CHARACTERIZATION OF MICROBEAD SUSPENSION LIMITS IN THE SOFTXS™ LOW SHEAR BIOREACTOR SYSTEM

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EXECUTIVE SUMMARY

Maintaining homogeneous suspension while minimizing hydrodynamic stress remains one of the major challenges in modern cell culture systems. Conventional stirred-tank bioreactors rely on impellers that generate localized turbulence, shear gradients, and heterogeneous mixing environments that can negatively impact sensitive biological materials such as stem cells, organoids, immune cells, and microcarrier cultures.

The SoftXS™ platform was developed to address these limitations through an impeller-free inclined rotation system generating continuous suspension under low-shear conditions.

This application note demonstrates that:

Reproducible Fluid Performance: SoftXS™ provides reproducible homogeneous suspension across all investigated scales.

Predictable Hydrodynamics: The system ensures robust suspension performance driven by particle size and operating conditions (tilt angle and speed).

Unrivaled Low-Shear Environment: Inclined rotation enables efficient low shear mixing without impellers.

Together, these results position SoftXS™ as a scalable low-shear bioreactor platform for sensitive mammalian cell culture applications

WHY CONVENTIONAL SUSPENSION SYSTEMS ARE LIMITING

Many advanced bioprocesses require the suspension of fragile biological structures such as stem cells, organoids, and immune cells. Conventional stirred-tank bioreactors typically rely on impellers to generate mixing, which can create localized high shear stress hydrodynamic and heterogeneous environments.

systems often generate:

Localized high shear stress	Turbulent eddies	Spatial heterogeneity	Sedimentation zones	Complex scale-up behavior

The SoftXS™ platform was developed to address these limitations through an inclined rotating vessel design generating chaotic advection and continuous resuspension without internal impellers. This approach enables efficient mixing and homogeneous suspension while preserving a low-shear environment compatible with sensitive mammalian cell systems.

SOFTXS™ MIXING PRINCIPLE

The SoftXS™ bioreactor operates using an inclined rotating cylindrical vessel without internal impellers.

The combination of:

- Rotation speed
- Tilt angle
- Free-surface dynamics
- Vessel geometry

generates continuous suspension and chaotic advection throughout the culture volume. Unlike conventional stirred systems, suspension is achieved without:

- Impeller-induced shear
- Localized turbulence
- Sparging-associated hydrodynamic stress.

This creates a more homogeneous and mechanically gentle environment suitable for sensitive mammalian cultures.

DEMONSTRATION OF PREDICTABLE SUSPENSION ACROSS SCALES

Fluorescent polyethylene microbeads with a density close to biological aggregates ($\rho \approx 1{,}05 g/cm3$) were used as model particles. Three bead size populations (~100µm, ~400µm, ~1300µm) were evaluated. These size ranges are representative of commonly encountered biological systems,including:

- Organoids and cell aggregates, which typically span from ~100µm to >1000 µm depending on culture conditions and maturation stage
- Microcarrier-based cultures, where carrier diameters usually range from ~100 to 300 µm, with effective aggregate sizes increasing further upon cell colonization

Experiments were conducted in SoftXS™ vessel formats corresponding to nominal working volumes of 10 mL, 50 mL, and 250 mL. Suspension studies were performed using a constant tilt angle of 25° and an H/D ratio of 0.7 in order to directly compare hydrodynamic behavior across scales. Within this evaluated range of vessel volumes and particle sizes, the optimal tilt angle remains constant, though it can be dynamically adjusted for larger containment formats in accordance with scale-up hydrodynamic models.

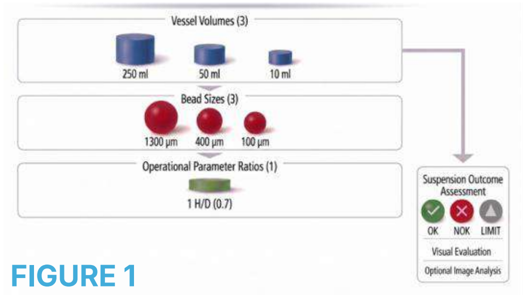

FIGURE 1

Scalable experimental framework.

