

Automated Media Exchange for Spheroid Cultures Using a Novel MultiFlo™ FX Accessory

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Abstract

Three dimensional (3D) spheroidal cell models have become a mainstay in life science research due to the ability to mimic *in vivo*-like environments. Performing media exchanges and washes with spheroids in cell repellent microplates can be problematic due to the risk of accidental spheroid removal. By incorporating a novel peristaltic pump-based tool, these procedures can be carried out in a controlled manner that eliminates spheroid disruption and removal, enabling long-term 3D experimental procedures requiring multiple media exchanges.

Introduction

Spheroidal 3D cellular structures are a mainstay in many research areas, including oncology and toxicology¹. Culturing cells in 3D provides a more *in vivo*-like environment, allowing cells to maintain high viability when cultured for extended time periods. To maintain the highest levels of viability within untreated cells and ensure that observed effects are solely from treatment, media exchanges and re-dosing are necessary throughout the experiment, particularly *in vitro* tests lasting weeks. Media exchanges with cell models that do not rely on adherence to labware can be daunting if performed by hand, even when performed on a single plate. Multichannel pipettes must remove and dispense media at an extremely slow rate, and care must be taken to keep pipette tips away from the actual spheroids.

Through incorporation of the AMX Media Exchange Module on the MultiFlo FX, risk of accidental spheroid removal from wells is eliminated. Spent media is automatically removed and replaced with fresh media only, or fresh media containing treatment concentrations.

Materials and Methods

Materials

Cells

U-87 glioblastoma cells were generously donated by Dr. Sachin Katyal (University of Manitoba, Winnipeg, Manitoba, Canada). HT-1080 fibrosarcoma cells (Catalog No. CCL-121) and PANC-1 carcinoma cells (Catalog No. CRL-1469) were obtained from ATCC (Manassas, VA).

Experimental Components

The known topoisomerase I inhibitor, camptothecin (Catalog No. 208925) was purchased from EMD Millipore (Billerica, MA). DMEM, low glucose, pyruvate, HEPES (Catalog No. 12320-032), advanced DMEM (Catalog No. 12491-015), fetal bovine serum, 10% (Catalog No. 10437-036), and penicillin-streptomycin (10,000 U/ml) (Catalog No. 15140-122), and penicillin-streptomycin-glutamine (100x) (Catalog No. 10378-016) were purchased from ThermoFisher Scientific (Waltham, MA).

Microplate Consumables

96-well, cell-repellent, polystyrene, round bottom, clear, sterile, microplates with lid (Catalog No. 650979) and 384-well, cell-repellent, polystyrene, round bottom, clear, sterile microplates with lid (Catalog No. 787979) were donated by Greiner Bio-One (Monroe, NC). 96-well, clear round bottom, sterile, ultra low attachment microplates with lid (Catalog No. 7007) and 384-well, black/clear round bottom, sterile, ultra low attachment microplates with lid (Catalog No. 3830) were donated by Corning, Inc. (Corning, NY). PrimeSurface® 96U clear round bottom 96-well microplates (Catalog No. MS-9096UZ) and PrimeSurface 384U clear round bottom 384-well microplates (Catalog No. MS-9384UZ) were donated by S-BIO (Hudson, NH).

Key Words:

AMX

MultiFlo AMX

Spheroid

3D

Spheroid Proliferation

Spheroid Media Exchange

Instrumentation

Cytation™ 5 Cell Imaging Multi-Mode Reader

Cytation 5 is a modular multi-mode microplate reader combined with an automated digital microscope. Filter- and monochromator-based microplate reading are available, and the microscopy module provides up to 60x magnification in fluorescence, brightfield, color brightfield and phase contrast. The instrument can perform fluorescence imaging in up to four channels in a single step. With special emphasis on live-cell assays, Cytation 5 features shaking, temperature control to 65 °C, CO₂/O₂ gas control and dual injectors for kinetic assays and is controlled by integrated Gen5™ Microplate Reader and Imager Software, which also automates image capture, analysis and processing. The instrument was used to kinetically monitor 3D tumoroid activity over the incubation period.

MultiFlo™ FX Multi-Mode Dispenser

The MultiFlo FX is a modular, upgradable reagent dispenser that can have as many as two peri-pump (8 tube dispensers), two syringe pump dispensers and a strip washer. The syringe and washer manifolds can be configured for plate densities from 6- to 384-well. The MultiFlo FX was equipped with the AMX Media Exchange Module.

