Constructing vesicle-based artificial cells with embedded living cells as organelle-like modules

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There is increasing interest in constructing artificial cells by functionalising lipid vesicles with biological and synthetic machinery. Due to their reduced complexity and lack of evolved biochemical pathways, the capabilities of artificial cells are limited in comparison to their biological counterparts. We show that encapsulating living cells in vesicles provides a means for artificial cells to leverage cellular biochemistry, with the encapsulated cells serving organelle-like functions as living modules inside a larger synthetic cell assembly. Using microfluidic technologies to construct such hybrid cellular bionic systems, we demonstrate that the vesicle host and the encapsulated cell operate in concert. The external architecture of the vesicle shields the cell from toxic surroundings, while the cell acts as a bioreactor module that processes encapsulated feedstock which is further processed by a synthetic enzymatic metabolism co-encapsulated in the vesicle.

The construction of membrane-encapsulated artificial cells from the bottom up is one of the cornerstone themes in biomimetic biotechnology. One avenue of research centres on functionalising lipid vesicles with biological and synthetic machinery in order to engineer artificial cells that resemble their biological counterparts in form and function1–6. Due to their biocompatibility and ability to incorporate biological components to impart function, the potential of vesicle-based artificial cells as soft-matter microdevices is considerable, with applications in directed evolution, protein synthesis, in vivo diagnostics, biosensing, drug delivery, and in situ drug synthesis7–15.

Biological cells, in contrast to their artificial counterparts, have evolved a complex set of biochemical pathways, which makes them capable of dynamic behaviours and of performing an array of tightly regulated functions. They exhibit defined responses to a range of diverse stimuli, and have access to a collection of metabolic pathways. The capabilities of biological cells are thus inherently more advanced than synthetic ones generated from the bottom up.

Herein, as a key step to bridge this divide, we present a cellular bionics approach where living and non-living components are integrated to yield hybrid systems. We apply this approach to vesicle-based artificial cells: whole biological cells are embedded inside functionalised vesicles for them to perform functions as organelle-like modules. We thus create a new breed of artificial cells that are constructed by fusing cellular and synthetic components in a single self-contained vesicular entity (Fig. 1). Crucially, the encapsulated living cell and the artificial cell host are chemically as well as physically linked together by coupling cellular reactions to enzymatic reactions co-encapsulated inside the vesicle.

Although vesicles have previously been functionalised with biological and synthetic machinery (including membrane channels15,16, enzymes17, DNA origami18, quantum dots19, and cell-free protein expression systems20–22), functionalisation with whole, intact, biological structures (i.e. cells and organelles) has not been achieved. There have been many efforts at encapsulation of cells in droplets23, but this is not true of cell-mimetic vesicles. This is an important milestone as vesicles, unlike droplets, have the potential to be used in physiological (aqueous) environments as artificial cells and soft-matter micro-devices with functionalised membranes. The presence of a lipid membrane as an encapsulating shell also paves the way for the incorporation of membrane-embedded machinery (e.g. protein transporters, mechanosensitive channels, photopolymerisable lipids) and for the utilisation of membrane phase behaviour to impart functionality.

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Technologies for efficient encapsulation of large, charged chemical species in vesicles have been developed in recent years using the strategy of using water-in-oil droplets as templates around which vesicles are assembled. This principle has been extended to encapsulate nano- and micro-sized particles, including proteins, beads, and cells, although characterisation of particle encapsulation number and vesicle size distribution was limited. Crucially, these investigations did not involve a demonstration of the use of the encapsulated materials as active functional components in the context of artificial cells.

Others have engineered communication pathways between co-existing populations of biological and artificial cells, an approach which allowed the sensory range of bacteria to be expanded to detect molecules they would otherwise be unable to detect. A similar effect was achieved by engaging the quorum sensing mechanism of bacteria. However, although these demonstrate the potential of linking artificial cells to biological cells for expanded functionality, there have still not been any demonstrations of living and synthetic cells operating in concert within a single hybrid structure.

