

Comparison of Ultra-Low Attachment Spheroid Microplates and Hanging Drop Microtissue Formation for High Content Screening

SnAPPShots

A brief technical report from the Corning Applications Group

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Introduction

In vitro three-dimensional (3D) cell cultures more accurately reflect the complex *in vivo* microenvironment than traditional two-dimensional (2D) cell monolayer cultures. Spheroid formation utilizing the hanging drop technique, whereby cells are suspended in droplets of medium to promote cell aggregation, is a popular 3D cell culture choice due to its cost effective cell usage and scaffold-free nature. Corning® spheroid microplates have opaque side walls, a unique clear round well-bottom design, and an Ultra-Low Attachment surface coating that enables the formation and growth of a single spheroid per well with reproducible size.

Here we compare human liver microtissues (hLiMTs) formed using the hanging drop technique or Corning spheroid microplates and assess the two approaches for their ease of imaging using high content screening.

Materials and Methods

- ▶ The following cell culture plates were used:
 - 96-well black with clear round bottom Ultra-Low Attachment surface spheroid microplates (Corning Cat. No. 4520)
 - 96-well black with clear flat bottom polystyrene TC-treated microplates (Corning Cat. No. 3603)

- Hanging drop microtissues were formed using specialized hanging drop cell culture plates of which there are multiple manufacturers.
- ▶ Dextromethorphan and midazolam substrates were used to study CYP2D6 and CYP3A4 activity, respectively, in hLiMTs. Formation of dextrophan and 1-OH hydroxymidazolam was analyzed by LC-MS/MS using standard Cyprotex methods.
- ▶ Microtissue ATP content was measured using CellTiter-Glo® Luminescent cell viability assay (Promega Cat. No. G7571).
- ▶ Monochlorobimane (Sigma Cat. No. 69899) was used to stain microtissues for GSH content with Hoechst (ThermoFisher Cat. No. H3570) nuclear counterstain. The microtissues were then imaged using the ArrayScan™ XTI system (ThermoFisher Cat. No. ASN00004F) in confocal mode. Bright field mode was utilized for microtissue diameter analysis.

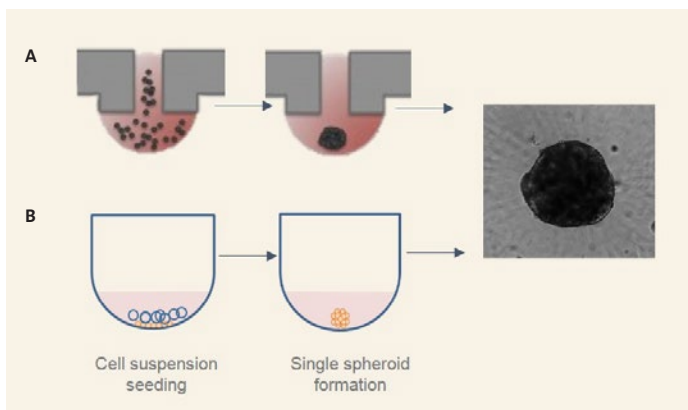


Figure 1. Spheroid formation in (A) hanging drop technique and (B) spheroid microplate with Ultra-Low Attachment surface coating.

Results

Hanging drop technique (HD), as well as the proprietary Corning spheroid microplates with proprietary round well bottom geometry, and Ultra-Low Attachment surface coating promote self-aggregation of cells into a single spheroid per well (Figure 1). hLiMTs display uniform size of 350 µm diameter and shape across the two approaches that can be maintained for up to 4 weeks (Figure 2), with comparable high levels of CYP2D6 and CYP3A4 activity shown on day 28 (Figure 3).

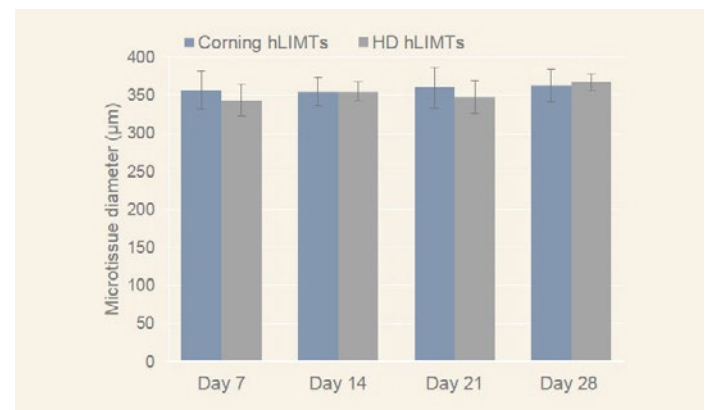


Figure 2. Microtissue uniformity and longevity. Time course measurement of microtissue cultured with the hanging drop technique (HD) and in Corning spheroid microplates. Microtissue diameter is shown with mean and standard deviation (SD) of triplicate samples shown on days 7, 14, 21, and 28 in culture.

