Microcarriers Suspension Characterization in a Rotating 3D Bioreactor

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Abstract: Three-dimensional bioreactors are increasingly employed for stem cell expansion and cultivated meat production, as they provide controlled environments with enhanced scalability compared to conventional 2D cultures. Microcarriers play a pivotal role in scaling up adherent cell cultures in bioreactor but the scale-up remains limited by shear stress, which can compromise cell viability and function. Here, we investigated microcarrier suspension in Cellura's SoftXS™ bioreactor, a system specifically engineered to generate chaotic mixing with minimal shear stress. Using Corning® polystyrene microcarriers, we experimentally determined the critical suspension speed (N_c) under varying vessel diameters, tilt angles, and aspect ratios. Results show that an optimal aspect ratio of ~0.9 and tilt angles ≥4° significantly improved suspension efficiency. Microcarriers were successfully resuspended at very low rotation speeds (30–60 rpm), with shear forces levels 12 to 50-fold lower than those typically observed in stirred-tank or fixed-bed bioreactors. These findings demonstrate that the SoftXS™ enables gentle and homogeneous suspension conditions suitable for fragile cells, suitable for use with microcarriers and offering a robust platform for applications in regenerative medicine and cultivated meat biomanufacturing.

Introduction

Three-dimensional bioreactor systems have become essential for the large-scale culture of stem cells, providing a controlled environment that supports proliferation, differentiation, and tissue formation. In regenerative medicine and cell-based therapies, stem cells require scalable and reproducible culture platforms that preserve their functional properties while enabling clinical translation. Similarly, in the context of cultivated meat, pluripotent and adult stem cells serve as the foundation for generating muscle and fat tissues, and their efficient expansion in 3D culture is essential to achieving industrially relevant yields. By combining the principles of stem cell biology with the engineering capabilities of bioreactor design, researchers are bridging fundamental science with applications that span sustainable food production and advanced medical treatments.

The shift from traditional two-dimensional culture systems to three-dimensional bioreactor addresses key challenges of scale, nutrient delivery, oxygenation, and shear-stress. For stem-cell–based cultivated meat, 3D bioreactors enable continuous expansion of myogenic progenitors and adipogenic cells, while also providing the mechanical and biochemical cues necessary for their maturation into edible tissue. In therapeutic contexts, bioreactors are designed to minimize shear stress while maintaining cell viability and potency, ensuring that clinical-grade stem cells can be produced under Good Manufacturing Practice conditions. Moreover, innovations such as microcarriers, dynamic scaffolds, and controlled biophysical stimulation are increasingly employed to tailor the cellular microenvironment in both fields.

Microcarriers have become a key platform for scaling up adherent cell cultures. Initially designed to support the growth of anchorage-dependent cells in stirred-tank bioreactors, they now serve as a critical link between conventional laboratory methods and industrial-scale production. The advantages of microcarriers—high surface-to-volume ratio, compatibility with bioreactor systems, and the ability to tailor material properties make them especially attractive for both food and biomedical applications. In cultivated meat production, microcarriers provide a scalable platform to grow animal cells efficiently, whereas in regenerative medicine and cell therapies, they enable the robust expansion of therapeutic cells under controlled, reproducible conditions.

As these industries move toward commercialization, understanding the principles, limitations, and innovations surrounding microcarrier-based systems has become essential. This convergence of fields highlights the versatility of microcarriers and underscores their importance as a key enabler of next-generation biomanufacturing.

Three-dimensional production not only increases yield and consistency but also facilitates better control over culture conditions such as nutrient distribution, and oxygenation. However, shear stress is known to have a strong influence on viability and gene expression of fragile cells and remains one of the major limitations over the industrialization of fragile cells.

Previous studies on embryonic (ESC) and mesenchymal (MSC) stem cells have demonstrated that shear forces above 250mPa increase the expression of tissue-specific gene and cell damages (Teo *et al.*, 2012; Lecina *et al.*, 2010; Kim *et al.*, 2011). In a recent study, Rohani *et al.*, considered that 600mPa is an adapted shear stress for iPSCs cultivation because it facilitated the formation of aggregates with a healthy morphology and an average diameter below levels where we would expect necrosis to occur. Therefore, it should be emphasized that these shear stress thresholds are cell-type and study-dependent but indicate an order of magnitude of shear-sensitivity.

To solve this major limitation, Cellura's bioreactor was specifically engineered to allow gentle suspension through a technology inspired by the Earth rotational movement. The low shear forces generated by this principle are compatible with the culture of fragile cells such as pluripotent stem cells or immune cells. The innovation relies on creating a so-called turbulent or chaotic mixing with very low shear in a culture vessel without an internal mixing system (i.e. : impellers). This relies on three principles: a rotation speed adapted to the vessel volume, a tilt angle greater than or equal to 3° and a specific aspect ratio (medium height / vessel diameter).

