Analyzing 3D Cell Culture using the Corning® Cell Counter

Application Note

Zhang Linyu, Wang Xuebin, and Chen Rui
Corning Incorporated, Life Sciences, Asia Technology Center
Shanghai, China

Introduction

Organoids, spheroids, and three-dimensional (3D) cell culture models show great potential in many applications, including disease modeling and regenerative medicine[1,2]. Organoids are complex clusters of organ-specific cells. These structures are derived from stem cells or progenitor cells and self-assemble in a scaffolding extracellular matrix. In such an environment, groups of cells proliferate into microscopic versions of parent organs and are viable for 3D studies. Spheroids are simple clusters of varying cell types that do not require any scaffolding to form 3D cell cultures; however, congeal via cell adhesion. Both spheroids and organoids are composed of multiple cells and have the characteristics to be the baseline for 3D cell culture research.

Currently, manual organoid or spheroid counting is a routine procedure for many research laboratories; however, it is time-consuming and user-dependent. In addition to counting irregular three-dimensional objects, collecting size and size distribution data of such samples are subject to user variability. Corning's automated solution described herein provides an organoid counting feature that accurately measures organoids or spheres. The Corning Cell Counter (Corning 6749) can rapidly capture concentration and surface area data of 3D objects by adding the Organoid Counting software extension (Corning 6749-OC).

Materials and Methods

Spheroid formation

MRC5 cell line was used to generate spheroids in a 6-well round bottom Corning Elplasia® plate (Corning 4440). MRC5 cells were cultured and passaged in DMEM (Corning 10-013-CV) with 10% FBS (Corning 35-081-CV). For the detachment of adherent cells, 3 mL of 0.25% Trypsin-EDTA (Corning 25-053-CI) was added to the culture in a T-75 flask (Corning 430641U); subsequently, an equal volume of serum-containing medium was used to terminate digestion.

Corning Elplasia round bottom plates with Corning Ultra-Low Attachment surface contained multiple microcavities and allowed cells to form large-scale consistent spheres in a well. Up to 2,885 spheres can be formed in one well of a 6-well round bottom Elplasia plate. By adjusting the initial cell concentration, spheres of various sizes were obtained. In this study, 50, 100, or 200 MRC5 cells for each microcavity (approximately 1.44 x 10^6, 2.89 x 10^6 or 5.77 x 10^6 cells for one well) were used to generate spheres of different sizes (defined as small, medium, and large spheres, respectively). Cells were plated in the wells and cultured at 37°C with 5% CO_2. After 2 days of culturing, spheres were formed in each microcavity (Figures 1A-1C).

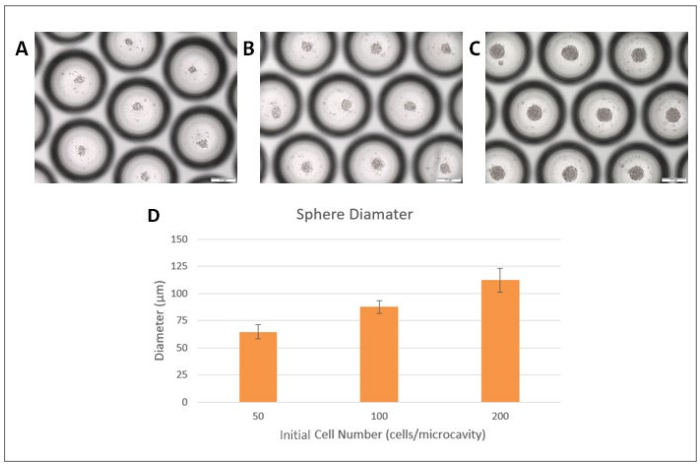

Figure 1. Consistent spheroids formed in the Corning Elplasia plate. After plating and culturing MRC5 cells for 48 hours in an Elplasia plate, the cells aggregated and formed into spheres. The sizes of spheres varied depending on the initial plating densities that were 50 (A), 100 (B), and 200 (C) per microcavity. (D) The diameter for each sphere was measured with ImageJ software. Images were captured at 100X magnification with an Olympus IX53 microscope. Scale bars are 200 µm. Data represent mean ± standard deviation (SD).

Sphere counting

To acquire a sphere suspension for counting, the spheres were suspended by gently aspirating and dispensing the medium then transferred to a 15 mL centrifuge tube. Spheres from two wells were harvested (approximately 5,000 spheres), centrifuged at 200 x g for 3 min., and resuspended in 100 µL of PBS.