AMX Media Exchange Module

Media exchange of spheroid cultures is accomplished through use of BioTek's patent-pending AMX Media Exchange Module, which consists of two unique, modified peristaltic pump cassettes with eight stainless steel tube aspirate (Figure 1, right arrow) and dispense (Figure 1, left arrow) heads. The cassette tubing is fed through the peristaltic pumps of the MultiFlo FX and into media bottles or tubes. Software allows the pumps to run slowly and gently so as to not disturb the spheroids during aspirate or dispense procedures. Each cassette is fully autoclavable, enabling sterile processing.

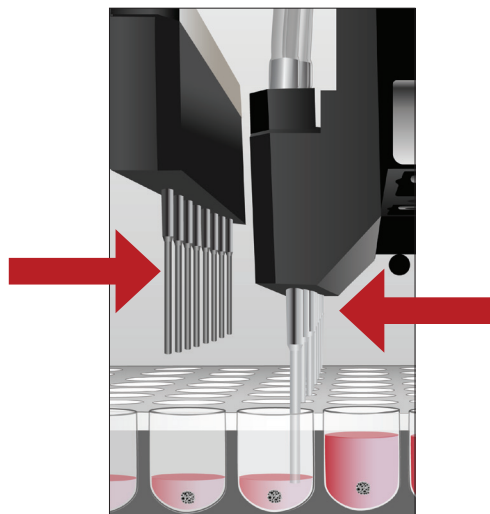


Figure 1. AMX Media Exchange Module with separate stainless steel tube aspirate (right arrow) and dispense (left arrow) heads.

Methods

Cell Preparation and Tumoroid Formation

Prepared U-87, HT-1080, or PANC-1 cells were harvested and diluted in complete media and dispensed into all microplate wells in a volume of 100 µL for 96-well microplates, and 50 µL for 384-well microplates. A total of 1000 cells was dispensed to all wells of each test spheroid plate. The microplates were incubated at 37 °C/5% CO₂ for forty-eight hours to allow cells to aggregate into single spheroids.

Media Exchange Method

The AMX Media Exchange Module aspirate tips were positioned at the back, right corner of each well in 96- and 384-well format, and the bottom of the tubes were elevated from the bottom of each well to avoid disturbing the spheroid (Figure 2). Media was removed from each well using a slow aspiration speed; with 15-20 µL of residual media volume in 96-well plate wells, and 10-15 µL of residual media volume in 384-well plate wells. When dispensing fresh media into 96-well spheroid plates, dispense tubes were positioned at the back, right corner of the well, away from the spheroid; whereas when dispensing into 384-well plates, the tubes were positioned directly above the spheroid to prevent disruption. In both microplate well densities, the bottom of the dispense tubes were elevated from the bottom of the well in a manner such that the media droplets contacted the existing well liquid to ensure equal volumes were dispensed to each well (Figure 3). The media dispense rate was optimized so that spheroids were not displaced from each well center.

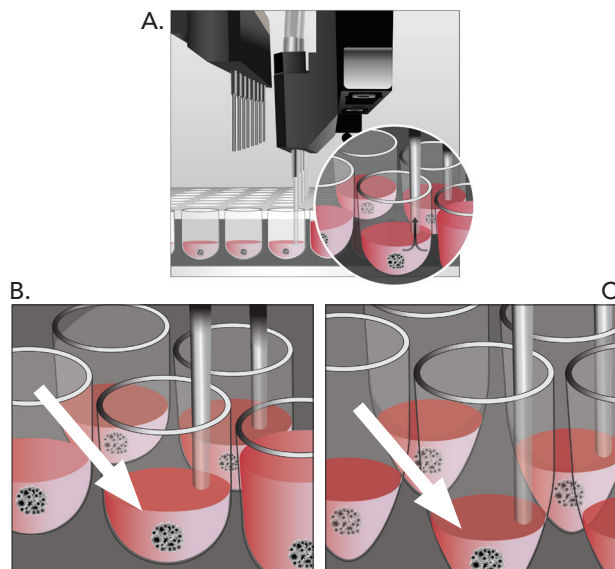


Figure 2. (A.) AMX Media Exchange Module aspirate tip positioning; illustrating intact spheroids in residual media using (B.) 96-well plates; and (C.) 384-well plates.