The tilt angle enables the medium to enter into resonance, inducing a gentle mixing effect starting at 3°. Without tilt angle, the mixing remains laminar and generates significant shear stress (as in an impeller-based bioreactor, for example). Beyond 3°, the tilt angle has a slight impact on shear stress. However, increasing the tilt may allow resuspension at lower rotation speeds by an increase of fluid instability, which could reduce global exposure to shear stress.

Thus in SoftXS, rotational speed is the main contributor to shear forces.

Turbulent mixing & Aspect ratio

Fluid dynamics and mass transfer in Cellura's bioreactors was previously characterized with a fixed tilt angle at 3° and an aspect ratio (*Height/Diameter*) of 1 in a 1L cylinder (Meunier, 2020; Lefranc *et al.,* 2023).

The fluid height (*H*) in a rotative cylinder with a diameter (*D*) is carefully selected to create a resonant flow generating efficient mixing within the culture vessel. In order to obtain the most efficient flow with the smallest possible angle, it is therefore interesting to choose the fluid height equal to one of these resonant heights Lefranc *et al.,* 2023. The first resonant height of the first mode is very close to *D.* Thus, for these experiments the optimal aspect ratio determined was near 1 (*H*=*D*).

Altogether, these parameters influence suspension capacity in the SoftXS bioreactor, allowing us to find the best culture conditions.

Suspension criterion

In microcarrier cultivation processes, selecting an appropriate stirring speed (*N*) is a complex task. To better describe the suspension behavior of microcarriers in stirred tank bioreactors (STBs), the $\boldsymbol{N_{s1}}$ **suspension criterion** was established. Within this framework, the N_{s1} criterion, initially introduced by (Zwietering, 1958), defines the stirring speed at which no particles remain in contact with the bottom of the bioreactor for longer than one second. In the literature, the N_{s1} criterion is also referred by alternative terms, such as the *"just suspended"* [N_{js}] (Ibrahim and Nienow, 2004; Rafiq *et al.,* 2017; Lawson *et al.,* 2017) or "critical" stirring speed criterion [N_c] (Petry and Salzig, 2021). Here we decided to use the definition "critical speed criterion N_c".

Objectives

In this work, we determined experimentally the critical speed N_c required to achieve suspension of microcarriers, by varying all parameters involved in mixing: vessel volume, rotation speed, tilt angle and aspect ratio. This N_c permits to calculate the minimal mean shear-stress which microcarriers and cells were exposed to and compare these results to other technologies.

Materials & Methods

SoftXS Bioreactor

Critical speed N_c was determined in different conditions using the SoftXS™ bioreactor (Cellura, FR) compatible with several sizes of vessels (**Fig. 1**).

Figure 1: *High angle view of a SoftXS base unit with Ø64, Ø84, and Ø114mm vessels.*

The tilt angle required to induce chaotic mixing was adjusted from 3 to 8° by step of 1° measured through the internal inclinometer. The rotation speeds ranged from 20 to 95rpm depending on vessel volumes (smaller vessels require higher speed to induce chaotic mixing).

Four vessel sizes were used for this study with different intern diameters respectively Ø56/64/84/114mm. This impacted working volumes assessed according to aspect ratio. Different Aspect ratios (*Height/Diameter*) were assessed from 0,7 to 1.

Fluid regime - Reynolds number

The Reynolds number (*Re*) is a fundamental dimensionless criterion used to predict whether a fluid flow will remain laminar or transition to a turbulent regime (chaotic).

$$\mathbf{Re} = \frac{\rho V L}{\mu}$$

Where:

- Re = Reynolds number (dimensionless)
- ρ = fluid density (kg/m³)
- V = characteristic velocity of the fluid (m/s)
- L = characteristic length (m)
- μ = dynamic viscosity (Pa·s)
- $\nu = \mu/\rho$ = kinematic viscosity (m²/s)

In a cylinder, typical values of Reynolds were determined to predict fluid regime:

- **Re < ~2000** → laminar regime.
- **2000 < Re < 4000** → transitional regime.
- **Re > ~4000** → turbulent regime

Importantly, this definition does not depend on whether the cylinder is filled, partially filled, or closed with a gas-permeable lid. In all configurations, the same fluid properties and geometric parameters determine *Re*.

