Application note

Influence of shear stress on long-term 3D construct performance: Comparison of orbital shaker and ClinoStar® system

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Introduction

The physical microenvironment plays a critical role in 3D cell culture performance. In addition to biochemical signals, forces such as fluid movement causing shear stress, directly influence construct formation, morphology, viability, and differentiation. While certain cell types benefit from controlled mechanical stimulation, many mammalian cells derived from low-shear tissue environments are highly sensitive to shear stress.

Dynamic suspension culture systems are widely used to improve nutrient exchange, reduce diffusion limitations, and prevent construct sedimentation. Orbital shakers remain a common and accessible solution due to their simplicity and scalability. However, orbital motion generates fluid forces that vary spatially and temporally across the culture vessel, creating differences between wells and requiring careful optimization of speed, vessel geometry, and media volume to maintain acceptable culture conditions. This can result in a relatively narrow operating window between insufficient mixing and excessive shear stress [1].

The Celvivo ClinoStar® system was developed to provide continuous dynamic suspension under homogeneous ultra-low shear conditions. By minimizing hydrodynamic variability while maintaining efficient nutrient exchange, the system is designed to support reproducible 3D culture for sensitive cell models and long-term applications.

In this application note, we compare 3D construct culture in orbital shaker and the ClinoStar® system, evaluating growth, morphology, viability, and reproducibility. The main experiment was performed based on pre-aggregated HepG2/C3A cells, while additional examples from embedded Caco-2 cells and and brain organoids further illustrate how hydrodynamic culture conditions can influence model quality across diverse applications.

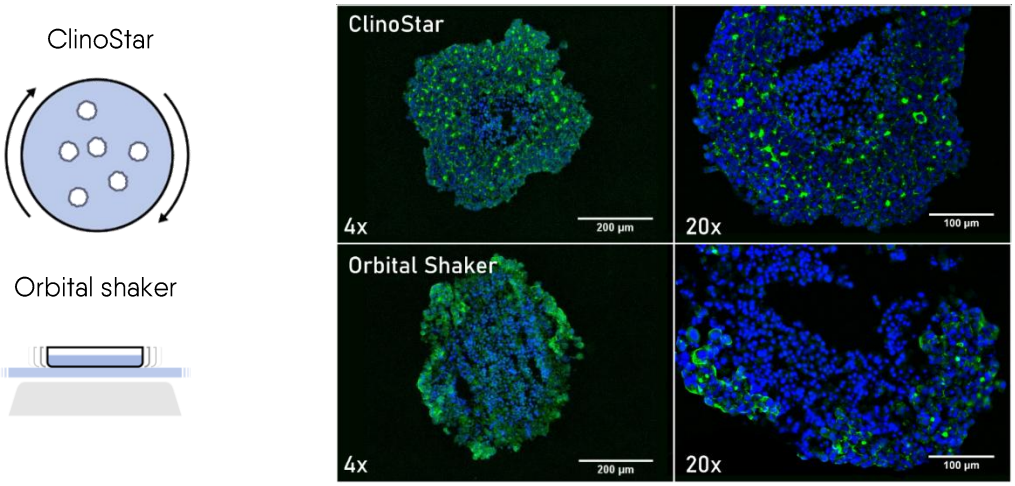


Figure 1: Comparison of cytoskeletal organization and structural integrity in 3D constructs cultured under ClinoStar® and orbital shaker conditions.
Schematic overview of ClinoStar® and orbital shaker culture conditions (left). Fluorescence imaging of day 15 constructs stained with phalloidin (F-actin, green) and DAPI (nuclei, blue). Constructs cultured in ClinoStar® exhibited compact morphology and preserved cytoskeletal organization, whereas orbital shaker cultures showed reduced structural cohesion. Scale bars: 200 µm (4x), 100 µm (20x).

