration resulted in similar power input values to those determined for Corning spinner flasks that are well established in human mesenchymal stem cell cultivations.

So it was no surprise that the flow cytometric analysis showed that the hBM-MSCs maintained their morphological and phenological (CD34⁻, CD45⁻, CD73⁺, CD90⁺, CD105⁺) stem cell properties during the cultivation (Figure 7B).

Conclusion & future perspective

A redesign of the commercially available UniVessel[®] SU bioreactor has been accomplished by means of CFD and experimental investigations. We have demonstrated that the numerical models in an Euler– Euler framework that consider the MC as a separate disperse phase agree well with empirically generated data. Moreover, it suggests that CFD modeling is an effective technique for the characterization and optimization of fluid flow. Using CFD, the number of bioreactor vessel prototypes and their characterization, which is expensive and time consuming, can be reduced.

The approach also illustrates that the fluid flow in the UniVessel[®] SU can be easily adapted for the cultivation of hBM-MSCs on MCs. In fact, only slight modifications to the impeller blade angle and the off-bottom clearance were necessary to greatly reduce the impeller speeds to achieve the required suspension criteria (N_{S1u} , N_{S1}) and MC solid fraction. Furthermore,

Research Article Jossen, Kaiser, Schirmaier et al.



Figure 7. Results of hBM-MSC cultivations. (A) Typical cell densities, dependent on cultivation time and cultivation system, achieved in experiments with a microcarrier solid fraction of 0.3%. (B) Results of flow cytometric analysis (CD73⁺, CD90⁺, CD105⁺, CD34⁻, CD45⁻) performed with hBM-MSCs expanded in the UniVessel[®] SU (#2). Gates of the flow cytometric analysis were set based on the isotype control.

we could confirm that the fluid flow and MC distribution inside the single-use bioreactor influence the growth of hBM-MSCs.

It was demonstrated that the improvement in the fluid flow resulted in a reduction of shear stress levels in the modified UniVessel[®] SU. Peak cell numbers of 1.06×10^9 cells and expansion factors of up to 35, observed in low-serum hBM-MSC expansions, clearly indicate the superiority of the redesigned benchtop bioreactor compared with the standard version.

Subsequent studies with the modified UniVessel® SU will focus on further optimization of the hBM- MSC production process in order to create autologous cellular therapies. Furthermore, there is also growing interest in investigating the suitability and limitations of the modified bioreactor vessel for expansion processes with other stem cell types.

Financial & competing interests disclosure

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Executive summary

Computational fluid dynamics as a tool for time- and cost-efficient improvement of bioreactor design

- By performing computational fluid dynamics (CFD) simulations, the number of modified UniVessel[®] prototypes manufactured for expansion studies was kept low.
- Additional parameters for fluid flow that are hard to measure experimentally are available.
- Suspension criteria N_{s1u} /N_{s1}
- The suspension criteria (N_{s1u}, N_{s1}) were suitable to ensure a homogeneous microcarrier suspension dependent on the percentage of microcarrier solid fractions.
- CFD model validation by particle image velocimetry and expansion experiments
- Particle image velocimetry investigations showed good agreement with CFD model predictions, with deviations below 20%. As expected, cell expansions performed in the modified UniVessel® SU delivered higher cell densities than in the standard version, where the maximum specific power input was up to 4.6-times higher. Thus, the maximum and mean shear stresses were up to 2.75-times and up to 2.4-times higher, respectively, in the standard version. Cell expansion factors exceeding 30 and peak cell numbers >1 × 10⁹ cells were achieved using the improved bioreactor design, which corresponds to data obtained in optimized spinner flask cultivations.

Modification & qualification of a stirred single-use bioreactor Research Article

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Ethical conduct of research

The authors state that they have obtained appropriate institutional review board approval or have followed the principles outlined in the Declaration of Helsinki for all human or animal experimental investigations. In addition, for investigations involving human subjects, informed consent has been obtained from the participants involved.

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