In this paper, we develop microfluidic technologies to construct hybrid cells. These are composed of biological cells that serve an organelle-like function, encapsulated in artificial vesicle-based cells. We demonstrate a symbiotic relationship between the vesicle host and encapsulated cell.

We show that the cell is shielded from the external surroundings, and is viable in a solution of Cu²⁺ which would otherwise be toxic. Conversely, we demonstrate that the cell can be used as a bioreactor module to process chemical feedstocks in the vesicle interior. A reaction sequence composed of three cellular and non-cellular steps is employed (Fig. 1), with the cell performing the first step and co-encapsulated enzymes performing the second and third steps. The reaction employed is as follows: (i) hydrolysis of lactose feedstock by the cell to produce galactose, (ii) oxidation of galactose product, yielding H₂O₂, (iii) enzymatic oxidation of fluorogenic molecule by H₂O₂ for a fluorescent readout. In this way, the encapsulated living cell and the artificial cell host are chemically as well as physically linked together in a cellular bionic system.

**Results and Discussion**

**Microfluidic Generation of Hybrids.** We construct hybrid cells using droplet microfluidics, enabling the vesicle to have user-defined physical and chemical features and with a defined number of encapsulated cells (see Fig. 2 for schematic and microscopy images; see SI for details). We developed a two-module experimental setup to generate cells-in-droplets which were then converted into vesicles. The first module consisted of a microfluidic chip to generate water-in-oil (w/o) droplets with encapsulated cells. Droplets were generated on-chip in high-throughput (2200 droplets min⁻¹) using a hydrodynamic flow focusing geometry, leading to break up of the aqueous phase into discrete w/o droplets. The aqueous channel contained cells in medium (L-15 with 125 mM sucrose), and the oil phase contained dissolved 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine lipids (POPC; 2 mg ml⁻¹) in mineral oil, which self-assembled along the water-oil interface, encasing droplets in a monolayer.

Droplets flowed through a serpentine to allow sufficient time for effective droplet stabilisation by a lipid monolayer, otherwise they would merge upon making contact with one another. Droplets then entered an observation chamber for analysis before being expelled from the device, and fed into a second module consisting of an emulsion phase-transfer column, which used the droplets as templates around which lipid vesicles were assembled.

The emulsion phase transfer column consisted of a cylindrical PDMS well on a glass slide with a lower aqueous phase (100 mM glucose in L-15 media) and an upper oil phase (2 mg ml⁻¹ POPC in hexadecane). Again, a lipid-monolayer assembled at the interface. Due to the presence of sucrose, droplets generated by the device were denser than the aqueous and water phase of the column which contained glucose. They thus descended through the column under gravity and transferred from the upper oil phase to the lower aqueous phase (Fig. 2B). As they crossed the interface, a second monolayer wrapped around the lipid-encased droplets, resulting in a surrounding bilayer. In this way the w/o droplets were transformed into w/w vesicles, with the internal contents maintained.
Cell encapsulation. A bacterium and several eukaryotic cell lines were successfully encapsulated in vesicles, with various encapsulation numbers (Fig. 3), including *Escherichia coli* DH5α, BE colon carcinoma cells, HCT colon carcinoma epithelial cells, and suspension Toledo B lymphocyte suspension cells. As they are well characterised, and due to their large size (enabling single-cell encapsulation), BE cells were used for all subsequent...
and interference with important cellular events. Three-step biochemical pathway, with the first step being performed by the encapsulated cell, and the final two components, revealed a 3.5 fold increase in fluorescence over 300 minutes, further confirming the coupled reaction cascade was operational. This was attributed to trace amounts of lactose and β-galactose remaining in the medium, photo-activated oxidation of AUR and non-specific light-mediated vesicle rupture on the surface. A viability reagent (NucGreen) was present in both the internal and external environments.

