An Innovative Cell Culture Platform that Enables the Generation of Thousands of Three-Dimensional Cultures in One Flask

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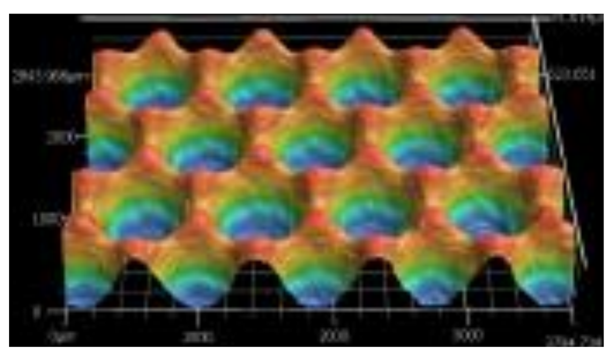
Introduction

Corning® Elplasia® Flask

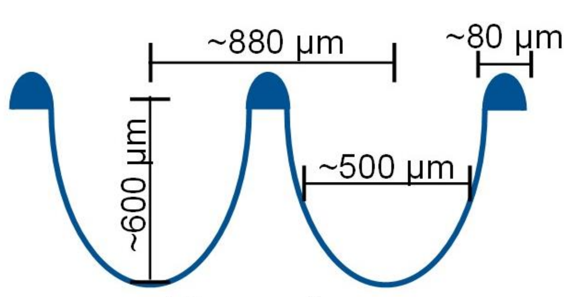
Three-dimensional (3D) cell culture models better mimic the *in vivo* environment compared with traditional monolayer culture models. As cancer research and advanced therapy development progress, production of greater numbers of spheroids are needed for more physiologically relevant applications. Current practices for producing high quality spheroids in bulk can be inefficient and/or highly variable. Here, we demonstrate the use of the new Corning Elplasia Flask for the growth of thousands of spheroids of consistent diameter and circularity in a footprint similar to a T-75 culture flask.

Microcavity Substrate

Schematic illustration of the microcavity substrate used to form the flask-bottom for the Corning Elplasia Flask. As cells settle into each microcavity via gravity, the microcavity geometry and gas-permeable substrate coated with Corning Ultra-Low Attachment (ULA) surface allow the formation of uniform size and shaped spheroids across the flask permitting long-term 3D culture. The substrate contains 152 microcavities per cm², which results in ~12,000 microcavities in the footprint of a T-75 flask.



Gas permeable



Dimensions
~500 µm wide and ~600 µm deep

Materials and Methods

HT-29 Spheroid Growth

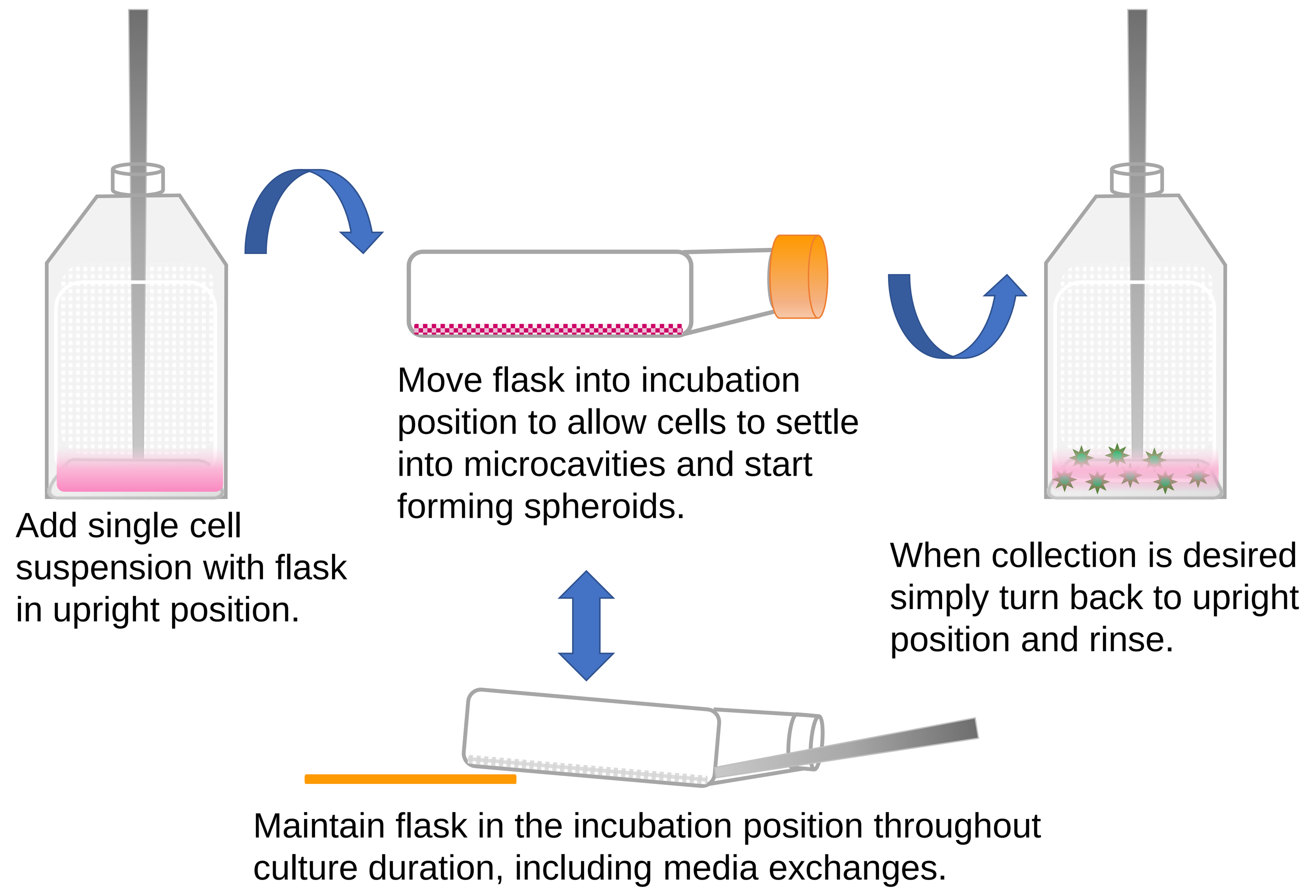
HT-29 GFP cells were seeded at 10 x 10⁶ cells per vessel in a Corning Elplasia Flask, a Corning ULA surface 75 cm² flask, and a Corning 125 mL disposable spinner flask.

