PLATFORM INSIGHT

SemaCyte® Cell Assaying Microcarriers: Turning Adherent Cells into Liquid Reagents to Enhance Screening Workflows

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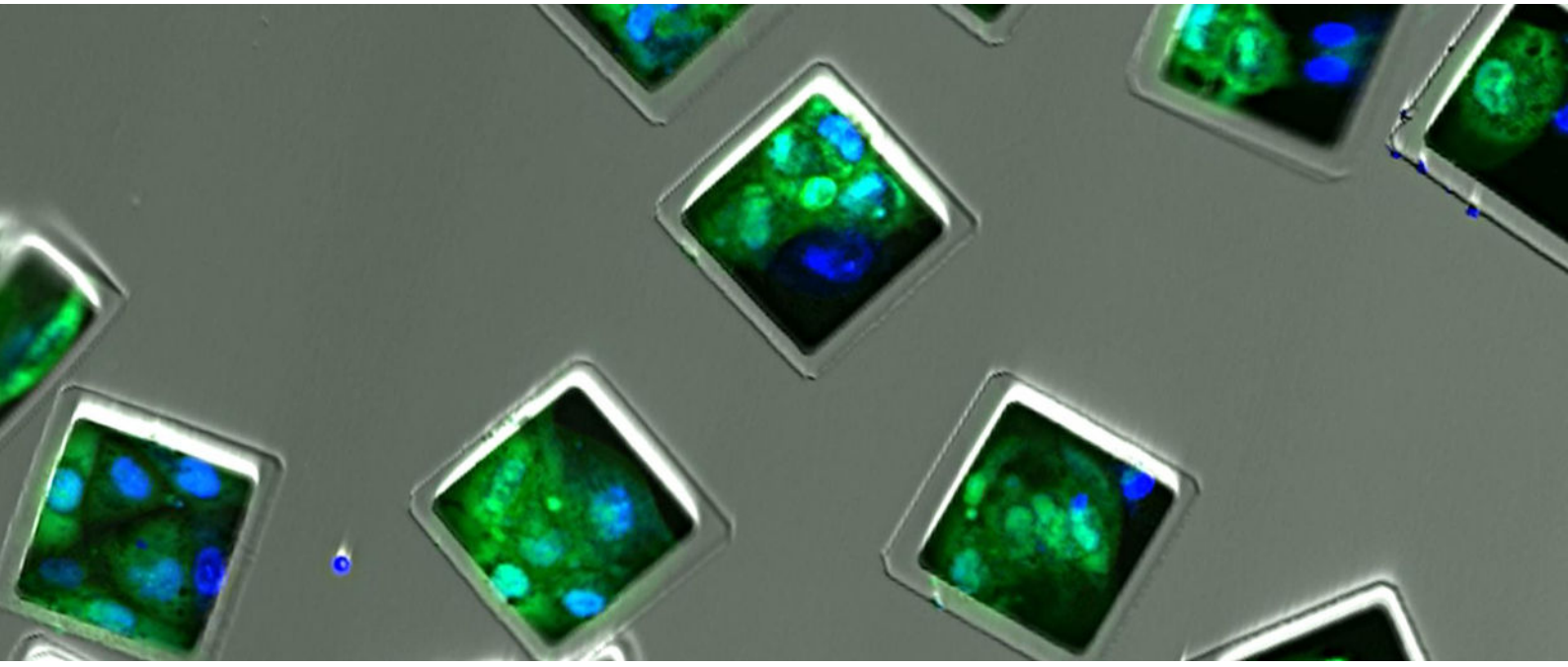
Abstract

Adherent cell assay workflows are often limited by the cells' anchorage dependence. SemaCyte® Cell Assaying Microcarriers can turn these cells into liquid reagents to facilitate novel experimental paradigms. These flat microcarriers function as ultra-miniaturized wells suspended in liquid. They can carry small colonies of adherent cells and are magnetically steerable. Using this platform technology, it is possible to create assay-ready adherent cells, multiplex multiple cell types together, and drastically reduce the number of cells per assay. These materials are compatible with typical cell handling and analysis equipment and facilitate better and faster data generation for cell screening workflows.



Limitations of Cellular Assays

Cellular assays are crucial in life science research. They offer an ex-vivo approach to study biological processes, to evaluate drug compounds for potency, toxicity, and mechanism of action, and to diagnose patients and evaluate drug responsiveness.