The Corning Cell Counter (Corning 6749) in conjunction with the organoid counting software (Corning 6749-OC) was used to automatically count the concentration and sizes of the spheres. The 3D counting chamber (Corning 480201) has a depth of 0.2 mm and is compatible with spheres or organoids with a diameter ranging from 10 to 200 µm. A 20 µL sphere suspension was added to the counting chamber, and placed on the stage for analysis. Corning's automated solution provides users an option to select the version of algorithm based of the contour of their 3D harvest. Version 1 is optimal for use with irregular morphologies whereas, Version 2 of the organoid counting software is for best use with spherical objects. Version 2 of the organoid counting software was used to capture the data herein.

For manual counting, 10 µL of sphere suspension was added to a well of a 384-well high-content imaging microplate (Corning 4681); the spheres were viewed under a microscope and carefully counted. The concentration was calculated based on the results of the manual counting.

In this experiment, the Corning® Cell Counter multicount feature was used averaging eight individual 1.5 x 1.5 mm fields as part of one count. While three wells of a 384-well microplate for each sample was counted for the manual method.

Results and Discussion

MRC5 cells were plated onto a 6-well Corning Elplasia® round bottom plate at a density of 50, 100, or 200 cells per microcavity. After 48 hours of culturing, the cells aggregated and formed consistent spheroids (Figures 1A-1C). The diameters (Figure 1D) of spheroids were measured to calculate corresponding sizes using the following formula: $S=\pi^*(d/2)^2$ (Figure 4).

More than 2,500 spheres were formed in one well of a 6-well Elplasia plate. Spheres in two wells were harvested and resuspended in 100 µL PBS. In theory, the concentration of the samples should be approximately 5 x 10^4 spheres/mL. The sample concentrations were measured using the Corning Cell Counter and manual counting, respectively (Figure 2).

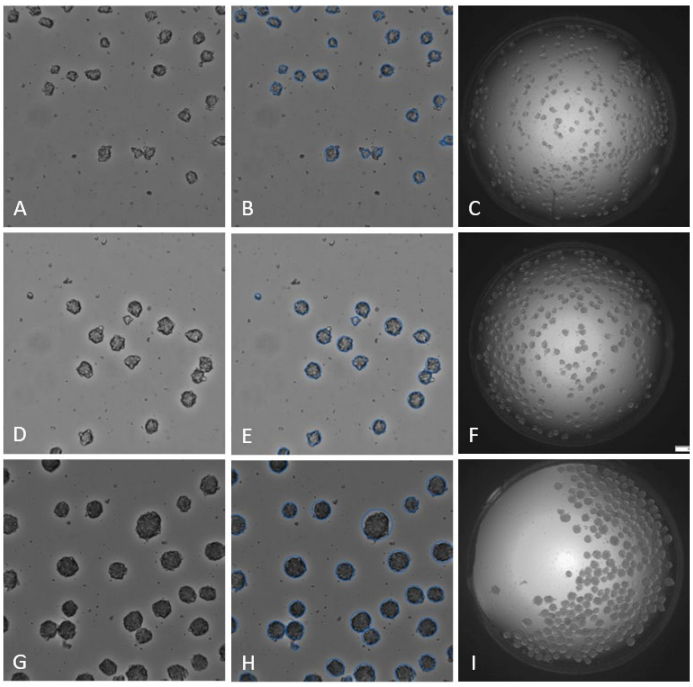

Figure 2. Representative counting images using the Corning Cell Counter and manual counting. (A-C) spheres formed at 50 cells/microcavity. (D-F) spheres formed at 100 cells/microcavity. (G-I) spheres formed at 200 cells/microcavity. (A, D, and G) Brightfield images of spheres prior to counting. (B, E, and H) Brightfield images of spheres were analyzed using an image analysis algorithm (in this application, the gates were set at 1,000 µm²). (C, F, and I) Brightfield images of spheres in the wells of a 384-well microplate under a microscope, images were captured with an Olympus IX53 microscope.

When comparing the two counting methods, the average concentrations measured by using the Corning Cell Counter were closer to theoretical values (Figure 3). The sizes of the spheroids were also measured by using the same instrument. As shown in Figure 4, sphere sizes measured by using the Corning Cell Counter and the calculated diameters are in agreement. These results indicate the suitability of the Corning Cell Counter for accurately counting spheroids.