Workflow

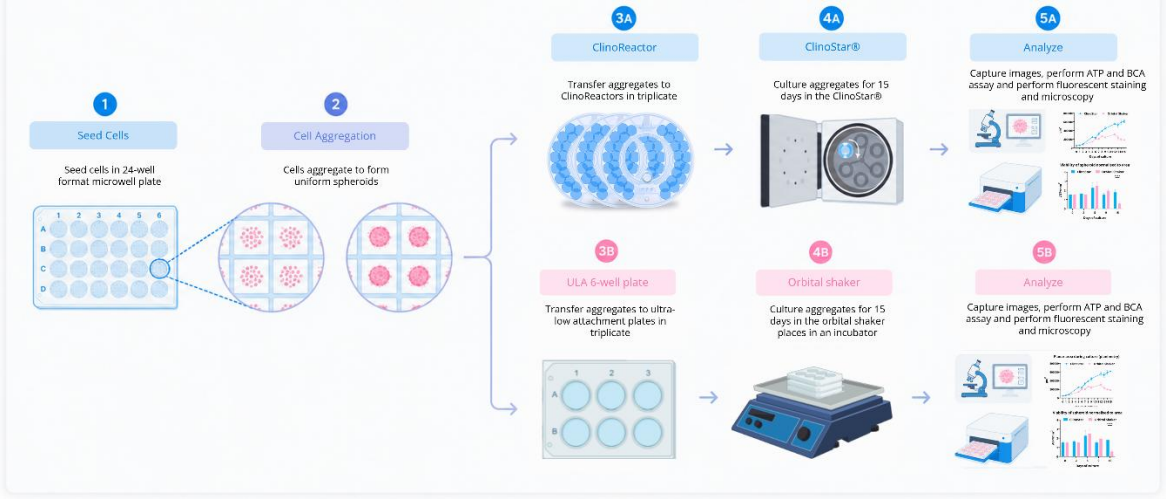


Figure 2: Experimental workflow for comparison of 3D aggregate culture in ClinoStar® and orbital shaker systems.
Schematic overview of the experimental design used to compare HepG2/C3A aggregate culture in ClinoStar® and orbital shaker conditions. Cells were seeded in a 24-well micropattern plate and allowed to aggregate into uniform spheroids. Aggregates were subsequently transferred either to ClinoReactor® vessels and cultured in the ClinoStar® system or to ultra-low attachment (ULA) 6-well plates cultured on an orbital shaker for 15 days. Endpoint analyses included image acquisition, ATP and BCA assays, and fluorescent staining followed by microscopy. Data were collected in triplicate for both culture conditions.

Materials

- Cells (e.g. HepG2/C3A (CRL-3581, ATCC))
- Growth media
- SP5D Spherical plate (SP5D-24W, Kugelmeiers)
- Syringe (B.Braun, 4616103)
- Needle (B.Braun, 466512)
- ClinoReactor (Celvivo, 10004-12)
- Ethanol 70%
- ClinoStar® (Celvivo, 30003)
- 6-well Ultra-low attachment plate (e.g. 174932, Thermo Fisher Scientific)
- Orbital shaker placed in incubator (SBS-LVS-100, Steinberg)

Methods

Experimental setup

1. HepG2/C3A cells were initially cultured as monolayer according to the manufacturer's instructions.
2. Cells were seeded in SP5D spherical plates at 1000 cells per aggregate as described previously in Celvivo 009_Protocol_Low_Adherent_Microwell_Plate_Setup_For_ClinoReactor.
3. Following overnight incubation, constructs were transferred either to ultra-low attachment 6-well plates for orbital shaker culture or to hydrated and equilibrated ClinoReactors according to Celvivo 003_Protocol_Preparation_of_ClinoReactor
4. Orbital shaker culture was performed at 75-100 RPM, using 3 ml medium per well in 6-well format, selected to maintain construct suspension without aggregation [2-3]. ClinoStar® culture was performed at 8,5-26,5 RPM according to manufacturer recommendations for this model.
5. Cultures were maintained for 15 days under standard incubator conditions (37 °C, 5% CO_2) with media exchange performed with 48/48/72 hours intervals.

Analysis

The following endpoints were assessed:

1. Construct area (planimetry): Celvivo 019_Protocol_Automatic_and_Manual_Planar_Area_Measurement.
2. Viability (ATP content): Celvivo 017_Protocol_Viability Determination of Constructs Cultured in the ClinoStar.
3. Biomass (protein content): Celvivo 018_Protocol_Protein determination of Constructs cultured in the ClinoStar.
4. Structural integrity: Fluorescence imaging with phalloidin and DAPI staining.