Most mammalian cell types relevant for life sciences research are adherent in nature, i.e. they need to be attached to a surface to remain viable and healthy. In typical cell culture, cells form a 2D layer by attaching to the bottom of well plates (**Figure 1**). Cells are loaded into the wells in a suspension format and are then allowed to attach to the plate surface over the course of 24-48 hours.

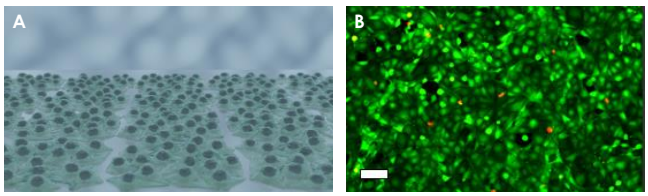


Figure 1. Standard Cell Assays in Wells. *(A) Adherent cells as currently used in life sciences research. (B) RCC-FG2 cells after 48h in well plates, stained with AM-Calcein (viable, green) and EthD1 (dead, red). Scale bar: 100 µm.*

Relevant experimental conditions, e.g. drug molecules, are added to the wells and then the cells' response is tested in the form of an assay, i.e. the addition of a reagent that is sensitive to a phenotypic (e.g. cell death) or molecular change (e.g. protein activation) in the cell. Generally, cell types studied include immortalized cell lines, disease models, and patient-derived primary cells.

Currently, the 384-well plate based assay workflow dominates drug discovery. The drive to optimize this workflow has generated a suite of automated liquid handling tools to streamline the culturing and plating of cells and the addition of drug molecules, as well as automated imaging, plate reader, and cytometry tools for the rapid assaying of each well in a 384-well plate.

Despite large gains in efficiency through automation, there are fundamental bottlenecks to further improving drug discovery workflows with adherent cells. The core pain point hampering efficiency improvements is the need for adherent cells to be attached to a surface to be in their native state. This anchorage dependence causes workflow challenges in three core application areas:

(1) a minimum of 24 hours is required in cell preparation in well plates, i.e. cell seeding, plate attachment, and morphological change, which severely limits the throughput of rapid cell assays,

(2) the need to plate different cell types in different wells makes conducting drug screens against large cell panels extremely labour and resource intensive, and

(3) experiments on more biologically relevant, but often scarce, cells such as patient-derived biopsies or induced pluripotent stem cells (iPSCs) cannot be done at scale because culture plates, even 384-well plates, require large amounts of cells.

The SemaCyte® platform aims to address these key challenges of adherent cell assaying workflows by turning adherent cells into liquid reagents and usher in a new cell screening paradigm.

Turning Adherent Cells into Liquid Reagents

SemaCytes are flat microcarriers which function as ultra-miniaturized wells suspended in liquid. At ~100 µm in lateral size, they can carry small colonies of adherent cells, typically about 10 live cells each.

The size and shape of these microcarriers is tailored through precise microfabrication techniques. This process also allows for the incorporation of optical barcodes, which can be read with a 10x optical microscope, for true cell multiplexing. Cell-loaded SemaCytes can be magnetically steered in liquid because they contain a magnetic heterostructure. Using an external magnetic field, the microcarriers can be tilted, sorted, and immobilised, without any magnetic aggregation (**Figure 2**).

Adherent cells loaded on the microcarriers experience the same local confluency environment as they would in a well plate, but can now be pooled, split, frozen, and titrated in their adherent state. This simple yet elegant approach addresses the most fundamental bottleneck to the workflow inefficiencies associated with this class of cells - the static nature of their attachment to a surface.

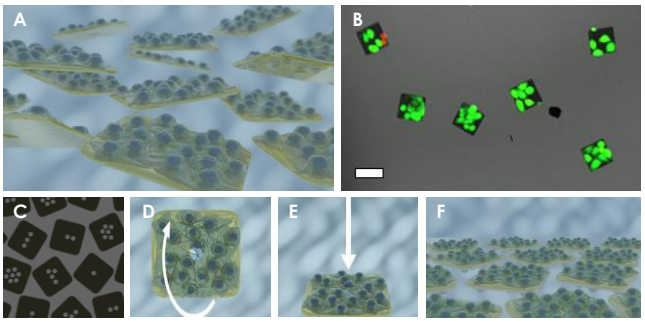


Figure 2. Cell Assays on SemaCytes. *(A) Adherent cells as liquid reagents on steerable microcarriers. (B) RCC-FG2 cells after 48h on SemaCytes®, stained with AM-Calcein (viable, green) and EthD1 (dead, red). Scale bar: 100 µm. Cell-containing microcarriers can be (C) optically barcoded, (D) tilted, (E) sorted, (F) and immobilised into well plates.*