However, while the numerical value of *Re* remains unchanged, the resulting flow field is strongly affected by boundary conditions. In a completely filled and sealed cylinder, the flow is dominated by wall-bounded layers and secondary recirculation. In a partially filled open vessel, the presence of a free surface introduces surface waves and sloshing, amplifying local shear stresses.

Here, in the case of a closed vessel with a gas-permeable lid, the situation is intermediate: the liquid retains a free surface, which can deform and generate surface instabilities, while the mechanical closure constrains the global motion.

Thus, although the Reynolds number provides a consistent global measure of inertial versus viscous forces, the nature of the flow at a given *Re* depends sensitively on the filling and boundary conditions.

Shear forces calculation

The shear forces in a rotating cylinder were measured and compared between Rushton turbine and Soft Mixers (Cellura's technology) in previous studies (Meunier *et al.,* 2020).

It is of the order of 30 % of the angular velocity *Ω* for the soft mixer whereas it is equal to approximately 10*Ω* for the Rushton turbine.

In the SoftXS, the shear forces τ could be approximated as follow:

$$\tau = 0.3.\mu\gamma_{wall} \approx 0.3.\mu\Omega$$

Where μ is the dynamic viscosity and *Ω* the rotation speed in *rad/s* depending on vessel radius.

In the SoftXS with a tilt angle of 1° and a Reynolds of 2440 (laminar flow) the wall shear forces were determined to be ~3.*Ω* (**Fig. 2**).

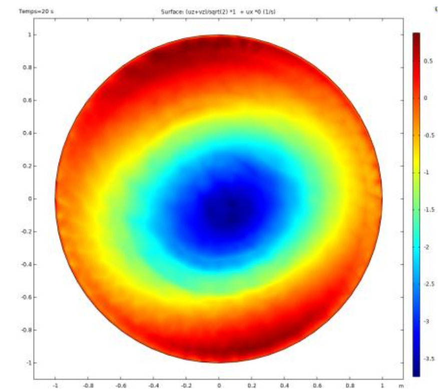

Figure 2: *Computational simulation of shear distribution in a rotating cylinder with a tilt angle of 1° and a laminar flow (Re=2440)*

Based on these results, for a tilt angle of 3°, the wall shear forces are about 3-fold higher, thus it could be approximated thanks to:

$$\tau_{max} \approx 10\Omega \approx 30\tau$$

This small difference is explained by the fact that the vessel is rotating at the same speed as the fluid providing a small speed gradient between particle speed and the wall reducing therefore the maximal shear forces.

Microcarriers suspension

SoftXS vessels were filled with pure water (room temperature relative density: 0.9982) to a volume corresponding to an aspect ratio of 0,7. Notably, pure water density is lower than the usual culture media and that increases the difficulty to suspend dense particles. Thus, results presented in this study therefore correspond to the worst-case scenario.

Corning® polystyrene microcarriers non-treated (relative density of 1.026 ± 0.0004 g/cm^2) were added. Quantity was adjusted only to clearly visualize microcarriers suspension in the different volumes.

The evaluation of mixing quality will mainly be subjective and therefore qualitative (whether the microcarriers are resuspended with chaotic mixing or not within 5 minutes). Suspension was considered validated when microcarriers exhibit chaotic mixing without particles staying at the bottom of the vessel.

After each run, rotation was stopped for 5 minutes to allow the microcarriers to sediment. The rotation speed was adjusted by steps of 5rpm to identify the lower limit for each tilt angle.

If suspension is not achieved or microcarriers stay on the bottom of the vessel, the test is considered invalid. This allows us to determine the critical suspension speed Nc for each condition.

When the speed limit was reached, the tilt angle was increased by 1° and the run was restarted to measure the impact of the tilt angle on microcarrier resuspension.

Once all data were collected for one aspect ratio, a small volume of pure water was added to reach the next aspect ratio and start a new run of assays.

Since rotation speed is one of the main factors responsible for shear forces, identifying critical speed N_c is important to determine the lowest shear level possible in each condition.

Results

Optimal aspect ratio

In a rotating cylinder with a tilt angle of 3° filled with 1L of distilled water, the optimal aspect ratio was previously determined as ~1 (*H*=*D*) (Lefranc *et al.*, 2023).

Based on these experiments, we explored in this study, the impact of tilt angle and rotation speed on microcarrier suspension in several vessel sizes between values of aspect ratio between 0.7 and 1.

For each vessel diameter, we determined that the highest percentage of validated conditions corresponds to an optimal aspect ratio near 0,9 (*H/D*) (**Fig. 3-6**).