LINEAR SCALE-UP & CRITICAL SUSPENSION SPEED (N_C) PROFILES

The generated dataset demonstrates that the SoftXS™ platform produces :

- Homogeneous suspension maintained from 10 mL to 250 mL
- Predictable scale-up behavior
- No internal impeller required
- Stable suspension achieved at low rotational energy input
- Consistent hydrodynamic behavior across vessel formats

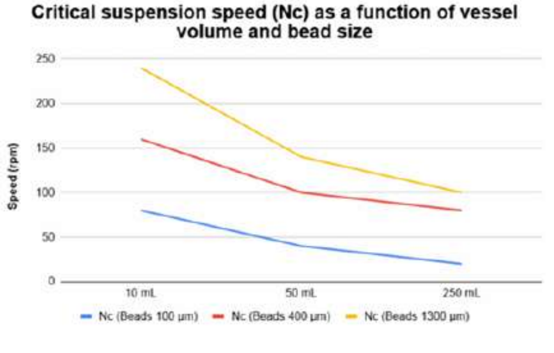

FIGURE 2

Predictable suspension behavior across SoftXS™ vessel scales.

Critical suspension speed (Nc) measured at H/D = 0.7 and 25° tilt angle for multiple particle sizes across 10 mL, 50 mL, and 250 mL SoftXS™ vessel formats.

IMPACT OF SCALE: Smaller vessels required higher rotational speeds to maintain equivalent suspension conditions. Despite these differences, suspension trends remained highly consistent across all vessel formats, confirming predictable process transfer and scale-up behavior.

IMPACT OF PARTICLE SIZE: Larger particles (~1300 µm) required higher rotational speeds to overcome faster sedimentation, consistent with classical sedimentation theory.

GEOMETRIC OPTIMIZATION (H/D = 0.7): Across all vessel sizes, an H/D ratio close to 0.7 minimized the critical suspension speed (Nc) required to maintain stable suspension at a 25° tilt angle.

These results demonstrate that the interaction between vessel geometry, free-surface dynamics, and inclined rotation generates efficient suspension patterns, enabling suspension of large aggregates at low energy input and reduced shear stress.

IDENTIFICATION OF OPERATING WINDOWS

HOMOGENEOUS REGIME

- Complete particle suspension
- No visible sedimentation
- Stable full-height mixing

LIMIT REGIME

- Partial sedimentation
- Reduced suspension efficiency
- Transitional suspension behavior

NON-HOMOGENEOUS REGIME

- Significant particle accumulation
- Strong spatial heterogeneity
- Incomplete suspension

The transition between these regimes was strongly influenced by: Particle size, rotational speed and vessel geometry.

