3D cell spheroid growth using Sphericalplates 5D® on the Fluent® Automation Workstation.

Application Note

AUTOMATED PRODUCTION AND MAINTENANCE OF 3D CELL SPHEROIDS

INTRODUCTION

There has been growing interest in the use of in vitro three-dimensional (3D) cell culture in cancer research and tissue biology over the last couple of decades. 3D spheroids are more physiologically relevant compared to conventional 2D cell culture, making them more suitable for in vitro efficacy testing and analysis of drug penetration, bridging the gap between traditional monolayer cultures and animal testing. Furthermore, 3D cell models are promising tools in personalized medicine, as tumor spheroids can be obtained from most tumors, and can be used to screen drugs to find effective patient-specific therapies. Alternatively, healthy multicellular spheroids are potential organ building blocks to restore lost organ functions in regenerative medicine applications.

However, most available techniques for 3D spheroid generation and manipulations are tedious, show poor reproducibility, and require more sensitive handling than traditional approaches. Standardized and reproducible cell seeding – and spheroid maintenance during growth – is essential to enable robust data collection and analysis. The use of automated handling procedures ensures consistent operating conditions across multiple experimental iterations, microplates and wells. Combined with the innovative design of the Sphericalplate 5D (SP5D, Kugelmeiers Ltd.) microplates, automation enables the production of large amounts of spheroids for further investigation.

This application note describes the automated plating and maintenance of hundreds of single spheroids in SP5D microplates on the Fluent Automation Workstation, and compares the results obtained with manual handling.

MATERIALS AND METHODS

Automation platform

Experiments were conducted on a Fluent 780 system, which included an eight-channel Air Flexible Channel Arm™ (Air FCA), a long Robotic Gripper Arm™ (RGA) that can reach below the Dynamic Deck™, and a Multiple Channel Arm™ (MCA) (from left to right, Figure 1). The system was fitted with a vertical laminar flow HEPA hood with UV light to ensure a clean environment for sterile cell culture applications. Liquid handling tasks with the Air FCA were conducted with 1,000 µl filtered disposable tips, and a sterile 50 ml Falcon® tube was used for pipetting into a sterile SP5D with the Air FCA. Individual scripts for cell seeding and medium exchange with the Air FCA were developed using FluentControl™ software.

Figure 1: A Fluent Automation Workstation **(A)** fitted with a vertical laminar flow HEPA hood **(B)** used for the cell seeding and medium exchanges.

Cell culture

Human fibroblasts fetal lung cell line WI-38 (Sigma) was expanded in 2D conditions in Dulbecco's modified Eagle's medium high glucose (Sigma) supplemented with 10 % fetal bovine serum (Gibco), 1 % penicillin-streptomycin (Sigma) and 1 % L-glutamine (Sigma). Cells were cultivated at 37 °C and 5 % CO_2, and the medium was changed every second or third day.

Cell seeding

Prior to seeding the cells, two SP5D microplates were rinsed by pipetting 1 ml PBS into the wells, and centrifuging the microplates at 1,000 x g for two minutes to remove air bubbles. Cells were then seeded either manually in one SP5D microplate or automatically using the Air FCA in a second microplate. A total of eight wells per plate were used for seeding, each at a density of one million cells per well and a final volume of 1 ml. It is important to note that the SP5D microplates are treated with a special non-fouling coating that prevents cells from attaching to the surface, allowing the formation of uniform and single spheroids in each micro-well (Figure 2).

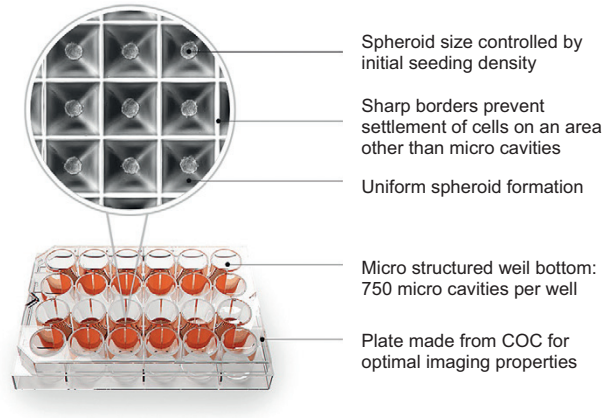

Figure 2: Each SP5D microplate provides 12 wells with microstructured well bottoms, allowing the cultivation of 750 uniform spheroids per well and 9,000 spheroids per plate.