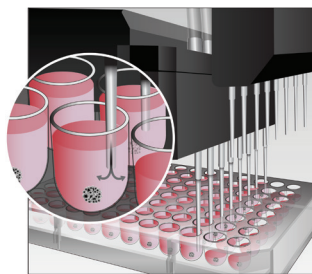


Figure 3. AMX Media Exchange Module 96-well plate dispense tip positioning.

Qualitative Validation of AMX Media Exchange Module

After U-87 spheroid formation, 96- and 384-well spheroid microplates were transferred to the MultiFlo™ FX with AMX module, and five media exchange cycles were performed concurrently to simulate a rigorous washing protocol. Once the media exchanges were complete, microplates were transferred to Cytation™ 5 for brightfield imaging of all wells. A 4x objective was used for all image capture. Due to the conical shape of the bottom of the well in 384-well microplates, a black ring is seen at the outer edges of each image (Figure 4B).

Quantitative Validation of AMX Media Exchange Module

Camptothecin was diluted in media to create an eight-point titration (10 µM - 2.4 nM) including a negative control without compound. Following aggregation, 96-well Greiner microplates containing U-87 spheroids, 96-well Corning microplates containing HT-1080 spheroids, and 384-well S-Bio microplates containing PANC-1 spheroids were placed into the BioSpa™ 8 Automated Incubator. At regular intervals, the plates were automatically transferred to the MultiFlo FX, where media was removed using the aspirate cassette and replaced with the various inhibitor concentrations using the dispense cassette. After each dosed media exchange, the plates were then transferred to Cytation 5 for kinetic brightfield imaging to monitor spheroid growth. Multiple images were captured in a z-stack using a 4x objective to ensure accurate calculation of spheroid volume. The “Object Size” value, which is the average diameter of the spheroid, was incorporated into a custom Gen5 metric using the mathematical volume of a sphere formula.

$$\text{Spheroid Volume} = (4/3) * \pi * (\text{Object Size}/2)^3$$

The process was repeated over the one or two-week spheroid proliferation incubation period.

Results

Qualitative Validation of AMX Media Exchange Module

After five cycles of 85% media exchange, brightfield imaging of the 96- and 384-well microplates (Figure 4) confirm that spheroids were not disturbed during the automated aspirate and dispense steps.

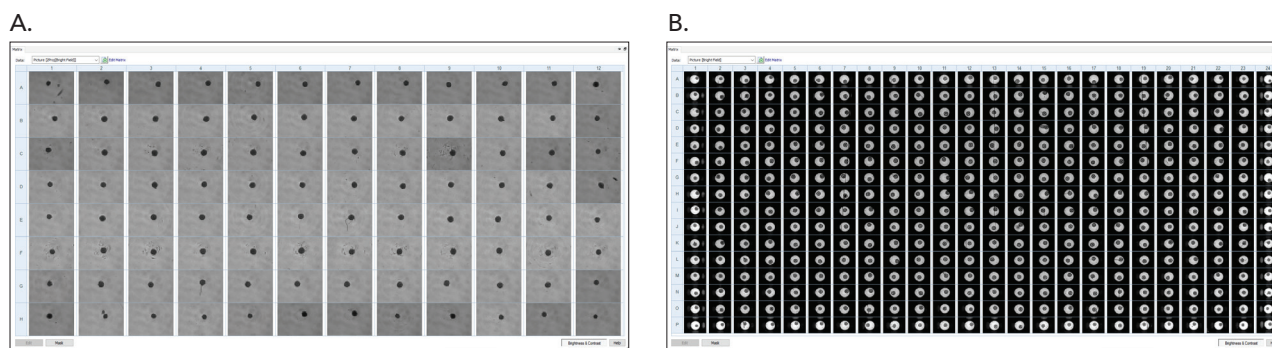


Figure 4. Brightfield images of (A.) 96-well, and (B.) 384-well Greiner spheroid microplates following 5x media exchange. Images from Corning and S-Bio microplates not shown.

Quantitative Validation of AMX Media Exchange Module

During the spheroid proliferation inhibitor dosing period, z-stacked brightfield images were captured kinetically using Cytation 5. Spheroid volume was then automatically calculated using Gen5™ Microplate Reader and Imager Software.

