semarion

APPLICATION NOTE

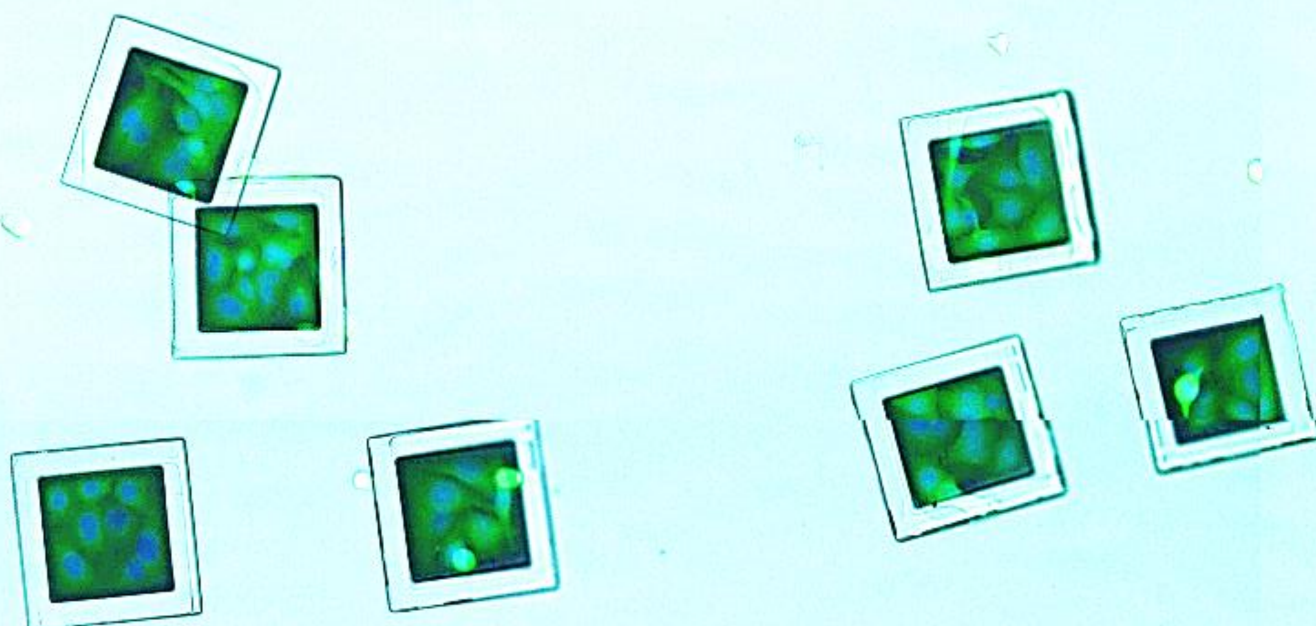
Enhancing Cell Assay Efficiency: Rapid Assessment of A549 Cells Frozen on SemaCyte® Microcarriers

Blaise Louis[1], Serena Belluschi[1], and Daniel Weekes[1]

[1] Semarion Ltd, UK

Abstract

The SemaCyte® microcarriers introduce a transformative approach to cell assay preparation. These microcarriers facilitate the cryopreservation of fully adherent A549 cells within standard cryovials. Upon thawing, these cells can be promptly dispensed into microplates and assayed within a mere 1-hour window, significantly streamlining cell assay setup. By minimizing cell preparation time, our method accelerates data throughput. Batch production of assay-ready cells ensures uniformity across longitudinal studies. A549 cells frozen on SemaCyte microcarriers exhibit robust post-thaw recovery rates and maintain healthy mitochondrial function. We demonstrate uniform and reproducible seeding onto SemaCyte® Seeding Dishes. Furthermore, our SemaCyte® workflow has been successfully deployed in various high-throughput (plate reader) and high-content (imaging) assays, as detailed in our other application notes.



Introduction

Cell assays typically utilise anchorage-dependent cells that are adhered to standardised multi-well plates. Frozen vials of cells are thawed and cultured to a desired confluency in tissue culture flasks, commonly over a period of multiple days or weeks (**Figure 1a**). This initial cell expansion phase and the requirement for surface attachment introduces a time-consuming and costly operational bottleneck to tissue culture workflows. Following expansion, cells are detached from the adhesive surface using a detaching solution such as trypsin or TrypLE™ and then seeded into microplates at a suitable density for the desired cell assay. As the cells typically need time to attach to the microplate and regain their native morphology after plating, this introduces further delay to the cell assay workflow. The process of culturing, detaching, and seeding cells between different cell assays furthermore leads to inter-experimental variability.