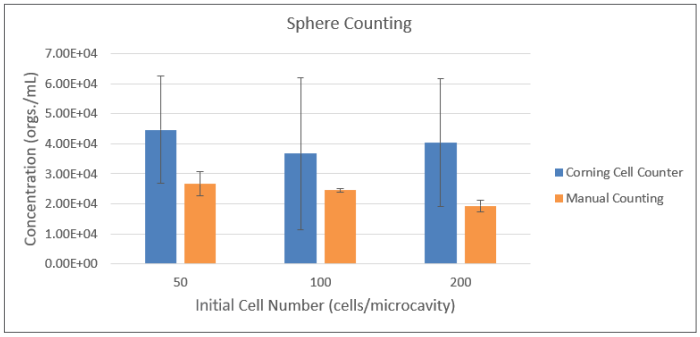

Figure 3. Sphere concentration calculated using the Corning Cell Counter and manual counting method. Data represent mean ± standard deviation (SD).

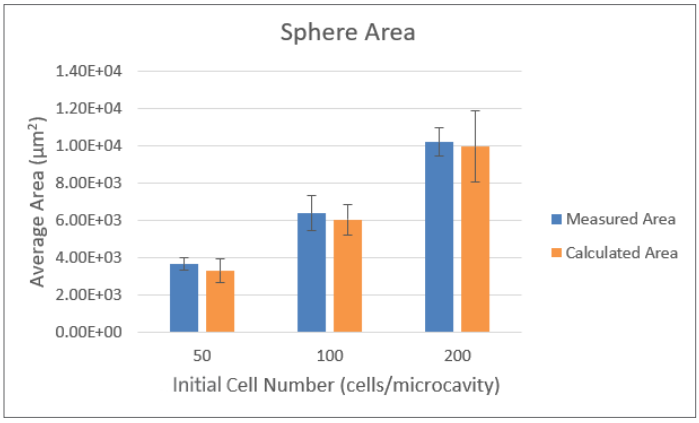

Figure 4. Sphere areas calculated using the Corning Cell Counter and manual counting method. Data represent mean ± standard deviation (SD).

Conclusions

- The Corning Cell Counter, with the organoid counting feature, enables quick and accurate counting of spheroids or organoids. The use of the Corning Cell Counter is intended to replace manual counting methods.
- The image analysis algorithm allows for the detection of organoids or spheroids with a wide range of sizes. The output of the analysis provides the user with an organoid size and count.
- The spheroid/organoid counting results acquired by using the Corning Cell Counter are more accurate than those obtained by manual counting.

References

1. Gunti S, Hoke ATK, Vu KP, London NR Jr. Organoid and Spheroid Tumor Models: Techniques and Applications. Cancers (Basel). 2021 Feb 19;13(4):874.
2. Fang Y, Eglen RM. Three-Dimensional Cell Cultures in Drug Discovery and Development. SLAS Discov. 2017 Jun;22(5):456-472.

Warranty/Disclaimer: Unless otherwise specified, all products are for research use or general laboratory use only.* Not intended for use in diagnostic or therapeutic procedures. Not for use in humans. These products are not intended to mitigate the presence of microorganisms on surfaces or in the environment, where such organisms can be deleterious to humans or the environment. Corning Life Sciences makes no claims regarding the performance of these products for clinical or diagnostic applications. *For a listing of US medical devices, regulatory classifications or specific information on claims, visit www.corning.com/resources.

CORNING

Corning Incorporated
Life Sciences

www.corning.com/lifesciences

NORTH AMERICA
t 800.492.1110
t 978.442.2200

ASIA/PACIFIC
Australia/New Zealand
t 61 427286832
Chinese Mainland
t 86 21 3338 4338
India
t 91 124 4604000
Japan
t 81 3-3586 1996
Korea
t 82 2-796-9500
Singapore
t 65 6572-9740
Taiwan
t 886 2-2716-0338

EUROPE
CSEurope@corning.com
France
t 0800 916 882
Germany
t 0800 101 1153
The Netherlands
t 020 655 79 28
United Kingdom
t 0800 376 8660
All Other European Countries
t +31 (0) 206 59 60 51

LATIN AMERICA
grupoLA@corning.com
Brazil
t 55 (11) 3089-7400
Mexico
t (52-81) 8158-8400