Data are presented as mean ± SD from three independent replicates per condition. The number of technical measurements per condition varied by assay: protein quantification (n = 3), planimetric analysis (n = 30), and viability assessment (n = 6). Statistical analysis was performed using two-way ANOVA followed by Šídák's multiple comparisons test, with significance set at $p < 0.05$.

Results & discussion

Low-shear ClinoStar® culture supports sustained growth and improved construct consistency

To compare long-term 3D culture performance, HepG2/C3A constructs were cultured in either the ClinoStar® system or orbital shaker conditions for 15 days.

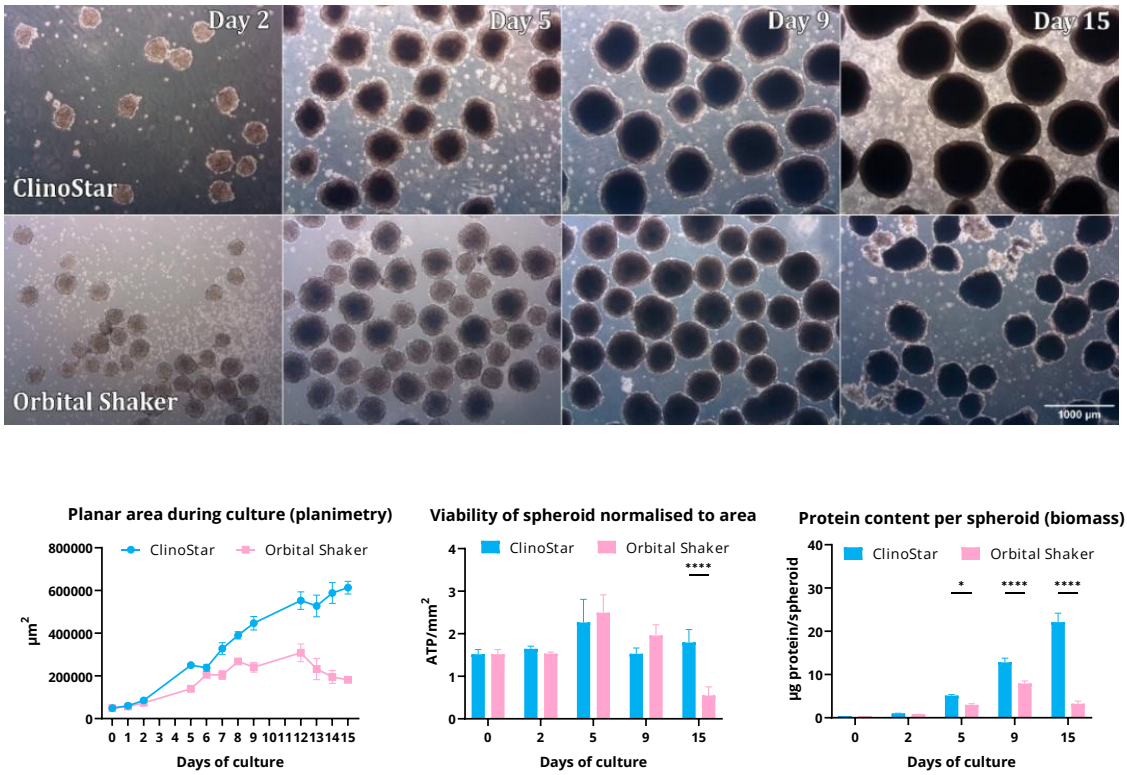


Figure 3: Comparison of 3D construct development in ClinoStar® and orbital shaker culture systems.
Representative brightfield images of HepG2/C3A constructs cultured in ClinoStar® or orbital shaker conditions at days 2, 5, 9, and 15. Quantitative analysis includes construct area, ATP content normalized to construct area, and protein content per construct. Data are presented as mean ± SD.

Constructs cultured in the ClinoStar® system maintained spherical morphology and continued to increase in size throughout the culture period. In contrast, orbital shaker cultures showed reduced structural stability at later time points, including peripheral cell detachment and decreased construct size after day 12.