The SemaCyte® platform offers a solution for overcoming these limitations by introducing mobility, speed, and miniaturisation to adherent cell assays. SemaCytes are flat microcarriers with a 100 x 100 µm2 cell-growth area and cell-repellent walls. They function as ultra-miniaturised wells suspended in liquid that carry small colonies of adherent cells which can be moved with standard manual and robotic liquid handling tools and can be spatially oriented due to their magnetic properties. The SemaCyte® Seeding Dish (SD20) is a 20 cm^2 dish with 50,000 arrayed SemaCyte® microcarriers onto which cells can be seeded using standard culturing methods. When the desired confluency and morphology is achieved, the microcarriers are dislodged from the array with agitation and purified.

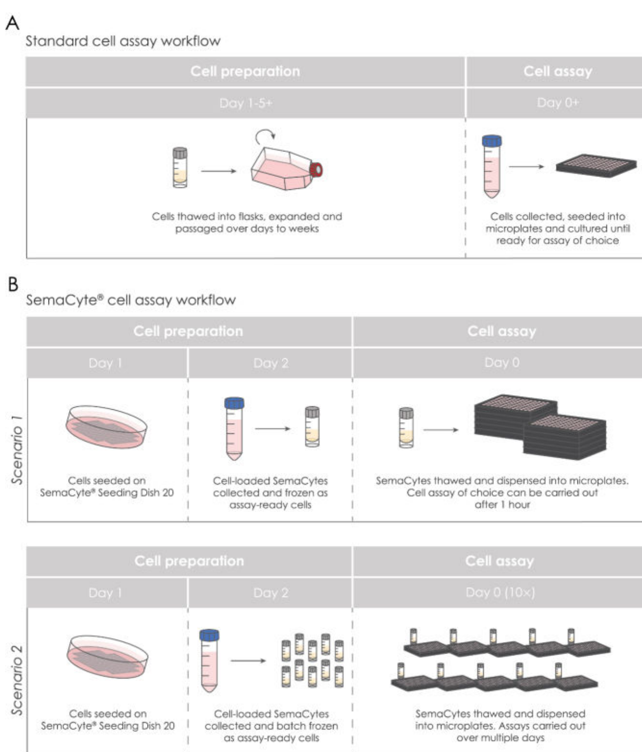


Figure 1. SemaCyte® microcarriers accelerate existing cell assay workflows and reduce variability. Schematic diagrams depicting (**A**) a standard cell assay workflow and (**B**) two SemaCyte® cell assay workflows. In a standard workflow, cells are thawed and passaged over a period of days to weeks before they are dissociated, collected, and seeded into microplates for the cell assay. Cells then need to attach and regain their native morphology, so they are cultured in the microplate until ready for the assay. In the first SemaCyte® cell assay workflow (scenario 1), cell-loaded SemaCytes are prepared by seeding cells onto an SD20 and then collecting and freezing them the following day as assay-ready adherent cells. To perform an assay, SemaCytes are thawed and dispensed into 2-10 microplates depending on the preferred density>They are assay-ready 1 hour post-thaw. In scenario 2, cell-loaded SemaCytes are batch frozen into multiple cryovials that can be thawed and dispensed into microplates over multiple days and cell-assays to ensure cells come from the same passage and seeding run.

Suspended, cell-loaded SemaCytes can be frozen in cryovials and then thawed as assay-ready cells that exhibit high post-thaw viability while maintaining their local confluency and adherent morphology. Thawed SemaCytes are dispensed into microplates and can be assayed within 1 hour, thus reducing cell preparation time and increasing assay throughput. SemaCytes from a single SD20 provide 2-10 microplates worth of experiments for high-throughput screening, high-content imaging, or other cell assays. Alternatively, cell-loaded SemaCytes can be aliquoted and batch frozen for future use across multiple experiments (**Figure 1b**).

Here, we demonstrate reliable and reproducible cell seeding, freezing, and thawing on SemaCyte® microcarriers using A549 cells. When seeded onto an SD20, A549 cells exhibit uniform loading into SemaCytes and can be frozen and thawed with high recovery rates and are assay-ready after 1 hour. This process reduces the total assaying time while enabling the generation of adherent cell batches deployed through longitudinal studies to reduce inter-experimental variability.

Key Advantages

Move Adherent Cells: Allow adherent cells to move without losing their natural morphology.

Ultra-Miniaturize Assays: Tailor the number of cells per well without compromising local confluency.

Assay-Ready Cells: Freeze and storing adherent cells in cryovials without detaching them.

