PLATFORM INSIGHT

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SemaCyte® Microcarriers Material Properties: Microfabrication, Nanomagnetism, Smart Materials, and Surface Engineering

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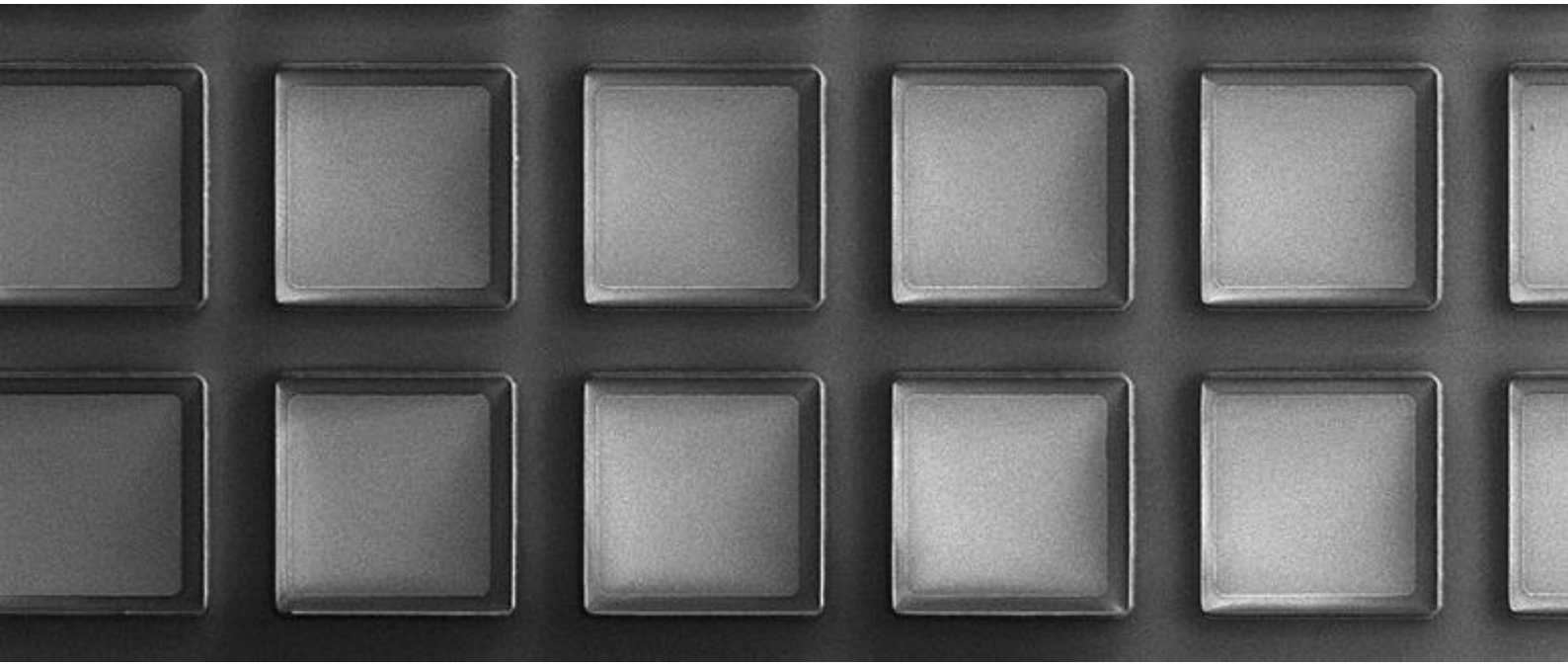
Abstract

Semarion has developed a new class of magnetic microparticle engineered with a range of unique physical and chemical properties that are ideally suited for carrying and assay adherent cells. By deploying microchip fabrication techniques, it is possible to make arrays of highly tailored microcarriers which can be conveniently loaded with live cells. The materials are biocompatible due to a gold cap and cells attach to a proprietary synthetic extracellular matrix components on top of the carriers. A smart release polymer dissolves in cell culture media to release cell-loaded SemaCyte® carriers into suspension. Once in liquid, they can be controlled with an external magnetic field due to a magnetic heterostructure inside the carriers. Together, this creates a carrier platform suitable to interface with cell screening workflows such as those in drug discovery.