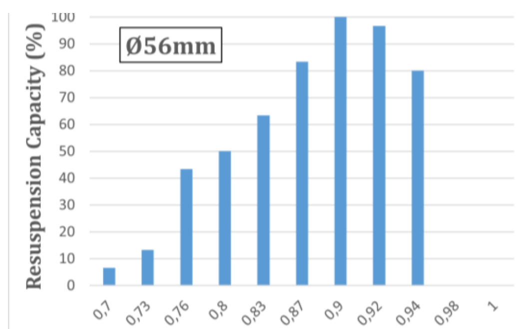

Figure 3: *Percentage of validated conditions according to vessel diameters and aspect ratio (Ø56mm).*

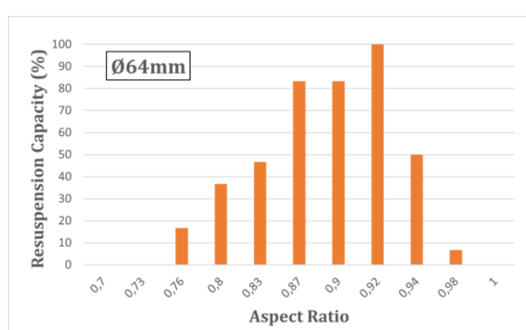

Figure 4: *Percentage of validated conditions according to vessel diameters and aspect ratio (Ø64mm).*

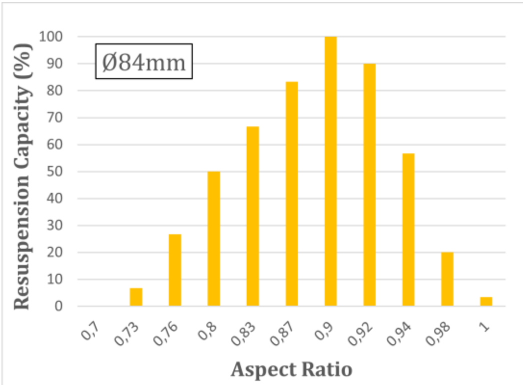

Figure 5: *Percentage of validated conditions according to vessel diameters and aspect ratio (Ø84mm).*

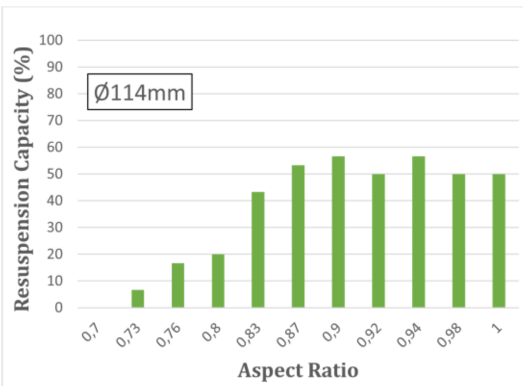

Figure 6: *Percentage of validated conditions according to vessel diameters and aspect ratio* (Ø114mm).

From the aspect ratio of 0,7 to 0,9 the number of conditions assessed allowing resuspension increase almost linearly, however, once the critical value of 0,9 exceeds, the resuspension capacity quickly decreases.

This phenomenon seems particularly linked to the small vessels as it is clearly visible with Ø56 to 84mm vessels (**Fig. 3 to 5**) but less on the Ø114mm vessel (**Fig. 6**) which confirm results obtained by Lefranc *et al.*, 2023.

The aspect ratio ~0,9 permits the gentle suspension of Corning® microcarriers with the tilt angles assessed from 3° to 8°.

Further experiments were carried out with a fixed aspect ratio of 0,9.

Impact of tilt angle on microcarriers suspension

As expected, and described in the introduction, tilt angle positively influences suspension capacity through an increase of fluid instability. More than 85% of speed conditions assessed with all vessels gave positive results with a tilt angle of 8° compared to 35% at 3° **(Fig. 7)**.

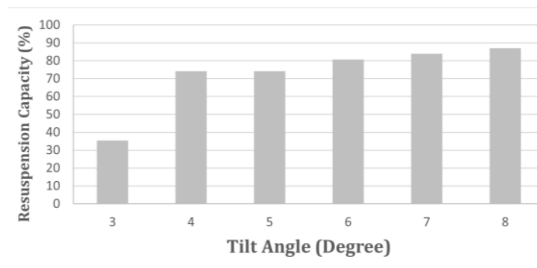

Figure 7: *Percentage of validated conditions with all vessels according to tilt angle with an aspect ratio of 0,9.*

A gap is clearly identified between the tilt angles of 3 and 4° which gave respectively 35% and 75% of positive results. That underlined that a tilt superior to 3° is necessary to maintain a homogeneous suspension of microcarrier in the SoftXS bioreactor.

