

PLA-based microcavity arrays – A practical alternative to PS, PC & Co with extended sustainability profile





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Optimal cell count and conditions for reproducible Hep G2 spheroids in PLA-based microcavity arrays

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Abstract

Poly(lactic acid) (PLA) microcavity arrays were evaluated as an alternative to conventional polymeric cell culture substrates. The PLA-based microcavity arrays combine optical transparency, precise cavity geometry, and industrial compostability (1) (2), thereby meeting the principal requirements for modern three-dimensional (3D) cell culture systems. Using the human hepatocellular carcinoma line Hep G2, optimal seeding conditions (1×10^5 cells per cavity) and surface-coating strategies were identified. BIOFLOAT™ coating yielded the most homogeneous spheroid morphology with minimal edge adhesion, whereas Pluronic® F-127 coating provided high cell viability ($\approx 80\text{--}90\%$). Collagen (rat tail) and uncoated surfaces promoted cell adhesion rather than aggregation and were deemed unsuitable for reproducible spheroid formation. Under the optimal conditions, uniform, viable spheroids were generated within 2–4 days, demonstrating that PLA microcavity arrays from the CaviSphere® platform can replace traditional plastics without compromising data quality while offering an improved sustainability profile.

Introduction

The most commonly used polymers for the production of cell culture materials are Polystyrene (PS), Polycarbonate (PC), Polyethylene (PE) and Polypropylene (PP) (3) (4). Three important material properties provide the basis for successful 3D cell culture platforms: optical clarity for reliable imaging, geometric precision of the microcavities to control spheroid size, and a favorable environmental footprint. PLA, a bio-derived polyester that is industrially compostable (1) (2), satisfies all three criteria. Its excellent formability enables the production of film-based microcavity arrays with reproducible cavity dimensions, and its intrinsic transparency facilitates microscopy. In addition to material selection, rapid identification of optimal assay parameters—particularly cell seeding density and anti-adhesion coating—is essential for establishing robust spheroid cultures.

Materials and methods (5)

Hep G2 cells (human hepatocellular carcinoma) were cultured in different cell numbers (5×10^4 , 1×10^5 , 5×10^5 , and 1×10^6) on MicroSphere-PLA arrays (CAVIGEN) coated with BIOFLOAT™ (according to the manufacturer), Pluronic® F-127 (2% w/v), rat tail collagen ($10 \mu\text{g}/\text{cm}^2$) or uncoated for 72 hours under standard conditions (37°C , 5% CO_2).

The development of the spheroids as well as their morphology and size were documented daily using bright field microscopy. To determine cell viability, cytotoxic LDH release (Cytotoxicity Detection Kit, Roche) in the culture supernatant was quantified after 72 hours and, in addition, a live/dead staining with SYTO™16 ($5 \mu\text{M}$) and propidium iodide ($3 \mu\text{M}$) was performed. The results were compared in terms of morphology, reproducibility of spheroid size, and cell viability.

Results

The results show that PLA-based microcavity arrays meet the requirements of modern 3D cell culture and offer an improved ecological balance. This makes PLA a practical alternative to PS/PC/PE/PP in the manufacture of cell culture articles without compromising the established requirements for sterility, handling and data quality.

The conditions described here have been optimized for the HepG2 cell line and provide a good basis for efficiently establishing other cell lines with minor adjustments.

The results in detail:

- Optimal cell count: 1×10^5 cells per MicroSphere-PLA array consistently produced uniform spheroids with high viability within 2–4 days (Fig. 1)
- Coating performance:
 - BIOFLOAT™: yielded the most homogeneous spheroids, minimal edge adhesion, and high reproducibility of sizes and numbers.
 - Pluronic® F-127: supported solid spheroid formation with viability $\geq 80\%$ but exhibited occasional multiple spheroids per cavity and slight variability in morphology.
 - Collagen (rat tail): increased surface adhesion, limiting aggregation; unsuitable for standardized spheroid generation.
 - Uncoated: resulted in significant adhesion and incomplete aggregation; unsuitable for reproducible 3D spheroids.
- Viability trends: High cell numbers ($\geq 5 \times 10^5$) generated overly large spheroids with reduced viability (70–80%) and suboptimal morphology, whereas the optimal density maintained viability in the range of 80–90%.

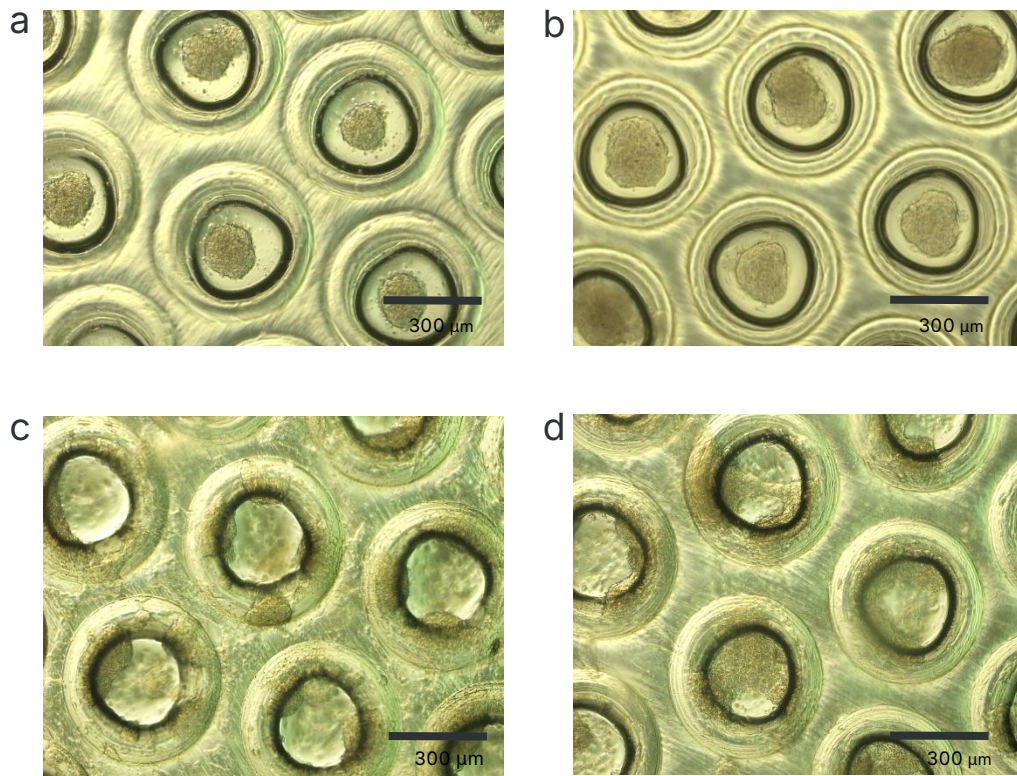


Fig. 1: Hep G2 spheroids in MicroSphere-PLA arrays, 1×10^5 cells; a: BIOFLOAT™-coated, day 3; b: Pluronic® F-127-coated, day 3; c: collagen (rat tail) coated, day 3; d: uncoated, day 3

Product note

PLA microcavity arrays (MicroSphere-PLA) from **CAVIGEN** are optimized for reproducible spheroid cultures. These are part of the CaviSphere® platform. A start protocol with coating and seeding recommendations (for Hep G2) is available and shortens the establishment phase of further cell lines to a few test rounds.

Information on this and other products can be found at <https://cavigen.eu/cavisphere/microsphere/>

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Version: v1.0
Date: December 2025

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