From Microcarrier Beads to SemaCytes

SemaCyte® cell assaying microcarriers are designed to carry small colonies of adherent cells into suspensions and to seamlessly integrate with existing cell assaying workflows. These planar microcarriers are multi-material composites made using microfabrication, nanomagnetism, smart materials, and surface engineering. These manufacturing techniques enable fine control over the carrier shape, structure, and behaviour. Arrays of SemaCytes can be loaded with adherent cells as per standard cell culturing protocols. Cell-containing carriers can subsequently be titrated into multi-well plates, making them compatible with most microscopy and plate-reader assay workflows.

Other microcarriers technologies are bead-based, for example Corning® Microcarriers or Cytiva's Cytodex. These colloidal microparticles typically have a diameter of over 100 µm and are made from polymers such as polystyrene or dextran. They capture adherent cells to increase the growth surface area in bioreactors for the manufacturing of biologics such as antibodies and enzymes. While well suited to upscale biomanufacturing, these microcarriers cannot facilitate cell assaying workflows. They are generally limited in compatibility with cell seeding protocols, cryostorage, multi-step liquid exchanges in assay protocols, and imaging endpoints for assays.

The SemaCyte® platform is specifically designed for integration with drug discovery cell screening workflows and provides unique benefits such as cell multiplexing, ultra-miniaturization of assays, and assay-ready adherent cell freezing.

Silicon Based Microfabrication

Manufacturing in the semiconductor industry is characterized by planar or two-dimensional patterning techniques on silicon wafers, i.e. large smooth discs of silicon. This fabrication approach creates miniaturized circuit components on the surface of the wafer which are then connected. These integrated circuits led to the development of the microchip and the computing revolution. Semarion uses the same principles of precise, two-dimensional patterning techniques to create the SemaCytes.

Patterned in arrays on silicon or glass slides called SemaCyte® Seeding Discs, the SemaCyte® microcarriers are carefully engineered to interface with living cells and perform bio-assays (**Figure 1**). Our materials system is built as a set of layers, each of which serves a crucial role in the platform's functioning. These layers include a smart release polymer, a structural photopolymer, a magnetic heterostructure, a functionalizable gold cap, and a cell-attachment polymer.

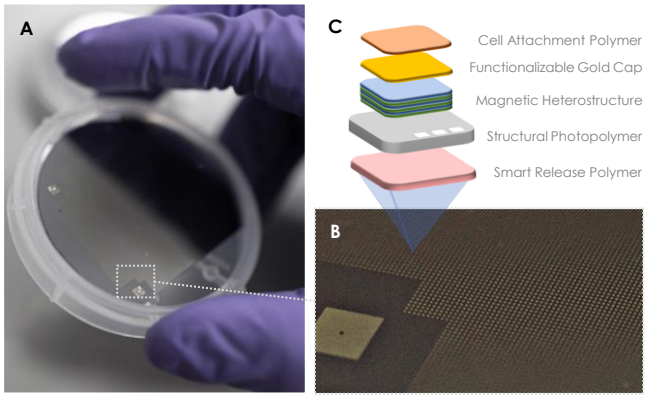


Figure 1. Microchip-based Fabrication to Create SemaCyte® Microcarriers. *(A) A 2- inch diameter silicon SemaCyte® Seeding Disc. (B) A zoomed in picture of the Seeding Disc shows the array of SemaCytes. (C) A schematic of the layered structure of each SemaCyte®.*

The SemaCyte® Seeding Discs can be manufactured in sizes ranging from 1 to 8 inches in diameter. A typical 2-inch disc contains ~50,000 SemaCytes with a 100 µm x 100 µm cell growth area each. This format provides enough materials to load a 96-well plate at a density of ~500 microcarriers per well, each carrying ~10 live cells for a total of 5,000 cells per well.

The Smart Polymer for Slow Release

The SemaCyte® microcarriers are anchored to the Seeding Disc with a smart release polymer. This bottom layer dissolves in cell culture medium during cell loading to release the SemaCytes into suspension after the cells have attached (**Figure 2**). This layer is applied via a process known as spin-coating, where a small amount of the polymer solution is added to the Seeding Disc. The Seeding Disc is then spun at high speeds (1,000-5,000 rpm) spreading the solution across the Seeding Disc surface using centrifugal force to create a uniform thin film (typically ranging from 0.5-2 µm thick) of the polymer. The solubilized polymer is fully biocompatible and does not affect cell viability. After the SemaCytes are released, any remaining soluble polymer is removed by medium exchange.

