INTEGR



APPLICATION NOTE Optimizing Methods in Microtissue Cultivation

Introduction

Cells grown in standard suspension or monolayer culture lose many features that are characteristic of cells in tissues and limit their use to study certain aspects of cell biology. Major differences to in vivo growth conditions of cells are:

- Mostly cell-substrate interactions, absence or limited cell to cell and cell to matrix interaction
- Flat cell shape rather than a three-dimensional architecture
- Altered hormonal, nutritional and physical environment
- Altered gene expression profile

InSphero has developed a hanging drop culture system to overcome these drawbacks of conventional cell culture technologies. As a result, the cells preserve their differentiated function and tissue like behavior. The microtissue system is based on the novel Gravity^{Plus} culture platform (patent pending) which is designed for the use of multichannel pipettes for efficient processing of the hanging drops such as cell loading, media exchange or compound supplementation.

Extensive working and testing of the hanging drop culture system revealed that the versatility and precision of the INTEGRA VIAFLO electronic pipettes allows a fast and economic production of microtissues in hanging drops.

The hanging drop culture

The hanging drop culture technology has shown to be a versatile method to generate microtissues with most cell types (primary cells or tumor cell lines) with inherent properties to reform a tissue with neighboring cells. The process is accelerated by gravity-induced cell accumulation at the bottom of the drop and yields microtissues of defined dimensions based on the number of cells initially inoculated into the hanging drop.

The interaction with any artificial surface which might impact cell growth and functionality is prevented. Scaffold-free microtissues show a higher level of functionality and biomimicry than conventional monolayer cell cultures and are therefore a valuable model for biomedical and pharmaceutical research.

Microtissue applications:

- Cell-to-cell interactions
- Cell-to-extracellular matrix interaction
- Compound efficacy testing
- Compound toxicity testing
- Stem-cell research
- Cell-invasion assays
- Study of transcriptomics, proteomics and metabolomics
- Tissue engineering
- Regenerative medicine

Gravity^{Plus} hanging drop technology

InSphero has developed a new microtissue culture system. The Gravity^{Plus} plate is designed in a standard 96-well format that allows full compatibility with manual and automated liquid handling systems. This compatibility makes the Gravity^{Plus} platform the first 3D cell-culture technology permitting high throughput screening with a single microtissue per well.

Working with microtissues in the Gravity^{Plus} format is as simple as working with current 2D multiwell culture systems. The well design consists of two opposed funnellike structures which are connected by a small capillary.



Fig. 1: Illustration of drop application on the Gravity^{Plus} platform by top loading for the formation of hanging drop microtissue culture.

Approximately 35-45 μ l of cell suspension is passed through the channel with a pipette which creates a hanging drop on the opposite side. The bore ensures a good fit of the pipette tip to the channel and guarantees reproducible drop size. Regular medium exchange can be performed by aspirating the medium from the top and adding fresh medium.

To assure a high throughput all steps are performed by a robotic liquid handling system or a multichannel pipette. The quality of the pipettor in this context is of high importance. It is crucial for all steps that the tips seal accurately on the eight bores of a row and the dispense volume is precisely the same for all channels. To fulfill this requirement the tips need to be exactly in line and sit precisely on the same height.

Insphero uses an INTEGRA VIAFLO 300 µI 8-channel electronic pipette for R&D purpose. The INTEGRA VIAFLO pipettors feature the Tri-Lobe tip fitting system which ensures that the tips snap into place, gripping the pipettor to deliver the highest lateral resistance.





Benefiting from unique Tri-Lobe fittings, GripTips deliver a precise and consistent tip seal ensuring that all tips on a multichannel pipette are precisely at the same height to increase the accuracy and precision of each multichannel dispense and at the same time, never accidently fall off.

An accurate pipetting system is crucial to produce microtissues containing the same amount of cells throughout the plate, to add the right concentration of testing compounds in each drop, and to have an equivalent medium exchange rate between the different wells to produce a native tissue model with the highest reproducibility.



Fig. 2: The unique tip mounting mechanism of INTEGRA VIAFLO electronic pipettes.

If the cell concentration and media is the same for the multiple columns, it is best dispensed with the repeat dispense mode. For certain assays different microtissues are grown on a single plate, usually in column patterns 1-12. This makes it necessary to handle different media. With the INTEGRA VIAFLO electronic pipettes this task is no problem because it lets the user fully customize his own programs, e.g. aspirating and dispensing a different volume for each column. Using an electronic pipette also eliminates human pipetting errors which could falsify the assay.



Fig. 3: Production of rat primary liver microtissues: top loading of hepatocyte suspension with INTEGRA VIAFLO pipettor (left); rows of hanging drops (right).

An additional very helpful feature of INTEGRA VIAFLO is the wide range of the adjustable pipetting speed. This allows efficient cell seeding at high speed, and ensures safe medium exchange without tissue loss at low speed. The mixing option is optimally suited for the addition of test compounds into the hanging drop, especially at low dosage volumes.

Conclusion

InSphero's Gravity^{Plus} technology (patent pending) enables the generation of various microtissue models using either cancer-derived cells to reconstitute tumor tissues or primary cells to mimic structure and function of diverse organs.



Fig. 4: Primary rat heaptocyte microtissues after 6 days in culture.

The advantages of microtissues produced with the Gravity^{Plus} technology are:

- Defined, uniform size and growth, highly standardized with superior assay reproducibility
- In-vitro culture method with highest biological relevance
- Spherical structure ideally suited for computational modeling

The INTEGRA VIAFLO electronic multichannel pipettes provide an optimal solution from the simplest to the most complex pipetting task. For InSphero the outstanding tip fitting providing precise positioning of the tips is one of the key features for using these pipettes, as it guarantees connection of all tips to the bores on their Gravity^{Plus} platform technology.

Authors

Bastien Machi is Biotechnology Engineer at InSphero AG, Technoparkstrasse 1, 8005 Zurich, Switzerland, phone: +41 44 515 04 90, email: bastien.machi@insphero.com

Michael Beier

is Product Manager for Liquid Handling at INTEGRA Biosciences AG, Tardisstrasse 201, 7205 Zizers, Switzerland, phone: +41 81 286 95 41, email: michael.beier@integra-biosciences.com