Robust Storage and Recovery: Preserve adherent cell morphology and achieve high recovery rates (over 85%) after freezing and thawing.

Accelerate Assays: Thawed cells can be promptly dispensed and assayed within just 1 hour.

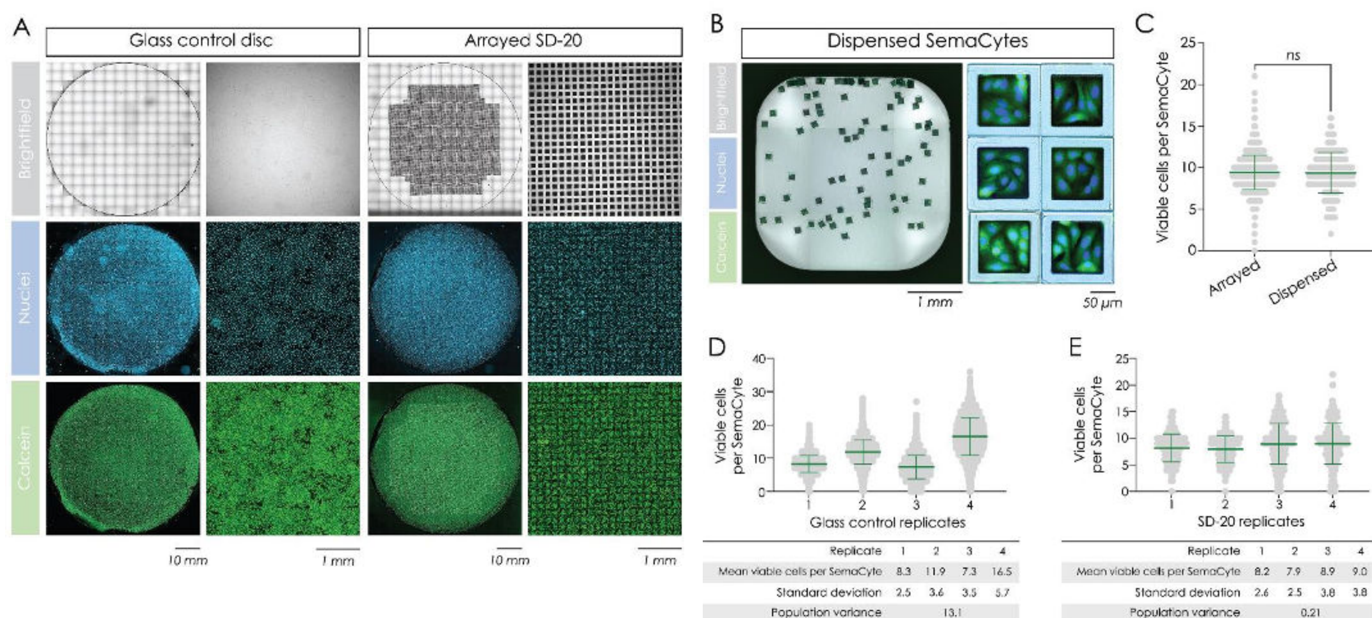
Mitochondrial Integrity: Preserve healthy mitochondrial function in frozen cells.

Consistent Performance: Batch production of assay-ready cells ensures uniformity across longitudinal studies.

Materials and Methods

A549 pulmonary adenocarcinoma cells were seeded into an SD20 at a density of 80,000 cells/cm^2 in RPMI-1640 cell culture medium supplemented with foetal bovine serum (FBS), sodium pyruvate, and penicillin/streptomycin and then cultured in a 37°C incubator for 24 hours. Cells were also plated onto blank glass discs of the same size as a seeding control. After 24 hours in culture, SD20s were agitated to release the cell-loaded SemaCytes into suspension, collected, and then purified by magnetic separation. SemaCytes were then resuspended in cryopreservation medium consisting of 10% DMSO in FBS, aliquoted into cryovials and frozen at a rate of 1°C/min in a -80°C freezer. The following day, some cryovials were transferred to liquid nitrogen storage and some were placed in cryovial storage boxes and retained in the -80°C freezer to allow for a direct comparison between storage methods.