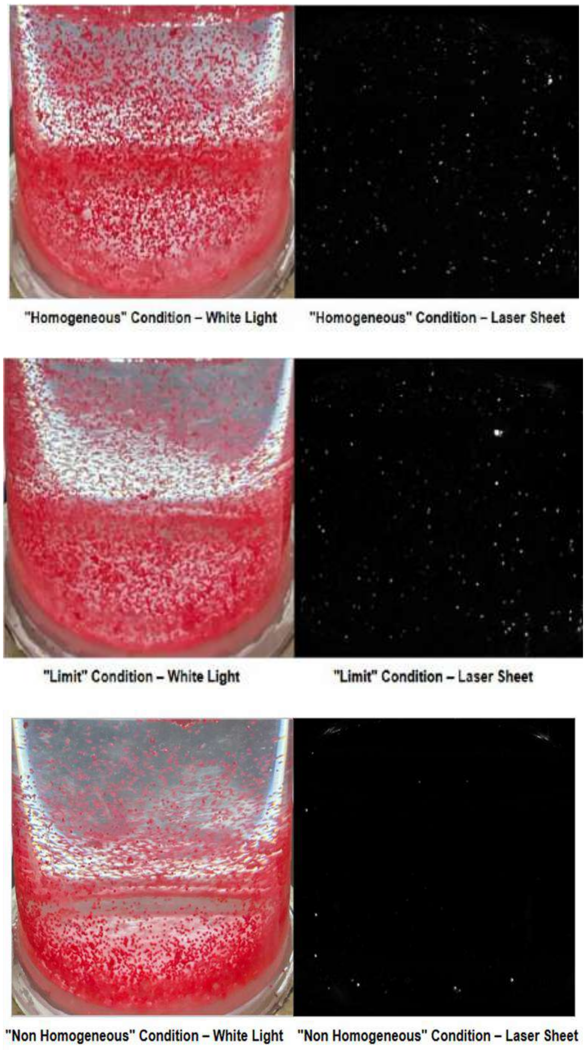

FIGURE 3
Illustration of the three visual conditions determined in the 45 mm vessel: Homogeneous, Limit, and Non-homogeneous. The left panel presents visual results and the right panel presents laser imaging results. The tests were conducted using model beads of 355–425 µm (mean size ~400µm), with a tilt angle of 25° and an H/D ratio of 0.7.

QUANTITATIVE VALIDATION USING LASER IMAGING

Laser-sheet imaging was used as an independent method to quantitatively validate suspension homogeneity. Two complementary metrics were combined: Coefficient of variation (CV) and Particle counting.

Vessel Volume	Vessel Diameter	Mixing Speed	Suspension Status	Spatial Variation (CV)	Active Fluid Particles	Sedimented Fraction
250 mL	76 mm	120 rpm	OK	6.9%	401,205	0%
250 mL	76 mm	80 rpm	LIMIT	9.4%	317,283	20%
250 mL	76 mm	40 rpm	NOK	5.6%	203,539	49%
50 mL	45 mm	140 rpm	OK	11.0%	185,361	0%
50 mL	45 mm	100 rpm	LIMIT	8.0%	168,351	9%
50 mL	45 mm	60 rpm	NOK	38.3%	23,175	87%
10 mL	26 mm	200 rpm	OK	12.8%	36,975	0%
10 mL	26 mm	120 rpm	LIMIT	17.7%	35,186	5%
10 mL	26 mm	80 rpm	NOK	61.9%	14,830	60%

TABLE 1
Multi-scale homogeneity matrix (Bead diameters: 355–425 µm, Tilt angle: 25°, H/D = 0.7). Green-shaded rows highlight optimized parameters where full particle suspension is securely achieved with zero sedimentation.

HOMOGENEOUS OPERATING CONDITIONS CONSISTENTLY EXHIBITED:

- Low CV values (<15%),
- Negligible sedimentation
- Uniform particle distribution throughout the liquid column

NON-HOMOGENEOUS CONDITIONS SHOWED:

- Elevated CV values
- Strong sedimentation
- Heterogeneous particle distribution throughout the liquid column

Importantly, some intermediate conditions displayed relatively low CV values despite partial sedimentation. These observations demonstrate that CV analysis alone may underestimate suspension heterogeneity if sedimented particles are excluded from the detected population.

Combining CV analysis with particle counting therefore provides a more robust framework for defining true suspension homogeneity.

Single-particle tracking experiments further confirmed the dynamic suspension capabilities of the SoftXS™ platform.

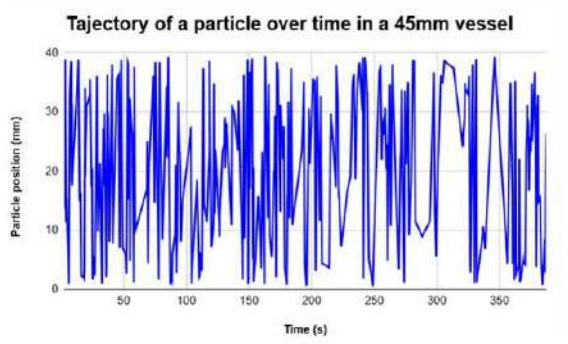

FIGURE 4
Trajectory of a single particle in the SoftXS™ in OK condition.

Tracked particles continuously explored the accessible vessel volume without prolonged confinement in localized regions.