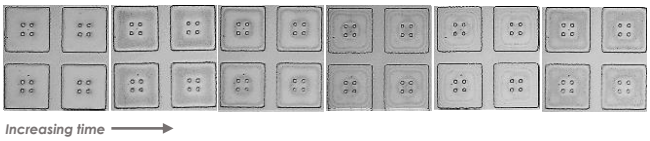


Figure 2. Releasing the SemaCytes from the Seeding Disc. *The slow dissolution of a smart release polymer allows adherent cells to settle and attach before the carriers are released. The dissolution of the polymer is characterized by a changing light scattering pattern under each SemaCyte®, here observed with a reflection microscope. These specific carriers are 100 µm x 100 µm in size and are barcoded with 4 holes.*

Optical Lithography to Define the Shape and Barcode

The microcarrier structure is built atop the smart release polymer and uses a structural photopolymer backbone. The photopolymer allows for the lateral definition of the SemaCytes and the incorporation of optical barcodes (indicated by the three holes in this layer in **Figure 1**) using a technique known as photolithography. The photosensitive polymer is spin-coated onto the smart release polymer, and the lateral size, shape, and barcodes of each carrier is patterned using light, akin to how a film negative is used to develop a picture. The areas of the polymer exposed to light are crosslinked, while the unexposed areas are removed in a solvent known as a developer. This leaves behind an array of identical microcarrier structures on the Seeding Disc that are then engineered into SemaCyte® arrays in subsequent processing (**Figure 3**).

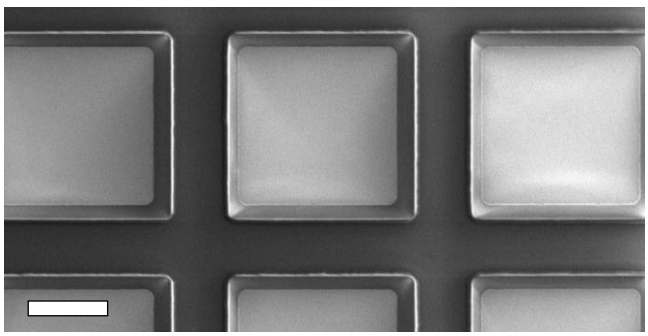


Figure 3. Identical Size and Shape of Each SemaCyte® in the Array. *A scanning electron micrograph of an array of SemaCytes on a Seeding Disc shows the precisely defined 2-dimensional shape of the SemaCytes. Scale bar: 50 µm.*

SemaCytes can be made into a variety of shapes with lateral sizes of 20-200 µm, allowing them to either carry single cells or small colonies. Depending on the barcoding protocol, 1,000s of uniquely marked SemaCytes can be created. These codes can be viewed using a simple brightfield microscope at 10x magnification and do not interfere with cell attachment or assaying (**Figure 2**, **5**).

Thin Film Nanomagnetism to Steer the Microcarriers

A set of metallic layers are incorporated onto each microcarrier using a thin film physical deposition technique known as magnetron sputtering. A target material is bombarded by ions from a plasma, which ejects atoms from the material. These atoms are uniformly deposited on nearby surfaces, in this case the patterned array of SemaCytes. This allows control of the thickness of each layer deposited to ~0.5 nm (**Figure 4**). By depositing sub-nanometre layers of various metals in a thin film heterostructure, unique magnetic properties are achieved. Each SemaCyte® has a North and South magnetic pole which correspond to its top and bottom face, set with a high coercive field akin to a hard magnet. This perpendicular magnetic anisotropy arises from spin-orbit coupling at the interfaces of the magnetic and non-magnetic thin films in the heterostructure.