After snap thawing the cryovials in a 37°C water bath, the cryopreservation medium was carefully removed and cell medium was added. SemaCytes were transferred to a 15 mL falcon tube and then placed in the incubator to allow the cells to recover for 1 hour. Cell-loaded SemaCytes were then dispensed into 384-well



Replicate	1	2	3	4
Mean viable cells per SemaCyte	8.3	11.9	7.3	16.5
Standard deviation	2.5	3.6	3.5	5.7
Population variance	13.1			

Replicate	1	2	3	4
Mean viable cells per SemaCyte	8.2	7.9	8.9	9.0
Standard deviation	2.6	2.5	3.8	3.8
Population variance	0.21			

Figure 2. Uniform and reproducible cell seeding on SD20s. A549 cells were seeded at a density of 80,000 cells/cm^2 and their viability assessed after 24 hours by labelling with AM-Calcein (green) and HCS Nuclear Mask (blue). Representative fluorescence images of cells on (**A**) a blank glass control disc, an unreleased, arrayed SD20, and on (**B**) SemaCytes dispensed into a 384-well plate. The number of viable cells per SemaCyte® was determined for (**C**) arrayed and dispensed microcarriers and the P-value determined using an unpaired t-test with Welch's correction (ns = $P > 0.05$). For (**D**) blank glass controls and (**E**) released microcarriers, the mean viable cells per SemaCyte was assessed over four experiments and the replicate standard deviation and population variance determined. A minimum of 200 SemaCytes were analysed for each replicate. For glass controls, repeated 100 x 100 µm^2 areas were analysed to determine the viable cells per SemaCyte® area. Lines and error bars represent the mean and the standard deviation for all graphs.

microplates and labelled with AM-Calcein and HCS Nuclear Mask Blue (both ThermoFisher Scientific) to determine the number of viable cells per microcarrier or PhenoVue 641 Mitochondrial stain (Revvity) to assess mitochondrial health. Cells were imaged using the ImageXpress® Pico Automated Cell Imaging System (Molecular Devices, 10× objective) and analysed with ImageJ processing software. For whole-disc and whole-well images, images were acquired at 4× and 10× respectively and then digitally stitched using CellReporterXpress Image Acquisition and Analysis Software (Molecular Devices). For blank glass control discs, ImageJ was used to generate masks of SemaCytes which were overlaid over the images to generate ROIs for analysis and comparison.

Uniform and reproducible cell seeding on SemaCyte® Seeding Dishes

To demonstrate the reproducibility of cell loading on SemaCyte® microcarriers across SD20s, we conducted benchmarking experiments using A549 cells. Blank glass discs were used as controls to assess cell seeding variability. After 24 hours in culture, we labelled the cells and then imaged the SD20 with arrayed, cell-loaded SemaCytes and the glass control disc to determine the distribution of AM-Calcein-positive viable cells across the entire surface (**Figure 2a**). We then released the SemaCytes into suspension and dispensed them into a 384-well microplate to determine the number of viable cells per 100 x 100 µm^2 microcarrier post-release (**Figure 2b**, **2c**). We did not observe a difference in cell numbers on arrayed and dispensed SemaCytes (P value = 0.7523), indicating that cells remain strongly adhered to the microcarriers after being released from the SD20, magnetically purified, and dispensed into microplates. For blank glass controls, we observed higher variability in the number of viable cells per 100 x 100 µm2 SemaCyte® areas across loading experiment replicates (Figure 2d) compared to dispensed SemaCyte® microcarriers (Figure 2e). This demonstrates a more uniform loading of cells onto SD20s than blank glass controls. SD20s reproducibly generate microcarriers loaded with healthy, viable cells that can be released and immediately used for cell assays or frozen for future use to produce assay-ready adherent cells.

SemaCyte® microcarriers generate assay-ready adherent cells 1-hour post-thaw

Cell-loaded SemaCytes can be batch frozen and thawed as assay-ready adherent cells for accelerating cell assay workflows and improving experimental reproducibility. To demonstrate this, we loaded an SD20 with A549 cells and determined the number of viable cells per SemaCyte® after 24 hours and then froze aliquots of cell-loaded microcarriers. We then thawed the cryovials and assessed the number of viable cells 1-hour post-thaw (**Figure 3a, 3b**). The cells maintained an adherent, spread morphology, and the average number of viable cells per SemaCyte® pre-freeze and post-thaw was 10.1 and 9.3, respectively. The recovery rates of the cells were consistently high (over 85%) for cryovials frozen from the same SD20 and for multiple SD20 replicates (**Figure 3c**).

To determine the impact of different cryogenic storage methods over longer periods of time, we compared post-thaw recovery rates of A549 cells at weekly intervals over a 4-week period of storage in a -80°C freezer and in liquid nitrogen. For both storage methods, the cells maintained their morphology at each thaw interval (2 and 4 weeks shown) and displayed high recovery rates (over 70%) on SemaCyte® microcarriers (**Figure 3d, 3e**).