Protein accumulation increased progressively in ClinoStar® cultures and remained lower under orbital shaker conditions. ATP content normalized to construct area remained stable in ClinoStar®-derived constructs but declined in orbital shaker cultures by day 15, indicating reduced long-term metabolic performance under the tested conditions.

ClinoStar® culture improves reproducibility of dynamic 3D culture

Constructs cultured in the ClinoStar® system showed lower variability in construct size and ATP measurements over time compared with orbital shaker cultures. Protein variability was similar between systems, though greater fluctuations were observed in orbital shaker cultures at select time points.

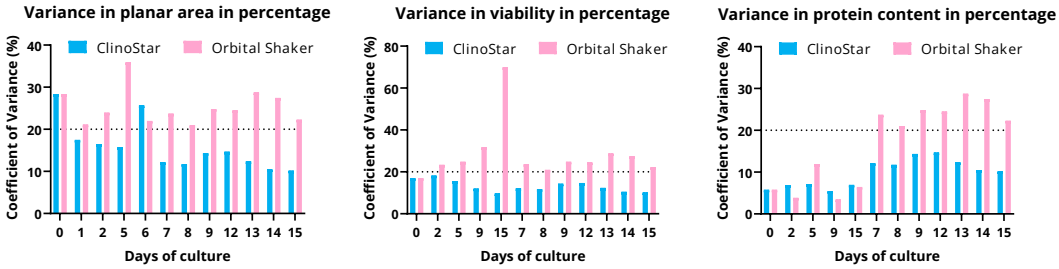


Figure 4: Comparison of reproducibility in 3D construct culture using ClinoStar® and orbital shaker systems.
Coefficient of variation (CV%) for construct area, ATP content, and protein content in ClinoStar® and orbital shaker cultures over 15 days. Dashed line indicates 20% CV threshold where applicable.

Low-shear culture preserves structural integrity of 3D constructs

Fluorescence imaging revealed marked structural differences between culture conditions (Fig 1). Constructs maintained in ClinoStar® exhibited compact architecture, uniform nuclear distribution, and preserved F-actin organization. Orbital shaker cultures displayed reduced structural cohesion and localized construct disruption, consistent with increased mechanical stress exposure.

Shear Stress Effects Across Culture Systems

To investigate whether the effects of shear stress are specific to HepG2/C3A cells, we also examined its impact on other culture systems. This section presents two additional examples: one in-house experiment using gel-embedded constructs and one independent comparative study using iPSC-derived brain organoids.

Hydrodynamic effects extend to hydrogel-embedded 3D culture models

To assess whether hydrodynamic culture conditions also influence embedded 3D constructs, a limited comparison was performed using Matrigel-embedded Caco-2 cultures.

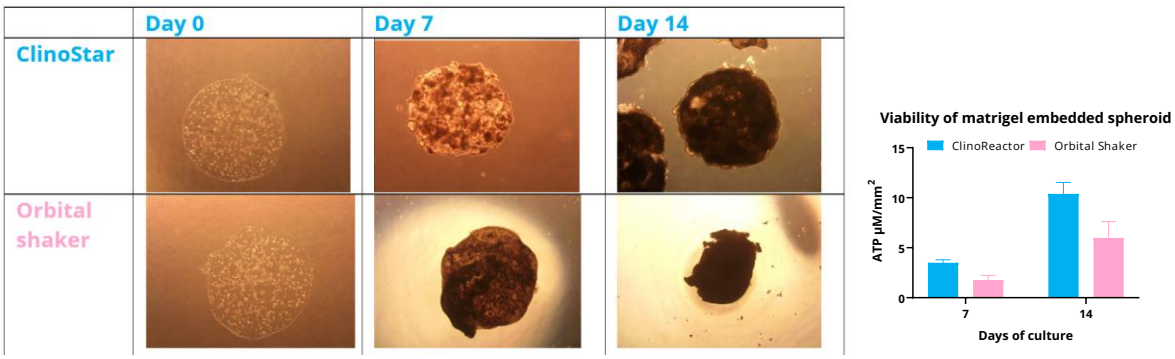


Figure 5: Hydrodynamic culture conditions influence hydrogel-embedded Caco-2 constructs.
Representative brightfield images of Matrigel-embedded Caco-2 constructs cultured under orbital shaker or low-shear dynamic conditions at days 0, 7 and 14. ATP content normalized to construct area is shown as mean ± SD.