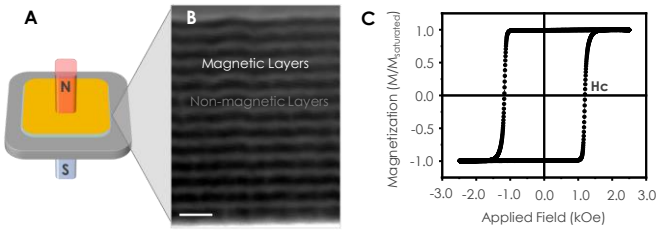


Figure 4. Magnetic Properties of the SemaCytes *(A) Each SemaCyte® behaves as a strong permanent magnet with a defined pole corresponding to the top and bottom face. (B) A transmission electron micrograph cross-section of a thin film structure like that used in the SemaCytes. Thin films of alternating magnetic (light) and non-magnetic (dark) sets of layers create a "magnetic heterostructure". Individual layers can be as thin as 0.5 nm. Scale bar: 20 nm. Figure courtesy of the Cowburn Lab, University of Cambridge. (C) A magnetic hysteresis loop of a magnetic thin film like that used in the SemaCytes. A high coercive field (Hc) and strong remanent magnetization allow SemaCytes to be uniquely magnetically manipulated in liquid.*

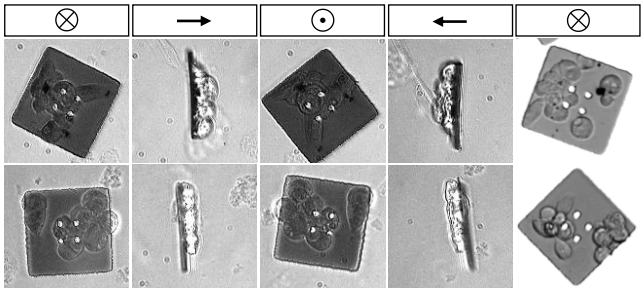


Figure 5. Orientating Cell-Containing SemaCytes with an External Magnetic Field. *The magnetic heterostructure allows unique 3D orientation of the carriers in suspension. Imaging was done with a standard inverted (row 1-4) or confocal (row 5) microscope in bright field at 10x magnification. SemaCytes were 100 µm x 100 µm and loaded with RCC-FG2 cells.*

While responding strongly to external magnetic fields, they do not magnetically interact with each other. This allows magnetic tilting, sorting, and immobilising while preventing carrier-to-carrier magnetic aggregation. These ground-breaking features facilitate careful control of the carrier orientation and location to enable SemaCyte® assay workflows (**Figure 5**).

Top Surface Designed to Interface with Cells

A gold capping layer on the microcarriers is also deposited via magnetron sputtering and renders each SemaCyte® biocompatible. It furthermore enables convenient functionalization with a variety cell attachment polymers and bio-molecules, such as poly-D-lysine (PDL) and antibodies, via physisorption or covalent binding of amine or thiol group to gold. The gold layer provides significantly higher fluorescent signal compared to well plate plastic due to its reflective nature (**Figure 6**).

Semarion developed its own proprietary Synthetic Extracellular Matrix (ECM) polymer which is used to coat the SemaCytes. Compared to well-plates or slides coated with PDL, it provides significantly faster and stronger cell attachment while facilitating a more rapid change to an adherent morphology (**Figure 6**).

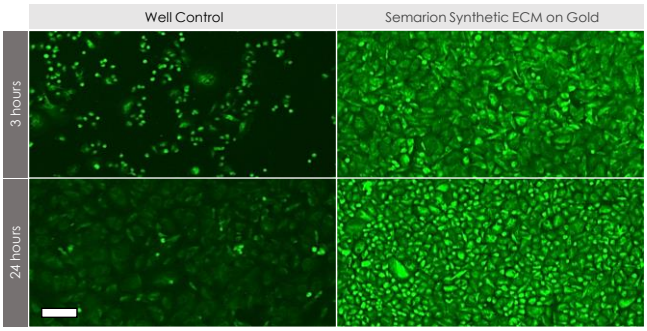


Figure 6. Rapid Cell Attachment and Morphology Change on Semarion's Proprietary Cell Attachment Polymer. *Beta actin staining (green) of RCC-FG2 cells grown on well plate plastic and Semarion's Synthetic ECM on gold. In addition to excellent cell attachment and morphology after simply 3 hours, Semarion's cell interfacing materials also provide significantly higher fluorescence signal.*

Conclusion

The SemaCyte® technology platform leverages 2D optical patterning techniques from the semiconductor industry combined with novel nanomagnetic materials, smart materials, and surface chemistry to create microcarriers uniquely suited for interfacing with cells. This platform serves to overcome key bottlenecks for cell assays including adherent cell freezing, assay miniaturization, and cell-multiplexing.

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