This fundamental change to adherent cell handling gives the SemaCyte® platform the potential to offer a 10x improvement to the three key workflow challenges in this space:

(1) frozen assay-ready adherent cells to reduce the cell preparation time per assay by >10x to less than 2 hours,

(2) cell-multiplexing or the ability to run an experiment on 10 cell types in the same culture well as opposed to a single cell type, and

(3) a reduction in cell numbers needed per assay by 10x from ~5,000, typical for 384-well plates, to 200-500.

SemaCytes are compatible with typical liquid handling tools, plate readers, and microscopy systems. Standard cell-culturing protocols and well-plate based assays can be performed using this microcarrier platform, with the benefit of producing better data, faster. The platform also makes it possible to develop completely novel cell assaying methodologies, e.g. those using flow-based methods.

Cell Loading and Assaying with SemaCytes

Immobilized as arrays on slides called Seeding Discs, SemaCytes can be loaded with cells as per standard cell culture protocols. Briefly, cells are cultured in flasks and stripped to create a single cell suspension. The desired density of cells is then added onto the SemaCyte® Seeding Disc where they attach to the immobilised microcarriers. The cells are incubated until they achieve the desired confluency and morphology. Through slight agitation of the Seeding Disc, the cell loaded SemaCytes are released into liquid and then collected into a tube (**Figure 3**).

Semarion has developed its own proprietary cell-attachment polymer which is covalently attached to the SemaCytes for faster and more stable cell attachment. Other binding moieties can also be attached to the surface of the SemaCytes, e.g. poly-D-lysine, polyethylineimine, fibronectin, and antibodies.

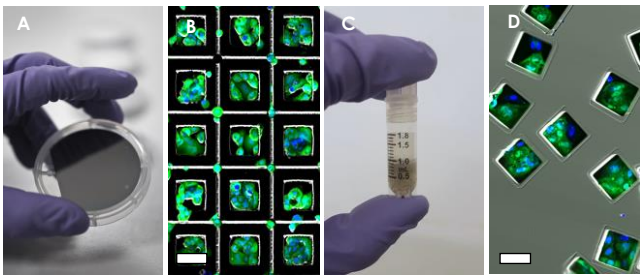


Figure 3. Loading and Assaying of SemaCytes. *(A) Seeding Discs are placed in a Petri-dish and (B) the cell type of interest is seeded on top. The microcarriers are immobilised and the cells grown until the desired confluency and morphology is achieved. Mild agitation releases the cell-containing SemaCytes from the Seeding Disc. (C) These are collected for freezing or titration into a multi-well plate for assaying. (B, D) RCC-FG2 seeded at 50K/cm² and released after 24h, stained with AM-Calcein (viable, green) and HCS Nuclear Mask (nucleus, blue). Scale bars: 100 µm.*

Cell-containing SemaCytes can be frozen as assay-ready adherent cells for later use or directly titrated into multi-well plates for assaying. Using a magnetic plate holder, the SemaCytes are pulled down to the bottom of the well where they are immobilised.

Initial validation work for the platform was done using different cancer cell lines, such as T98G (human glioblastoma), RCC-FG2 (human renal cell carcinoma), and MCF-7 (human breast cancer) cells. After release, SemaCytes were incubated for up to weeks and assayed using plate readers and microscopes. Cell proliferation, viability, and cytotoxicity assays on plate readers included MTT, MTS, LDH, AM-Calcein, EthD1, CellTitre-Fluor™, and CytoTox-Fluor™. Cell viability, morphology and phenotype were evaluated through brightfield and fluorescence microscopes on both live and fixated cells, e.g. by using immunocytochemistry stains for membrane, cytosol, or nuclear markers or viability stains such as TUNEL, AM-Calcein, and EthD1.

To improve image analysis, single SemaCyte® microcarrier images can be digitally isolated for downstream analysis and quantification. In this case, each carrier with ~10 cells represents one datapoint. When various cell types are multiplexed, software deconvolutes the optical barcode for each analysed microcarrier.