**Encapsulated cell as an organelle-like reactor.** Constructing vesicle/cell hybrids raises the possibility of using the encapsulated cells as a bioreactor modules within vesicle-based artificial cells. This would allow the coupling of cellular to non-cellular pathways co-encapsulated in the vesicle. To demonstrate this, we devised a three-step biochemical pathway, with the first step being performed by the encapsulated cell, and the final two steps performed externally from the cell by enzymes present in the vesicle interior (Fig. 5). The first step was the hydrolysis of lactose to glucose and galactose by β-Galactosidase (β-gal) expressed by an engineered BE cell designed to express β-gal. This was followed by translocation of glucose out of the cell, and its subsequent oxidation by Glucose Oxidase (GOx) in the vesicle interior, producing H2O2. The final step was oxidation of Amplex Ultra Red (AUR) by H2O2 in the presence of horseradish peroxidase (HRP) to yield the fluorescent resorufin end product. The use of AUR as opposed to its analogue, Amplex Red, was due to its membrane impermeability allowing its accumulation to be monitored in the vesicle.

Cell shielding by vesicle host. One prospective application of hybrid artificial cells relates to the vesicles capacity to shield its interior including the real cell from the external environment, which may be toxic. We explored this using a fluorescence assay to monitor the viability of cell-in-vesicles with Cu2+ present in the external solution (Fig. 6). Cu2+ was present at levels known to lead to cell death due to initiation of oxidative damage and interference with important cellular events.

Cells were generated as before, but with a passivating layer of BSA on the glass substrate to prevent ion-mediated vesicle rupture on the surface. A viability reagent (NucGreen) was present in both the internal and external environments.
external vesicle solutions, and CuCl₂ was added to the external solution after generation to a final concentration of 5 mM. Fluorescence was monitored over a period of 120 minutes to determine levels of cell death \( n > 120 \). Over 66% of cells-in-vesicles were viable, compared to 6% in the control scenario of unencapsulated cells, a statistically significant difference (unpaired t-test p-value = 0.0002), indicating that the vesicle effectively shielded the cell from membrane impermeable species⁴¹,⁴². Cells-in-vesicles had lower viability than those present in bulk solution without the presence of copper in identical conditions (98% viability), presumably due to the effects of confinement, including the build-up of waste products in the vesicle interior⁴³. This limitation can be addressed in future through the addition of selective protein pores that allow the expulsion of waste products, but prevent the inflow of harmful materials. The lack of influx of Cu²⁺ to the POPC GUV interior was confirmed through a permeation assay involving the Cu²⁺ sensitive dye calcein (see SI 9).

We note that the ability of vesicles to shield cells is highly dependent on the physicochemical properties of the molecule in question (e.g. the LogP), and of the permeability the vesicle membrane itself⁴⁴,⁴⁵. Large and/or charged biomolecules (e.g. enzymes, nucleic acids, DNA)⁴⁶ are largely impermeable to phospholipids which makes this system particularly useful in physiological settings.

**Longevity of hybrid cells.** A key indicator of cellular activity is their ability to replicate, a metric which was used to assess the potentially deleterious effects of confinement. Cells-in-vesicles were monitored over a period of 24 hours. Encapsulated _E. coli_ were seen to increase in number over this time (Fig. 6E). This ability to replicate shows that the effects of confinement, potential nutrient deprivation, and stress resulting from microfluidic generation do not significantly interfere with cell biology in this case. However, replication was not observed with the eukaryotic cell-lines despite them being metabolically active. This could be due to the higher sensitivity and reduced robustness of these cells compared to _E. coli_, which could lead, for example, to an arrest in the cell cycle and potential confinement-induced genetic re-programming via cellular self-signalling, as have been observed in other systems⁴⁷. Their effective concentration could also mean that they are also close to the cell growth curve plateau⁴⁸. The presence of cells inside the vesicles did not affect the stability or integrity of the membrane. The vesicle remained intact a week after generation, and still successfully carried encapsulated cargo over this time (SI S8).
Summary and Conclusion. We have developed and characterised microfluidic technologies to encapsulate biological cells in vesicle-based artificial cells, thus creating hybrid structures composed of synthetic and cellular modules. The use of microfluidics allows the design of hybrids with user engineered features. Tuning droplet features using microfluidics is well-established by altering parameters such as device geometries and flow rates, and as droplets act as templates around which vesicles are assembled, these features are transferred to the vesicle in turn. Hybrid cells were produced at high throughput and with control over vesicle size, biomolecular content, and cell encapsulation number. The experimental setup also exposes the technique to varied advantages of microfluidic technologies, including low sample size, increased efficiency, and amenability to multiplexing, automation, and translation into portable lab-on-chip devices.