This behavior demonstrates:

- Efficient temporal mixing
- Continuous spatial renewal
- Homogeneous environmental exposure throughout the reactor

Such suspension behavior is particularly relevant for cell structure sensitive applications requiring:

- Uniform nutrient exposure
- Controlled oxygen transfer culture
- Minimized hydrodynamic stress

HYDRODYNAMIC QUANTIFICATION OF SHEAR STRESS

While maintaining homogeneous suspension in small volumes (10 mL format) requires higher rotational speeds reaching 200 to 250 rpm (Figure 3), the mechanical shear stress experienced by the cells remains exceptionally low. To address potential concerns from users accustomed to conventional stirred-tank bioreactors, the hydrodynamic profile of the SoftXS™ system were calculated from the experimental Particle Image Velocimetry (PIV) given in Scollo (2025).

Figure 5 establishes a comprehensive side-by-side comparison on a log-log scale, mapping the strictly predictable shear stress profiles of the SoftXSTM against the operational shear stress signatures of its main market competitors.

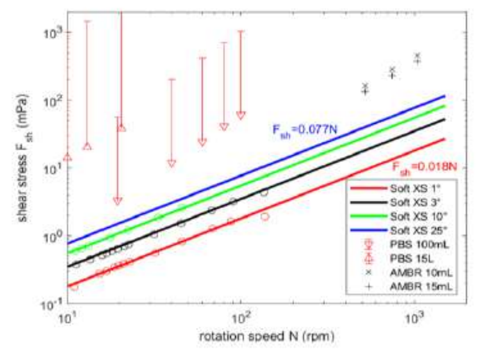

FIGURE 5
Hydrodynamic shear stress (Fsh, mPa) as a function of rotation speed (ω, rpm): Direct comparison between SoftXS™ ™ (lines) against AMBR®(crosses/pluses) and PBS Vertical Wheel systems (triangles/bars).

SoftXSTM Operating Window (< 20 mPa): For the validated standard tilt angle of 25°, the fluid dynamics model shows a strictly linear progression governed by the equation Fsh = 0.077 * ω.

Within the actual operational range required to achieve full homogeneous suspension in the SoftXSTM (70 to 250 rpm),the absolute shear stress remains exceptionally low, safely capping at 19.25 mPa.

The AMBR® High-Shear Zone (130 to >450 mPa): In traditional stirred microscale bioreactors, localized turbulence and high peripheral impeller tip-speeds generate severe mechanical stress. At standard operational velocities (520 to 1040 rpm), the AMBR® system exhibits a massive shear gap, exposing cells to time-averaged shear stresses ranging from 130 mPa to over 450 mPa—orders of magnitude higher than the SoftXS™ operating window.

Dismantling the "Low-RPM Illusion" with PBS Data (10 to >1000 mPa): Vertical Wheel systems (PBS 100 mL and 15 L) are often operated at low raw rotational speeds (10 to 100 rpm) under the assumption that low RPM guarantees low shear. However, as illustrated by the wide vertical error bars in Figure 5, the internal cutting-edge mechanics of PBS wheels induce massive shear stress fluctuations. Even at a mere 20–30 rpm, the local maximum shear stress peaks in a PBS 100 mL vessel can rapidly spike from 3 mPa to over 1000 mPa, heavily crossing into cell-damaging territory.

CONCLUSION

This study demonstrates that the SoftXS™ bioreactor platform enables robust and reproducible low-shear suspension across multiple working volumes (10–250 mL) without the use of internal impellers. Across all tested conditions, inclined rotation consistently generated stable hydrodynamic environments capable of maintaining homogeneous suspension of density-matched microbeads, confirming the effectiveness of the SoftXS™ mixing principle for sensitive particulate systems.

Three major outcomes emerge from this work:

SoftXS™ provides reproducible homogeneous suspension across all investigated scales, demonstrating reliable process transfer from 10 mL to 250 mL.

The system ensures robust suspension performance driven by particle size and operating conditions, aligning empirically with classical sedimentation physics.

Inclined rotation enables efficient low-shear mixing without impellers, achieving full-volume suspension while particle minimizing mechanical stress.

Beyond qualitative observations, quantitative laser-sheet imaging confirmed stable operating windows and validated that homogeneous suspension corresponds to continuous dynamic suspension rather than static particle distribution.

Overall, these results establish SoftXS™ as a scalable and controllable low-shear bioreactor platform for applications requiring gentle yet effective suspension of sensitive biological materials, including stem cells, organoids, immune cells, and microcarrier-based cultures.

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