Assay-Ready Adherent Cells for Rapid Screening

With the SemaCyte® Seeding Disc, it is possible to generate assay-ready adherent cells for rapid plate-reader based experiments. Users can now seed large batches of cells, grow them until the desired confluency and morphology is achieved, collect the cell-containing SemaCytes, and batch freeze them for later use. Frozen aliquots of adherent cells on SemaCytes can be snap thawed for accelerated rapid assay workflows and better experimental reproducibility (**Figure 4**). This approach negates the need to plate single cell suspensions and to wait for them to achieve the desired morphology and confluency. This can furthermore reduce batch-to-batch variance which can be seen when cells are seeded and grown. Although assay-ready cells have emerged recently to tackle this issue, no convenient solution is currently available to freeze and store cells in an adherent format.

This approach is particularly relevant for drug candidate profiling and structure-activity-relationship (SAR) studies during lead validation and optimisation. The key bottleneck in these workflows is the slowness and poor reproducibility of cell-based assays on newly synthesized compounds. These studies typically deploy rapid plate-reader based screens for potency, selectivity, toxicity, and mechanism-of-action.

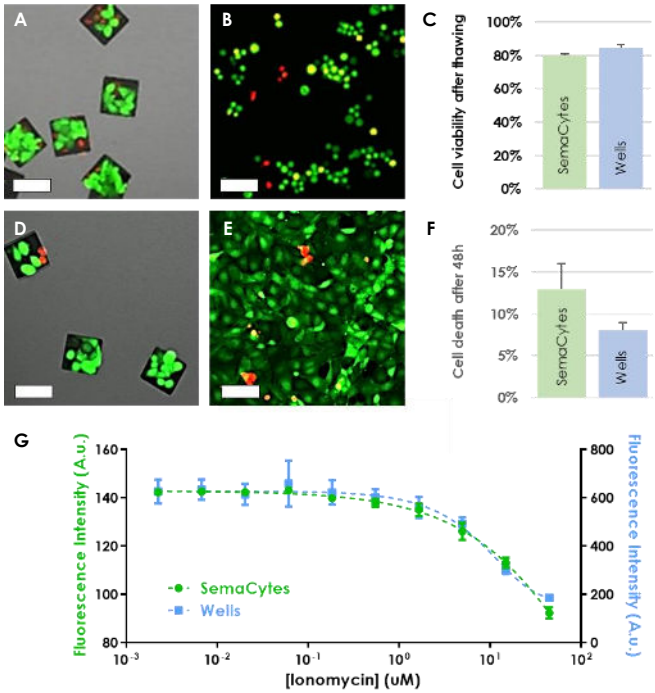


Figure 4. SemaCyte® Assay-Ready Cell Screening. *(A) RCC-FG2 cells on SemaCytes thawed and (B) compared to thawed suspension cells. (C) Post-thaw cell viability (AM-Caclein-EthD1 assay) was comparable. (D) Cells grown on SemaCytes and (E) in well plates had similar morphology. (F) The rate of cell death (LDH assay) was comparable. Image stained with AM-Calcein (viable, green) and EthD1 (dead, red). Scale bar: 100 µm. (G) 24h ionomycin dose-response on thawed SemaCytes vs standard wells (CellTitre-Fluor™ assay).*

True Cell Multiplexing for Screening of Cell Panels

Using barcoded SemaCytes, it is now possible to pool 10 different cell types together into a tube or well to accelerate screens on larger cell panels. Akin to trans-well systems, the different cell types remain spatially separated, although they can interact via paracrine signalling. Seeding Discs, each with differently barcoded SemaCytes, can be loaded with different cell types of interest. Once released into suspension, these barcoded cell-containing SemaCytes can be pooled together and stored at -80°C or used directly for assaying, e.g. drug incubation experiments. The optical barcodes are deconvoluted through microscopy and image analysis software (**Figure 5**). These cell multiplexing assays are thus designed for imaging-based workflows.