This phenomenon is not surprising as in a rotating cylinder an increase of the tilt angle favors vortex formation, fluid instability and therefore turbulent mixing.

Critical speed N_c

Previous results permit defining the best resuspension conditions as an aspect ratio of 0,9 and a tilt angle of 8° allowing us to determine experimentally the Nc for each vessel diameter. Then, we calculate the shear-stress and Reynolds value associated with each critical speed (**Table 1**).

Altogether, these results show that it is possible to resuspend Corning® microcarriers at very low speed (min. 30rpm) and in very low-shear conditions in the SoftXS bioreactors and with turbulent characteristics (Reynolds).

Vessel Diameter	Ø56mm	Ø64mm	Ø84mm	Ø114mm
N_c	60	45	35	30
Shear stress median (mPa)	1,88	1,41	1,1	0,94
Shear stress max (mPa)	56,4	42,3	33	28,2
Reynolds number	5500	5400	7250	11000

Table 1: *Critical speed criterion Nc and associated shear-stress and Reynolds in function of vessel size for a tilt angle of 8° and an aspect ratio of 0,9.*

Discussion

In this study, we explored suspension capacity of Corning® microcarriers in a SoftXS™ bioreactor in order to define the critical speed of suspension.

It is important to mention that Nc was determined as the speed where microcarriers are fully homogenized in the vessels, but lower speeds assessed also allowed suspension of microcarriers, however, small parts remain a few seconds at the bottom of the vessels and were considered excluded because these conditions are not fully homogeneous.

Moreover, experiments were carried out with pure water at room temperature to facilitate operating procedures, but dynamic viscosity decreases more than 25% at 37°C compared to room temperature resulting in an increase of Reynolds number and probably suspension capacity. Therefore, with cell culture media at 37°C resuspension of microcarriers should be even more facilitated in a SoftXS.

These experiments allowed us to assess the sensitivity of SoftXS technology to the aspect ratio. That underlines the need to adjust parameters in case of volume variation during the process to maintain microcarriers and/or cell suspension. In small diameter vessels from Ø56 to 84mm the impact of aspect ratio seems more critical than in bigger vessels (Ø114mm) probably due to the maintenance of turbulent mixing despite liquid volume variations underlined by a higher Reynolds number.

Tilt angle plays an important role in the suspension capacity as it allows chaotic mixing through fluid instability. Higher tilt angle provided higher percentage of validated conditions through liquid volume variation (aspect ratio), however, the increase of tilt angle slightly increased the shear forces, therefore, the angle of inclination does not necessarily have to be as high as possible, even though shear stress remains extremely low compared to others mixing solutions.

Shear stress conditions presented in **Table 1** provided by the SoftXS are far away from the critical thresholds of ~250-600mPa (Teo *et al.*, 2012; Lecina *et al.*, 2010; Kim *et al.*, 2011; Rohani *et al.*, 2020), noted to elicit the upregulation of tissue-specific genes, and the threshold of 10000mPa, known to trigger the detachment of adherently growing cells (Fuhrmann and Engler 2015).

Moreover, recent studies (Schneider *et al.*, 2025; Teale *et al.*, 2025) in the stirred tank bioreactor BioBLU-1 (BB1) (Eppendorf, AG) and the Ascent 1-m^2 fixed-bed bioreactor (AS-1) (Corning®, US) brought specific data about shear stress in both bioreactors.

In these later, cells faced values of shear forces ranging from ~25 - 550mPa (BB1) and ~200-2500mPa (AS-1) that are ~12 to 50-fold higher than in SoftXS.

It should be noted that over the scale-up with a SoftXS, shear forces remain constant. On the other hand, in a stirred suspension bioreactor the volume average shear rate changes linearly, whereas the volume average energy dissipation rate changes exponentially in response to changes in agitation rate.

This indicates that over the scale-up, the gap between Cellura's and Stirred tank bioreactors widens exponentially, in terms of shear stress and results reliability from lab to industrial production.

In conclusion, innovative mixing technology provided by Cellura ensures best suspension capacity with a very low shear stress exposure suitable for the culture of fragile cells under the form of aggregates, single-cell or on microcarriers.

Patents

In summary, this work demonstrates the successful application of the described methodology, which relies on patented technologies to achieve the reported results. The approach presented here benefits directly from the innovations disclosed in patents **[FR3048367B1/ Bladeless mixer and method]** and [**FR3117505B1/ System, method and assembly for cell culture**]. These protected technologies provided the necessary foundation for the experimental setup and data analysis. Future research will continue to build on these advances, exploring new applications and optimizing performance within this technological framework.

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