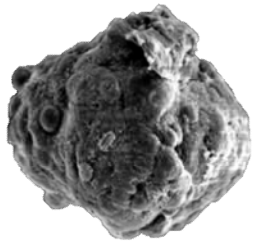
Accelerating 3D Microtissue Production

Fast and efficient 3D microtissue production with the Viaflo 96 channel hand-held electronic pipette

Preclinical drug development relies on advanced in vitro models with a high degree of standardization to achieve maximal predictivity for an efficient compound de-risking strategy. InSphero's scaffold-free organotypic three-dimensional (3D) microtissue spheroids fulfil these requirements as they can be produced and assayed by standard automated liquid handling systems. The Integra Viaflo 96 Channel Electronic Hand-Held Pipette is perfectly suited to produce 3D microtissues in InSphero's GravityPLUS™ hanging drop plate and to further culture and process them in the GravityTRAP™ platform. Ease of use, versatile programming, and highly accurate tip positioning are the key advantages of the Viaflo 96 pipette which guarantee to perform medium to high throughput screening campaigns with 3D microtissues successfully and with considerable time savings of up to 40% compared to manual pipetting with an 8-channel pipette.

3D microtissues for the advancement of cell-based assays



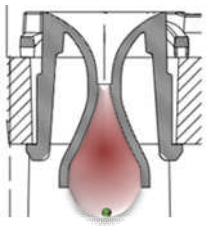
3D cell culture enables the investigation of cellular functions that are usually not observable in «petri-dish-based» culture formats. InSphero offers a variety of scaffold-free microtissues derived either from tumor cell lines, primary cells or iPSCs. Theses microtissues display a variety of organotypic features which underscores their biological relevance in predicting drug interaction far better than with commonly used cell monolayers:

- Enhanced formation of cell to cell contacts (tight junctions, desmosomes, etc.)
- Extracellular matrix formation
- Polarization of epithelial cells
- Organotypic cell arrangement in co-cultures of parenchymal and non-parenchymal cells
- Altered gene expression profile due to epigenetic regulation

Because of their more pronounced tissue-specific properties and functionality, 3D microtissues are perfectly suited for medium to large scale compound testing as required in the pharmaceutical, cosmetics and chemical industry.

InSphero's hanging drop microtissue production platform

InSphero's microtissue production system relies on the hanging-drop cell culture technology, which minimizes the interaction with artificial surfaces and maximizes cell to cell contact mediated by gravity-enforced cellular self-assembly. InSphero's patent-pending hanging-drop platform GravityPLUS™ is designed to produce and maintain standardized microtissues in a highly reproducible fashion using standard liquid handling equipment. Each individual well is designed in the shape of two opposed funnel-like structures connected by a capillary, thus creating sufficient adhesive and capillary forces to keep a drop of 35-45 µl stably in place. Microtissues are produced in the GravityPLUS™ plate upon application of a cell suspension of defined cell density from the top. Microtissue formation and maturation in the hanging drop occurs within 2-4 days after seeding depending on cell types used.

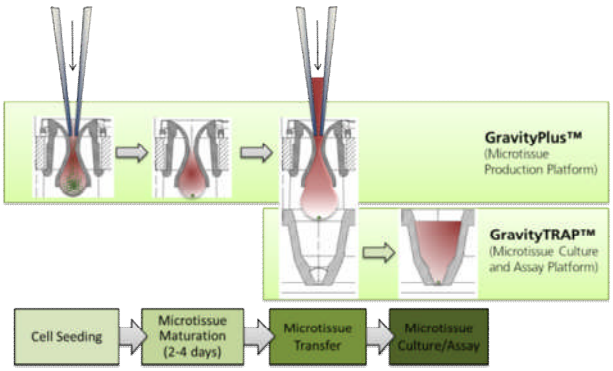


GravityTRAP™: 3D microtissue receiver and assay platform

For extended culture periods and downstream processing (compound treatments, assays), the microtissues can be transferred into the GravityTRAP™ format, which is a specifically designed multi-well plate to accommodate microtissues produced in the GravityPLUS™ system. The GravityTRAP™ offers several key advantages for culturing, manipulating and assaying microtissues:

- No attachment to plastic due to non-adhesive coating
- Transparent clear bottom for visual inspection by inverted bright field or fluorescence microscopy
- Safe medium replacement without risk of microtissue loss

The transfer of microtissues from the GravityPLUS™ into the GravityTRAP™ platform can be achieved simply by supplying sufficient medium to the already existing hanging drop, which eventually falls into the well of the aligned GravityTRAP™ underneath carrying along the microtissue.