Constructs maintained under low-shear dynamic conditions showed more uniform morphology and higher ATP levels compared with orbital shaker culture at the assessed time points. These observations shows that hydrodynamic environment remains an important experimental variable even in hydrogel-supported 3D culture systems.

Low-shear ClinoStar® culture supports improved brain organoid structural organization

Independent work from the laboratory of Liang Oscar Qiang, MD, PhD (Drexel University College of Medicine) demonstrated differences in brain organoid development between ClinoStar® and orbital shaker culture conditions.

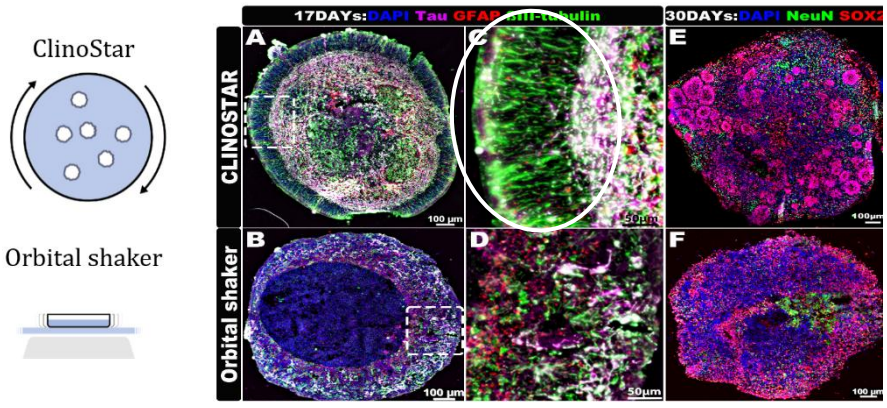


Figure 5: ClinoStar® culture supports improved structural organization of brain organoids compared with orbital shaker culture.
Immunofluorescence analysis of brain organoids cultured under ClinoStar® or orbital shaker conditions at days 17 and 30. Organoids cultured in ClinoStar® showed more defined structural organization and neural marker distribution compared with orbital shaker cultures. Markers shown include DAPI, Tau, GFAP, βIII-tubulin, NeuN, and SOX2. Scale bars: 100 µm (A, B, E, F), 50 µm (C, D).

Brain organoids cultured under ClinoStar® conditions exhibited more organized tissue architecture and clearer spatial distribution of neural markers compared with orbital shaker cultures, consistent with improved structural maturation under homogeneous low-shear conditions.

Conclusion

Hydrodynamic culture conditions influence the long-term performance of 3D cell models. In this study, HepG2/C3A constructs cultured under homogeneous low-shear conditions in the ClinoStar® system demonstrated improved structural integrity, sustained viability, continued biomass accumulation, and reduced variability compared with orbital shaker culture under the tested conditions.

Supporting observations from hydrogel-embedded epithelial constructs and external brain organoid studies suggest that hydrodynamic environment may similarly influence a broader range of 3D culture systems. These findings highlight fluid dynamic conditions as an important experimental parameter in the design of reproducible long-term 3D culture workflows. Hydrodynamic culture conditions play a central role in the long-term performance and reproducibility of 3D cell models. In the main study presented in this application note, HepG2/C3A constructs cultured under homogeneous low-shear conditions in the ClinoStar® system demonstrated improved structural integrity, sustained viability, continued biomass accumulation, and reduced variability compared with orbital shaker culture under the tested conditions. Similar trends were observed in other experiments where hydrogel-embedded epithelial constructs, and self-assembly brain organoids cultures shows that the hydrodynamic environment broadly influences the stability and development of diverse 3D culture systems.

Together, these findings emphasize fluid dynamic conditions as a critical experimental parameter for establishing robust, physiologically relevant, and reproducible long-term 3D culture workflows.

References

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