- **Media (DMEM + 10% FBS) volume**: 30 mL for the flasks, 60 mL for the disposable spinner flask.
- **Incubation settings:** 37°C, 5% CO_2 in a humidified incubator. Disposable spinner flask agitation set to 30 rpm, then increased to 60 and 90 rpm based on size of aggregate formations.
- **Culture conditions:** Maintained in culture for 11 days with complete media changes performed on days 5 and 7. Spheroid formation monitored and micrographs taken on days 1, 5, 7, and 11.
- **Analysis:** Images used for diameter and circularity analysis using ImageJ analysis software. Propidium iodide staining of 11-day-old HT-29 GFP spheroids cultures for live/dead staining analysis using EVOS® FL microscope (532 and 646 nm).

NOTE: Fluorescent imaging of spheroids from the disposable spinner flask was performed after spheroids were transferred to a 100 mm plate for live/dead staining.

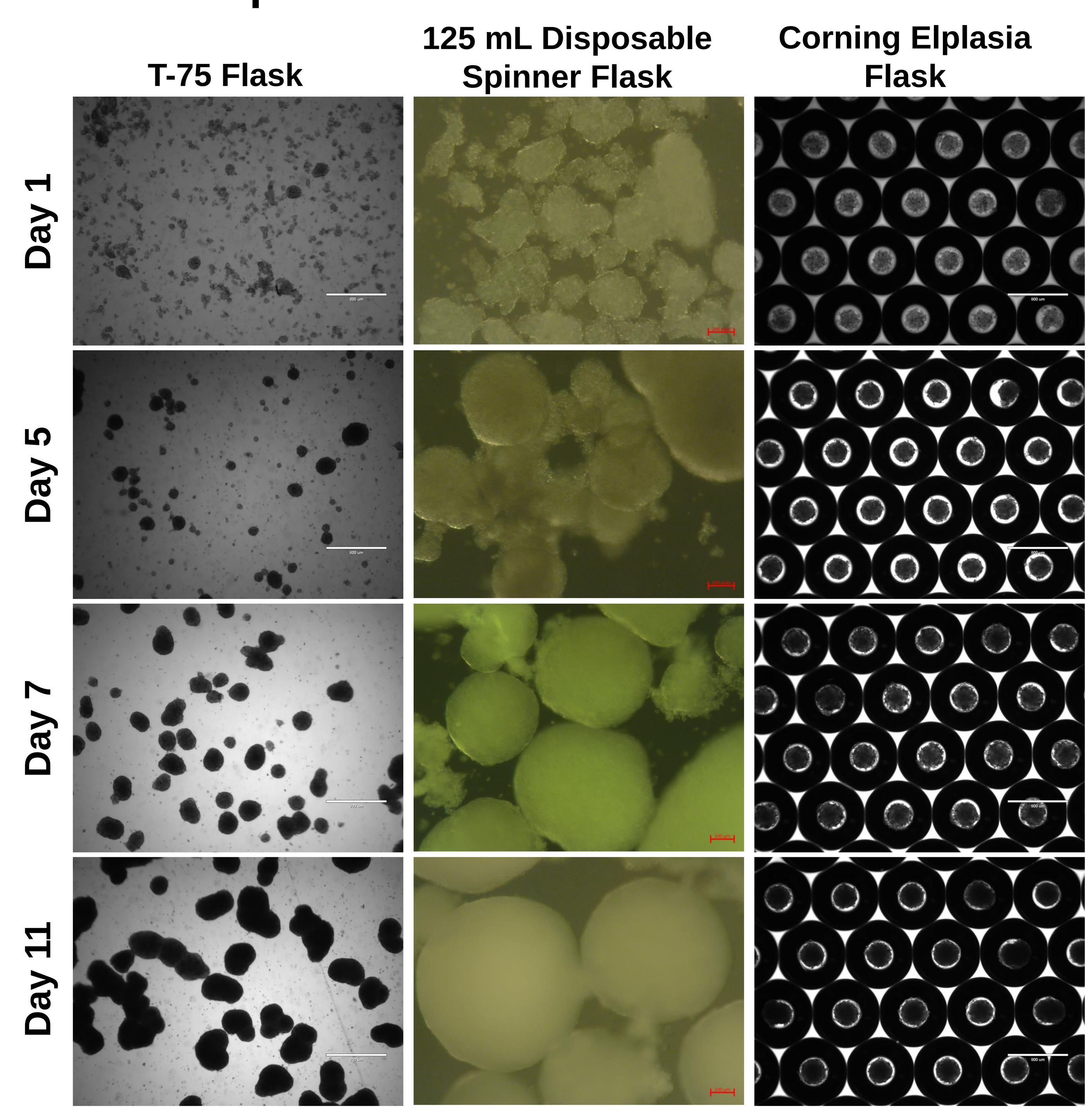
Flask Handling Overview

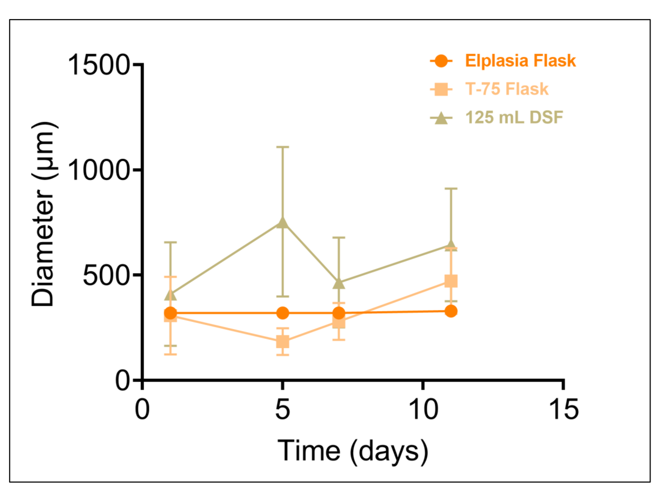
Corning Elplasia flasks are gamma irradiated and ready-to-use. Gravity along with the microcavity geometry and Corning ULA surface enable uniform spheroid formation. The microcavities are deep enough to permit media exchange with gentle handling, but not too deep that it is hard to remove the spheroids when desired. Below is a schematic representation of how the Corning Elplasia flask is used.

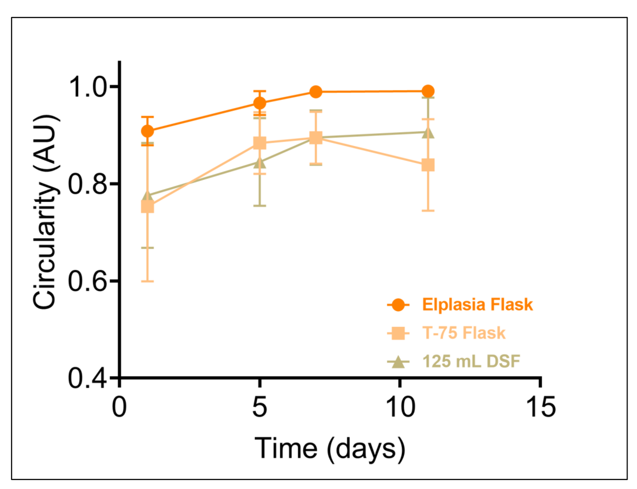


Results

Uniform Spheroid Production







Spheroid diameter (left) and circularity (right) was consistent in the Corning Elplasia flask while other methods generated spheroids with significant variability in both diameter and circularity throughout the 11 days. Data shown mean ± standard deviation, n ≥ 50 per group.