The above figure illustrates the sequence of 3D microtissue seeding, formation and maturation in the GravityPLUS™ plate with subsequent transfer into the GravityTRAP™ format for long term culture and experimental procedures.

Viaflo Electronic 96 Channel Hand Held Pipette

Viaflo 96 is a 96-channel pipette, which was designed to resemble hand held pipetting and to increase productivity at the same time. The pipette is guided by hand but movements are assisted by motors for effortless and ergonomic working.
Viaflo 96 is used to transfer reagents and samples from a reagent reservoir to 96 and 384 well plates or from plate to plate. Up to 96 samples can be transferred at once.
To reformat 96 well plates to 384 well plates, the plate holder can be shifted to quickly accommodate all wells of a 384 well plate.
Viaflo 96 covers a large volume range of 0.5 µl to 1250 µl with four interchangeable pipetting heads. These heads are changed within seconds to optimally adapt Viaflo 96 to the application currently performed.
For delicate pipetting operations, such as the transfer of microtissues, several user-defined position settings can be used. This allows automatic guidance into the wells but also to set a specific z-height. The z-height sets a minimum height above a plate and assures that all subsequent transfers are performed at the same height to guarantee highly reproducible pipetting results.

Microtissue production with the Viaflo 96 hand held pipette

The outstanding tip fitting of the Viaflo pipette which grants perfect tip positioning with the well inlets of the GravityPLUS™ plate was already described in a previous joint application note[1]. The following section compares the performance of the Viaflo electronic 96 channel hand held pipette in comparison to the Viaflo 8-channel pipette for the preparation and maintenance of microtissue culture with particular focus on production time and accuracy.

Material and Methods

Instruments

- Viaflo 96 (Integra Biosciences AG, Switzerland)
- Zeiss Axiovert20
- Canon Powershot G11

Microplates

- 96-well GravityPLUS™, hanging drop plate (InSphero AG, Switzerland)
- 96-well GravityTRAP™, clear with transparent optical bottom (InSphero AG, Switzerland)

Cells

- HCT-116 colon carcinoma cells (ATCC)

Reagents

- RPMI 1640, FCS, Penicillin/Streptomycin, Hepes, Trypsin (PAA Laboratories)

Procedure

Cell seeding: The HCT-116 cell suspension was adjusted to 12'500/ml and 62'500/ml corresponding to a final density of 500 and 2500 cells/drop, respectively. The cell suspension was mixed 3x by pipetting up and down (speed 3) and 40ul were dispensed (speed 3) into each well of the GravityPLUS™ plates either by a Viaflo 8-channel or Viaflo 96-channel pipette. The GravityPLUS™ plates were placed in a humidified CO_2-incubator.
Medium exchange: At day 4 after seeding, the medium was exchanged twice by aspirating 20µl (speed 1) of the drop and reapplying 20µl (speed 1) of fresh medium.
Microtissue transfer: A direct vertical transfer of the microtissue contained in hanging drops was achieved by applying 70µl of medium to the drops in the GravityPLUS™ plate. The weight of the 70µl volume surpasses the adhesive and capillary forces, thus dropping the microtissue into the GravityTRAP™ plate secured beneath the GravityPLUS™ plate by notches designed within the plates.

[1] http://www.integra-biosciences.com/sites/viaflo96_1_e.html

Microtissue size analysis: Pictures were taken from microtissues in the GravityTRAP™ and the size was assessed by determining the average diameter using an image processing program (ImageJ).