Medium exchange

To enable spheroid growth, a partial medium exchange was conducted 24 hours post-cell seeding, using the automated Air FCA or by manual pipetting. During automated medium exchange, 750 µl of spent medium was removed from the upper right corner at an aspiration speed of 20 µl/sec, and fresh medium was dispensed in the same location at 20 µl/sec.

Imaging and analysis

Imaging to analyze displaced spheroids was performed using an Olympus IX81 automated microscope (10x). Bright field overview images were taken before and after medium exchange, and empty micro-wells not containing a spheroid were counted by eye.

RESULTS

The automated and manual seeding approaches both enabled uniform spheroid formation within 24 hours of incubation after cell seeding, as confirmed by bright field images taken prior to the medium exchange. On average, eight spheroids per well were found to be displaced after the automatically performed medium exchange, compared to thirteen spheroids per well when handled with a single channel manual pipette (Table 1 and Figure 3). Results obtained by the automated approach were more consistent and reproducible, as indicated by a lower standard deviation between the wells and the affected zones of spheroid displacement, which were restricted to a more confined area. It was also found that the automated medium exchange could be performed twice as fast as manually.

	Automated	Manual
A2	9	25
A3	8	25
A4	9	6
A5	9	17
C2	5	22
C3	7	7
C4	8	0
C5	7	4
Total	62	106
Mean	7.8	13.3
STD	1.4	10.1

Table 1: Results showing the number of displaced spheroids per well and per plate, with mean and standard deviation for automated and manual approaches.

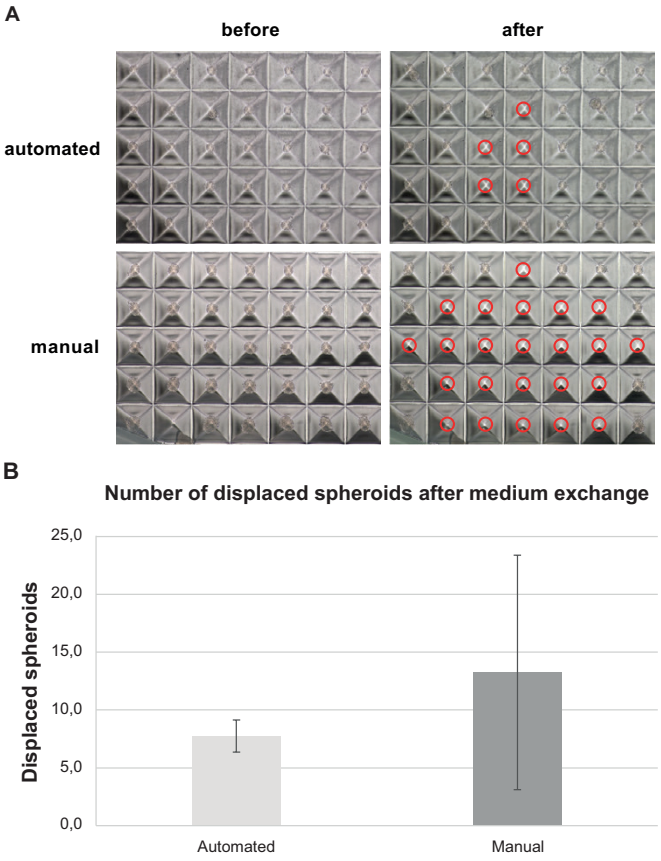

Figure 3: Image analysis before **(A, left)** and after **(A, right)** medium exchange revealed fewer displaced spheroids (○ red circles) per well using automation. Smaller error bars also indicate better reproducibility and consistency for the automated protocol **(B)**.

CONCLUSION

The data presented in this application note demonstrates the importance of standardized handling procedures for the cultivation of cell spheroids to increase reproducibility and throughput. The automated partial medium exchange for spheroids was more reliable when using the SP5D microplates on the Fluent Automation Workstation. This automated method caused less disturbance within the wells, reducing the number of spheroids being displaced. The automated approach was also demonstrated to be more reproducible and significantly faster compared with manual handling performed by an experienced operator. There is a high chance that the displacement of spheroids could potentially be eliminated entirely through further optimizations and fine tuning of pipetting speed and positioning.

About the authors

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