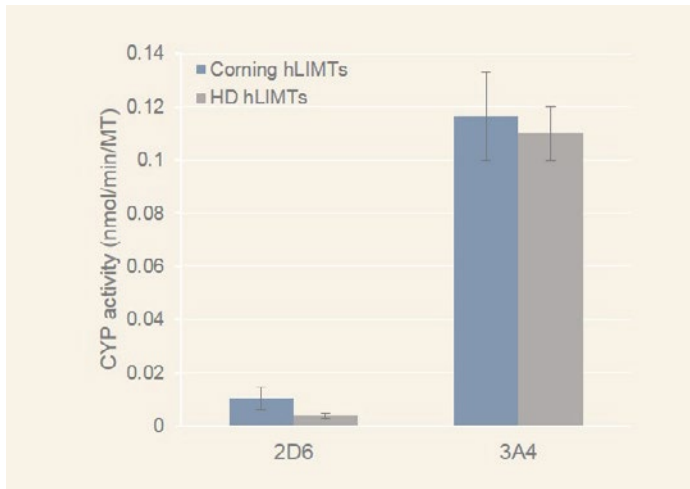


Figure 3. CYP2D6 (2D6) and CYP3A4 (3A4) activity in 28 day hLiMTs cultured with the hanging drop technique (HD) and in Ultra-Low Attachment surface Corning® spheroid microplates. Mean and SD of triplicate samples shown.

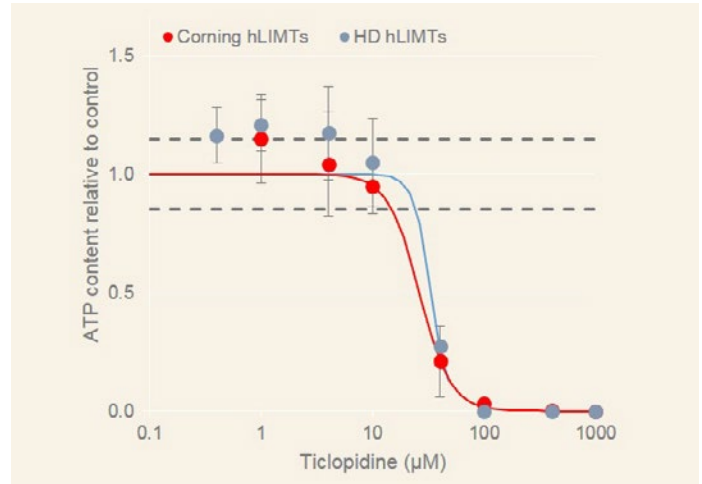


Figure 4. Sensitivity of hLiMTs to drug-induced liver injury. Concentration effect curves are shown for ATP content relative to control in microtissues cultured with the hanging drop technique (HD) and in the Ultra-Low Attachment surface Corning spheroid microplates following 14-day exposure. Mean and SD of triplicate samples shown.

hLiMTs formed from HD or Ultra-Low Attachment surface methods show comparable sensitivities to drug-induced liver injury (Figure 4). Ticlopidine, a known hepatotoxin¹, induces a dose dependent decrease in microtissue ATP content with IC₅₀ values of 33.5 µM and 26.1 µM in HD and Ultra-Low Attachment surface microtissues, respectively.