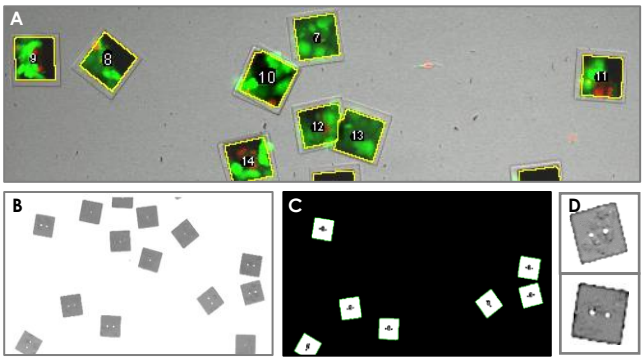


Figure 5. SemaCyte® Digital Isolation and Multiplexing. *(A) Individual microcarriers can be digitally isolated for image analysis. (B-D) SemaCytes are optically barcoded by incorporating holes. (B) Using brightfield images specific barcodes can be (C) digitally isolated and (D) the brightfield and fluorescence images individual microcarriers with that code be extracted.*

By combining 10 cell types into one well, e.g. of a 384-well plate, it is possible to increase the rate of data output for multi-cell screening workflows. It also drastically decreases the assay cost-per-datapoint which is particularly relevant for drug positioning studies and IND enabling studies, e.g. in oncology, where large panels of cells are typically screened. Other areas where this type of multiplexing could be beneficial is for complex multi-cell co-culture experiments.

Titrate Carriers to Reduce the Cells-per-Well

The number of cells added per well can be drastically reduced using the SemaCyte® technology. Using manual or robotic liquid handling tools, a specific amount of microcarriers, and thus cells, can be loaded into a well. This enables adding much less cells to a given well than typically necessary. Given that individual cells still see a locally high confluency, i.e. the confluency on a single microcarrier, they will behave like a normal cluster or colony. By carefully titrating cells into a multi-well plate it is possible to substantially reduce the number of cells per assay. For example, a 384-well plate typically contains 5,000 cells per well. For imaging end-points, less than 200 cells are typically imaged (**Figure 6**). With the SemaCytes it is possible to add ~200 cells per well, i.e. around 20 microcarriers, reducing the number of cells-per-well by 25-fold.

Ultra-miniaturization of cell assays can unlock broader screens for scarce cell types such as patient biopsies and iPSCs. This approach could, for example, enable genome-wide siRNA or CRISPR screens against patient-derived cells to identify novel drug targets for high unmet clinical needs. It could also facilitate the use of patient-derived cells during earlier phases of the drug screening cycle to provide more biologically relevant insight and ultimately reduce drug attrition rates.

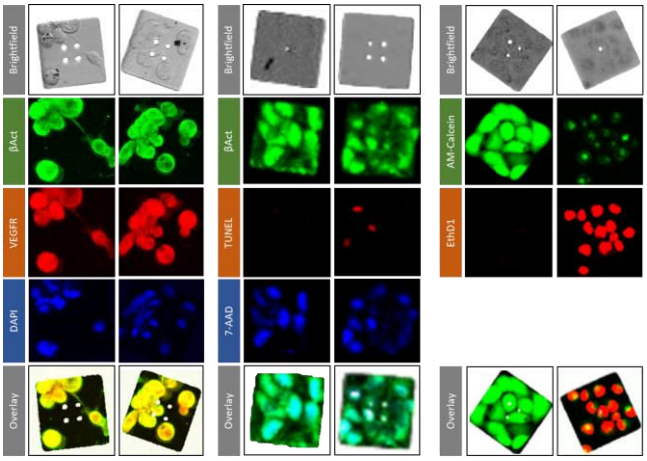


Figure 6. SemaCyte® Phenotypic Analysis of Small Cell Numbers. *RCC26-A cells were attached to SemaCytes, released into suspension, and titrated into 96-well plates. The image analysis software was able deconvolute optical barcodes, isolate individual microcarriers, and analyse fluorescence intensities across a variety of imaging channels. These assays included ICC staining against β-Actin and VEGFR, nuclear sta8ining using DAPI and 7-AAD, viability staining using AM-Calcein, and toxicity staining using TUNEL and EthD1. Each carrier is 100 µm x 100 µm in lateral size.*

Conclusion

SemaCyte® cell assaying microcarriers can overcome various bottlenecks in cell screening workflows. Specifically designed to integrate with existing assaying workflows, these materials enable novel features such as the assay-ready adherent cells for faster and more reliable outputs, true cell multiplexing to combine 10s of cell types into one well, and ultra-miniaturization to reducing the number of cells-per-assay. While the key features of the platform have been validated, Semarion is further developing specific applications.

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