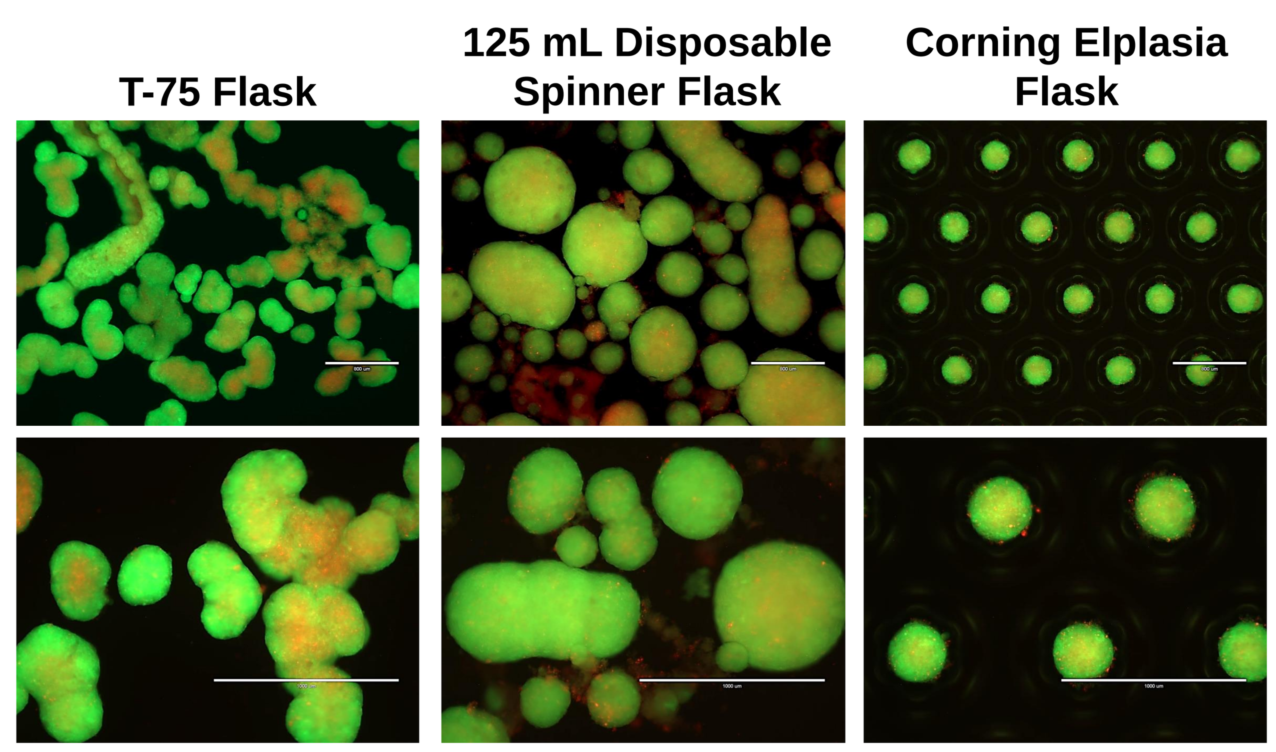
Bulk Spheroid Comparison

T-75 Flask with Corning ULA Surface

125 mL Disposable Spinner Flask

Corning Elplasia Flask

	T-75 Flask with Corning ULA Surface	Disposable Spinner Flask	Corning Elplasia Flask
Number of Spheroids	✓	✓	✓
Diameter Uniformity	✗	✗	✓
Circularity	✗	✗	✓
Viable Long-term Culture	–	–	✓



Representative images of HT-29 GFP spheroids at day 11 stained with propidium iodide for live/dead analysis. Microscopy images taken with EVOS FL microscope at 2X (top row) and 4X (bottom row) magnification.

Conclusions

Corning Elplasia flasks have been developed to support the bulk production of homogeneous spheroids in an easy-to-use flask design. The microcavity geometry, together with Corning Ultra-Low Attachment surface and gas permeable substrate, support long-term culture of spheroids that are easily visualized and collected for 3D cell culture applications such as:

- Compound screening
- Personalized medicine
- Biobanking
- Cell scale up
- Bioprinting
- Extracellular vesicle production

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