A conceptual parallel can be drawn between hybrid cells constructed here and eukaryotic cells, which originated through an evolutionary process of endosymbiosis where formerly free-living organisms were subsumed into their host. Likewise, the encapsulated cell and vesicle-based artificial cell host exhibit a mutually beneficial relationship: the vesicle shields the cell from toxic surrounding, and the cell provides the vesicles access to cellular biochemistry in a distinct organelle-like sub-compartment.

Viewed in the context of vesicle-based artificial cells, the encapsulation of biological cells allows them to serve modular functions. In the experiments above, the cell performed one step of a reaction sequence, with subsequent steps carried out by non-cellular components, thus combining cellular biochemistry with artificially devised chemistry in a compartmentalised system. The fact that vesicles remained intact days after they were generated, and cells were viable and able to reproduce inside the confined environment, further cements the potential of such hybrid systems.

This approach paves the way for the richness and versatility of cell biology to be interfaced with artificial cells generated from the bottom up, which could in turn lead a new generation of functional devices and materials. Such systems could find applications in cell therapy, in chemo-enzymatic hybrid cascades, in the study of confinement on biological systems, and in cell-based sensors.

Materials and Methods

Full experimental details are provided in the Supporting Information. Briefly, cells-in-vesicles were prepared using a PDMS-based microfluidic device that was fabricated using standard soft-lithography techniques. Water-in-oil droplets were formed where the oil phase consisted of 2 mg ml\(^{-1}\) POPC in mineral oil and the water droplets contained BE cells (ca. 4 \times 10^7 cells ml\(^{-1}\); L-15 media; 3 mM EDTA). Droplets were then converted into...
vesicles using the emulsion phase transfer method. This method relies on a density difference between the vesicle interior and exterior in order for vesicles to descend through the column under gravity. As such, vesicle interior contained sucrose (125 mM) and vesicle exterior contained glucose (100 mM), with the vesicles withstanding the resulting slight osmotic imbalance.

Unless otherwise noted, BE human colon carcinoma cells were used and cultured using Dulbecco’s Modified Eagles Medium (DMEM, no glucose) and 10% (v/v) foetal bovine serum (FBS) in a cell incubator. Other cells successfully encapsulated were HCT colorectal carcinoma cells, suspension Toledo B lymphocyte suspension cells, and E. coli. When conducting experiments cells were harvested up to four days following plating, and kept in L-15 media. In vesicle bioreactor experiments cells were engineered to express β-gal by transfection with Lipofectamine 3000, pSV-β-Galactosidase transfection vector, and Opti-MEM media, according to the supplier’s protocol.

For the organelle bioreactor experiments, BE cells expressing β-gal were mixed 1:1 with a reaction mix containing 125 mM sucrose, 100 µM lactose, 1000 µM AUR, 2 U ml⁻¹ GOx, and 0.2 U ml⁻¹ HRP. Fluorescence levels were monitored over time on a fluorimeter (Cary Eclipse; λex = 572 nm, λem = 583 nm). For fluorescence microscopy experiments, cells-in-vesicles were generated with the above reagent mix encapsulated in the vesicle interior, and monitored with 100 ms exposure. In the control experiments, lactose was absent from the reagent mix, and non-transfected cells were used.

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Author Contributions

Y.E., D.W., T.T., L.D., K.P., R.V.L. and O.C. designed the experiments and wrote the manuscript. Y.E. and D.W. carried out experiments with eukaryotic cells and Y.E., T.T., and L.D. carried out experiments bacterial cells. Y.E., T.T., and D.W. analysed the data.

Additional Information

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