HD microtissues were transferred into a Corning 96-well clear with flat black bottom polystyrene TC-treated microplate, while Ultra-Low Attachment surface microtissues were left in the Corning® spheroid microplate. HD and Ultra-Low Attachment surface microtissues were stained with Hoechst (nuclei) and monochlorobimane (GSH content). The inner 60 wells of each

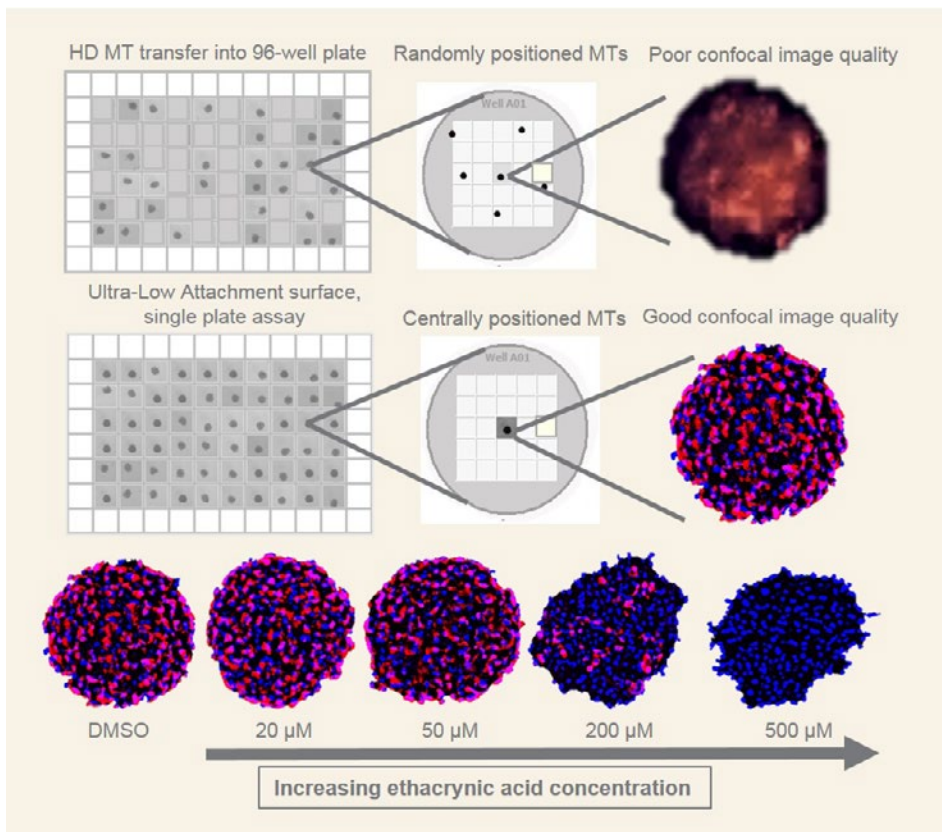


Figure 5. High content confocal imaging of microtissues (MT) cultured with the hanging drop technique (HD) and with the Ultra-Low Attachment surface Corning spheroid microplates. Microtissues were stained for nuclei and GSH content. HD MTs were transferred into 96-well flat bottom microplates for imaging.

plate were automatically imaged using the confocal mode of an ArrayScan XTI system (Figure 5). All 60 microtissues could be located and imaged at high definition in the Corning spheroid microplate, whereas the transfer approach of hanging drop cultures into a flat bottom plate resulted in high variability in microtissue location within the plate well and poor confocal image quality.

Advantages of Corning Spheroid Microplates for High Content Screening (HCS)

- ▶ Novel well geometry aids centering of microtissue position for automated high content imaging
- ▶ Single field of view
- ▶ Standard 96-well and 384-well microplate dimensions allow robotic automation
- ▶ Plates amenable to confocal high content imaging
- ▶ Opaque black side-walls minimize well-to-well fluorescent cross-talk

- ▶ Flexibility of antibodies and dyes
- ▶ Single plate assay (i.e., culture and assay of microtissues in the same microplate) allows for easier high throughput production and handling of microtissues

Conclusions

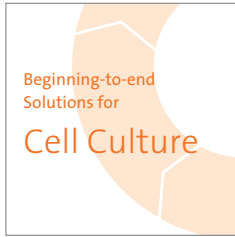
- ▶ The microtissues formed using either Corning spheroid microplates or the hanging drop technique display comparable characteristics in terms of size, longevity, and functional tissue characteristics.
- ▶ The novel well geometry of Corning spheroid microplates enable high quality automated imaging of microtissues for high content screening approaches, allowing multiplexed cell health analysis to be performed *in vitro* with *in vivo*-relevant 3D models.

Reference

1. Hirata, K., et al. Ticlopidine-induced hepatotoxicity is associated with specific human leukocyte antigen genomic subtypes in Japanese patients: a preliminary case-control study. *Pharmacogenomics J.* 8(1):29-33 (2008).

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