Finally, we labelled cell-loaded SemaCytes with PhenoVue 641 Mitochondrial stain pre-freeze and 1-hour post-thaw to identify healthy mitochondria (**Figure 3f, 3g**). We did not observe a difference

in PhenoVue 641 intensity between fresh and thawed SemaCytes (P value = 0.7267), indicating that cells on SemaCytes are viable and assay-ready 1-hour post-thaw.

Conclusion

In this application note we have demonstrated how SemaCyte® microcarriers can be used to reproducibly generate adherent assay-ready cells that exhibit high post-thaw recovery. SemaCytes can be loaded and used immediately or batch frozen, thawed, and then dispensed into microplates. Cell-loaded SemaCytes are assay-ready 1 hour after thawing, displaying high viability and healthy mitochondria, thus reducing cell preparation time, increasing assay throughput and experimental reproducibility. The SemaCyte® platform seamlessly integrates into existing cell assay workflows and their unique properties allow for increased workflow flexibility. Whilst adherent assay-ready cells exist in the form of frozen cells attached to whole tissue culture plates, this requires large amounts of freezer storage space. SemaCyte® microcarriers turn adherent cells into liquid reagents that can be frozen into cryovials therefore creating an efficient storage solution.

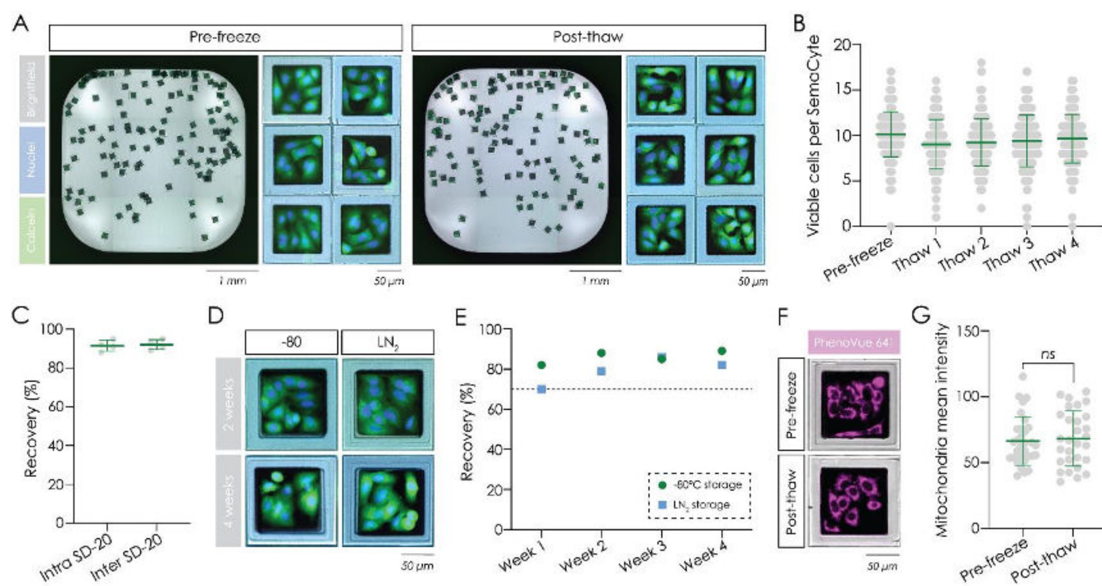


Figure 3. Frozen adherent cells on SemaCyte® microcarriers display high post-thaw viability and are assay-ready 1-hour post-thaw. (**A, B**) A549 cells were seeded on SD20s at a density of 80,000 cells/cm^2, released and purified after 24 hours and their viability assessed pre-freezing and 1-hour post-thaw by labelling with AM-Calcein (green) and HCS Nuclear Mask (blue). (**C**) The post-thaw recovery of cell-loaded SemaCytes from cryovials originating from the same SD20 and different SD20s was determined. Cryovials of SemaCytes were stored in a -80°C freezer and in liquid nitrogen (LN2) and thawed at weekly intervals over a 4-week period. (**D**) Cells on SemaCytes maintained their adherent and spread morphology after thawing (week 2 and 4 shown) and (**E**) displayed high rates of recovery (dotted line = 70%). (**F**) Cells were labelled with PhenoVue 641 (pink) pre-freeze and post-thaw to assess mitochondrial health and (**G**) the mean fluorescence intensity was plotted. The P-value was determined using an unpaired t-test with Welch's correction (ns = P > 0.05). Lines and error bars represent the mean and the standard deviation for all graphs.