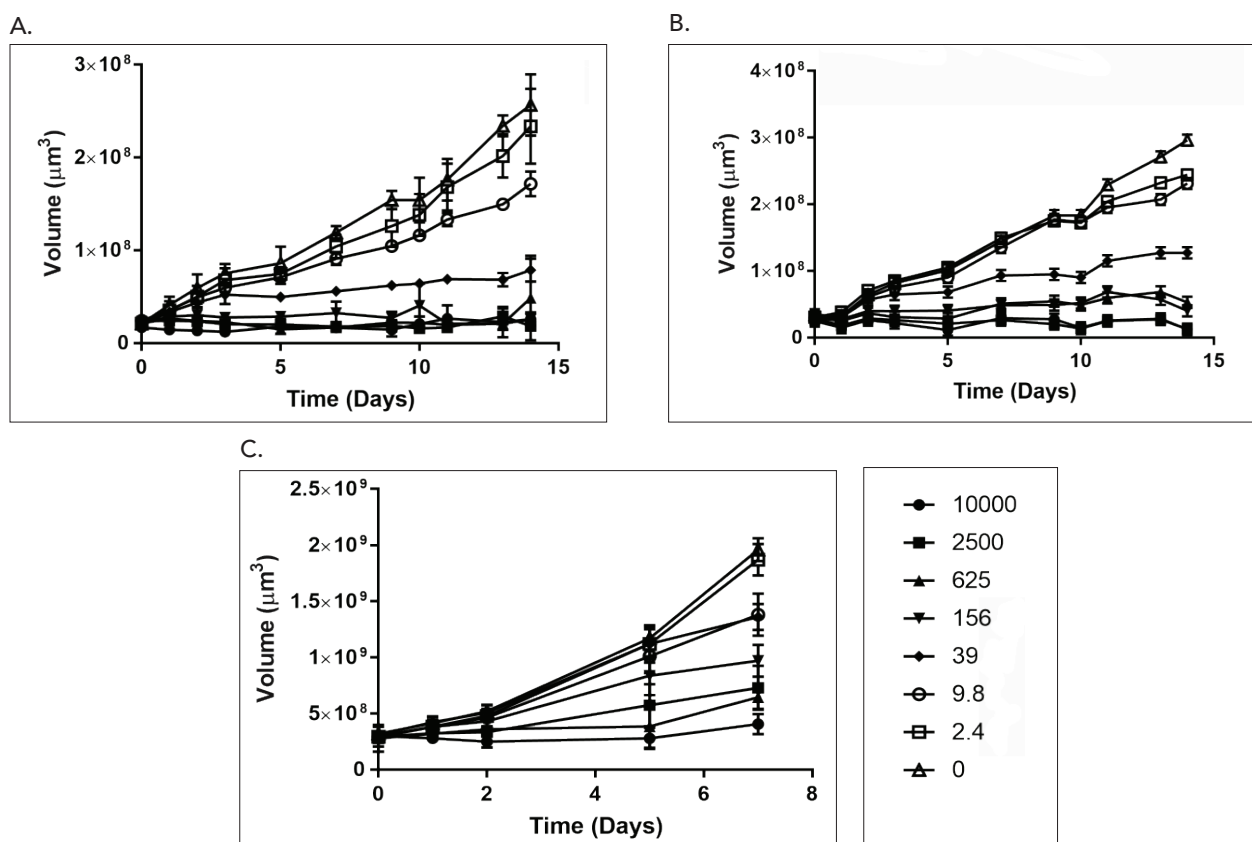


Figure 5. Automated kinetic spheroid proliferation results using AMX media exchanges. Calculated spheroid volumes following chronic exposure to varying camptothecin concentrations. (A.) U-87 glioblastoma spheroids in Greiner 96-well spheroid plates; (B.) HT-1080 spheroids in Corning 96-well spheroid plates; (C.) PANC-1 spheroids in 384-well spheroid plates.

Figure 5 demonstrates expected results with suitable experimental error where spheroids continue to propagate and increase in volume over time, in all cell models and microplate densities. Furthermore, the toxin camptothecin correspondingly interferes with spheroid propagation.

Conclusions

The MultiFlo™ FX with AMX Media Exchange Module effectively performs single and multiple media exchanges without disturbing unattached 3D spheroids in 96- and 384-well formats. When coupled with BioTek automation, the media exchange tool provides a walk away solution for long-term 3D experimental procedures.

References

1. Knight, E.; Przyborski, S. Advances in 3D cell culture technologies enabling tissue-like structures to be created *in vitro*. *J Anat.* **2015**, *227*, 746-756.