Results

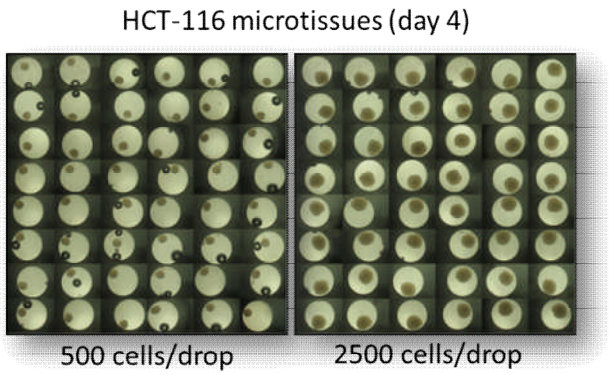
Processing time

The time requirement for the 3 microtissue processing steps – **1. Cell seeding** – **2. Medium exchange** – and **3. Transfer** into GravityTRAP™ was measured while processing 8 plates with either the Viaflo 96-channel or the Viaflo 8-channel pipette. The overall time for an entire microtissue production process could be reduced by 3:35 min per plate (-43%) using the Viaflo 96-channel pipette instead the 8-channel pipette. Major time saving could be achieved during medium exchange which involves 4 pipetting steps.

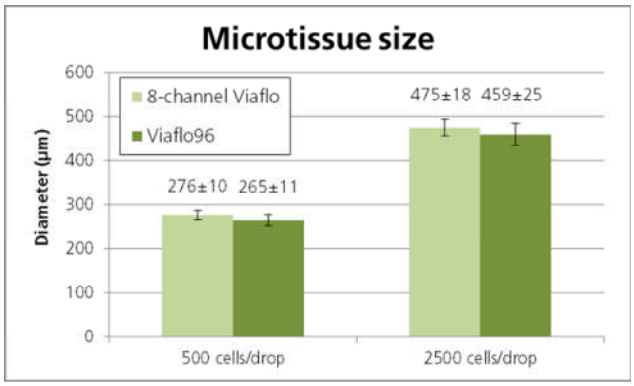
Process step	Conditions	8-Channel Viaflo	Viaflo 96
Seeding into GravityPlus™	mixing (3x), speed 3 asp./disp. , speed 3	02:36 min/plate	01:59 min/plate
Medium exchange in GravityPlus™	2x 50% asp./disp. , speed 1	02:47 min/plate	01:35 min/plate
Transfer into GravityTRAP™	with 70µl disp., speed 1	03:01 min/plate	01:15 min/plate
Total processing time		08:24 min/plate	04:49 min/plate (-43%)

Table 1: Processing time for the individual microtissue production steps. Pipetting modes and speed settings of both pipettors were identical. Data are mean out of n=8.

Pictures were taken from each microtissue and the individual size was measured by image analysis.



The resulting microtissue diameters were extremely homogeneous for both pipetting systems used, with very comparable CV of 3.7-5.5% for intra plate variations and 0.64-1.42% for inter plate variations (Table 2). Microtissues volumes calculated from the measured diameters while assuming a perfect sphere were 0.010mm^3 and 0.053mm^3, almost exactly reflecting the ratio of cell numbers at which the microtissues were seeded (500 and 2500 cells/drop).



Microtissue size variations (diameter)		8-Channel Viaflo (%CV)	Viaflo 96 (%CV)
Seeding density 500 cells/drop	intra plate	3.7	4.3
	inter plate	0.64	1.43
Seeding density 2500 cells/drop	intra plate	3.9	5.5
	inter plate	0.63	0.52

Table 2: Coefficient of variation of the diameter of microtissues processed with either the 8-channel or the 96-channel pipette. Data are obtained from 3 plates with a total of 138 microtissues per group. Inter-plate variations are based on size averages per plate.

Conclusions

The results clearly indicate that both pipetting systems, the 8-channel and the 96-channel Viaflo pipette, are highly accurate to produce microtissues of a homogeneous size. An intra-plate variation of roughly 5% is a remarkably low value considering the complex biology that affects 3D microtissue formation and cell proliferation.

As expected, the production time can be drastically reduced when using the 96-channel pipette saving up to 3.5 min per plate for the entire microtissue production process (including cell seeding, medium exchange and transfer into the GravityTRAP™ culture platform). For a typical screening campaign with 10,000 data points one could save approx. 6 hours of hands-on-time for microtissue production when using the Viaflo 96-channel pipette without compromising the spheroid size homogeneity. Thus, assuming an 8 hour labour day, with two hours of preparation the Viaflo 96 allows preparation of approx. 75 plates while only 43 plates could be processed with the 8-channel pipette.
In addition to the mentioned time savings, Viaflo 96 reduces strain on arms and wrists during pipetting due to the motor assisted movements.